joint meeting of the

International Society for Chemical Ecology

and the

Chemical Signals in Vertebrates group

# **ISCE-CSiV**

July 8–12, 2014

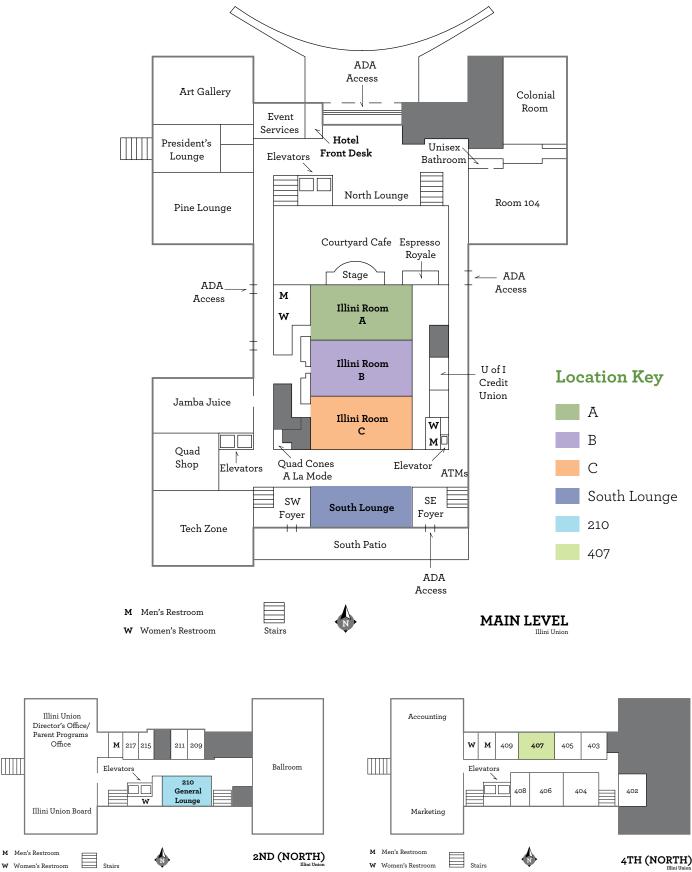
University of Illinois at Urbana-Champaign

www.life.illinois.edu/isce-csiv



### Illini Union

Meeting locations: Illini Rooms ABC, Room 210 (General Lounge), and Room 407



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International Society of Chemical Ecology

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> July 8–12, 2014 University of Illinois at Urbana-Champaign www.life.illinois.edu/isce-csiv

## Welcome!

Welcome to the twin cities of Urbana and Champaign, in the heart of east central Illinois. In case you're wondering, they're not identical twins; their births were separated by about 30 years. Urbana dates back to 1822, when William Tompkins built the first homestead on the site that 11 years later was officially platted as a new city, whereas Champaign didn't come into existence until 1852, when the Illinois Central Railroad laid tracks two miles outside of Urbana and residents who settled close by rejected annexation efforts and instead incorporated their own city a few years later. Despite the fact that the two cities now share many services (as well as Wright Street, the official dividing line) and are cooperative and cordial with each other, referenda to merge the two into a single metropolis have been, since 1855, consistently and resoundingly defeated. The courthouse in Urbana, the permanent county seat for Champaign County, was frequented in the 1840s and 1850s by a young lawyer who practiced law in the 8th Judicial Circuit. In 1854, this lawyer-named Abraham Lincoln-delivered one of his first public speeches against slavery in the county courthouse in downtown Urbana.

#### About the University of Illinois at Urbana-Champaign

The lives of the residents of Urbana and Champaign were changed forever when, in 1867, the Illinois Industrial University, later to become the University of Illinois at Urbana-Champaign, was chartered.

The University of Illinois at Urbana-Champaign is a comprehensive, major public university that is ranked among the best in the country. Founded in 1867 as a state-supported, land-grant institution under the terms of the Morrill Land Grant Act of 1862 with a threefold mission of teaching, research, and public service, the UIUC opened its doors to students in 1868 and rapidly earned a reputation as an educational institution of international stature. Today, scholars and educators rank it among a select group of the world's great universities. For more information, see

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the university Website (**www.illinois.edu/about/ about**).

UIUC is a residential campus of classrooms, laboratories, libraries, residence halls, and recreational and cultural facilities with, 200 major buildings on the central campus of 1,783 acres. Nearby are the University's 1,650-acre Willard Airport; Robert Allerton Park, the campus's 1,768-acre nature and conference center; and 3,600 acres of agricultural land. An additional 3,700 acres of farmland elsewhere in Illinois are used by the College of Agricultural, Consumer, and Environmental Sciences as experimental fields. The University also owns close to 1,000 acres of natural areas within a 50-mile radius of the campus, held for the express purpose of conducting ecological research.

#### About the 2014 ISCE-CSiV Meeting

The campus has a long history of hosting scientific meetings, dating back at least to 1890, when the first meeting of the American Association of Economic Entomologists was held in Urbana. The 2014 meeting of the International Society of Chemical Ecology is the 30th annual meeting of the society, the first held in the United States since the 2008 meeting at Pennsylvania State University and the first held jointly with Chemical Signals in Vertebrates group (www. wku.edu/csiv), which is holding its 13th triennial meeting. These two groups, dedicated to understanding the role of chemical communication in the lives of organisms, share a common history. The Chemical Signals in Vertebrates group originated in a 1976 symposium in Saratoga Springs, NY; individuals with a shared interest in the subject then began a series of triennial meetings that continues today. The idea for founding an international society of chemical ecology was proposed at the first Gordon Conference on Plant-Herbivore Interactions, held in 1980 in Santa Barbara, California, co-organized by Paul Feeny of Cornell University and Gerald Rosenthal of University of Kentucky. After the second Gordon Conference on Plant-Herbivore Interactions, in 1983,

the Executive Committee convened a meeting to define ISCE's functions and to plan the first meeting of the new Society in Austin, TX for 1984. At that meeting, vertebrates featured prominently; among the 130 participants at the first meeting was Dietland Muller-Schwarze, a founding member of CSiV. The 30th anniversary of the first meeting (and, as well, the 40th anniversary of the founding of the *Journal of Chemical Ecology*, long regarded as the definitive journal in the field) thus provides an opportunity to evaluate progress over the course of three generations of chemical ecology research.

As for the venue, UIUC has a special place in the history of chemical ecology. It was during his tenure as a professor of entomology at UIUC, spanning 1948 to 1972, that Gottfried Fraenkel recognized the significance of phytochemicals in the diets of herbivorous insects; in his landmark paper in Science, Fraenkel (1959) introduced the concept of chemically mediated reciprocal radiation (coevolution) and recounted seven decades of empirical support for the idea. As well, toxicologist and UIUC alumnus Robert Metcalf returned to the campus in 1968 and during his 30-year tenure pioneered ways of applying chemical ecology toward sustainable pest management, using kairomones to reduce insecticide applications for controlling corn rootworms from pounds per acre to ounces per acre. In the Department of Chemistry, William Pirkle's pioneering work on chromatographic enantioseparation provided new tools for understanding chiral pheromones and other natural products. Today, UIUC is home to life scientists across the campus using new genomic tools, computational methods and chemical analytical techniques to explore mechanisms underlying many phenomena of interest to chemical ecologists. In addition to departmental programs, the UIUC Institute for Genomic Biology, is an international center for insect genomics research. Other unique campus research resources include SoyFACE, a 60-acre facility instrumental in documenting the chemical ecology of global climate change in agriculture, the world's largest public university library (with 24 million-plus items in its catalogue), and Blue Waters, the petaflop supercomputer that is the fastest anywhere in the world on a university campus), capable of over 13 quadrillion calculations per second.

## The Story Behind the "Elemoth" Logo

The logo for the 2014 joint meeting of the International Society of Chemical Ecology and the Chemical Signals in Vertebrates (ISCE-CSiV 2014) is a fictional chimeric creature that could be called an "elemoth" -- part Asian elephant (Elephas maximus) and part cabbage looper (Trichoplusia ni). The elemoth embodies the fact that there are constraints on structure and composition of signal chemicals such that the same substance may be produced by wildly disparate organisms in very different ecological contexts. (Z)-7-dodecenyl acetate, a component of the sex pheromone of over 140 species of moths, including the cabbage looper Trichoplusia ni, also is produced as a urinary preovulatory femaleto-male pheromone in the Asian elephant (Rasmussen et al. 1997). Subsequent analyses of urine from African elephants (Loxodonta africana) have detected the bark beetle aggregation pheromones frontalin, exobrevicomin, and endo-brevicomin and the aphid alarm pheromones (E,E)-[]-farnesene and (E)-[]-farnesene (Goodwin et al. 2006). The basic requirements for life are in force irrespective of the environment—obtaining nutrients, escaping predation, and reproducing-and most primary metabolic pathways are shared across organisms, so biosynthetic starting materials tend to be conserved throughout the living world. Moreover, all organisms are constrained and directed by the same basic ecological and evolutionary processes. Thus, chemical identities and transmission dynamics can converge across taxonomic gulfs and many basic principles pertaining to chemical signaling emerge. The elemoth seemed to us to be an apt symbol for a gathering of scientists dedicated to identifying the substances, characterizing the transmission dynamics and elucidating the mechanisms underlying chemical mediation of interactions among organisms.

Goodwin, TE, MS Eggert, SJ House, ME Weddell, BA Schulte and LEL Rasmussen, 2006. Insect pheromones and precursors in female African elephant urine. J. Chem. Ecol. 32: 1849-1853.

Rasmussen, LEL, TD Lee, A Zhang, WL Roelofs, and GD Daves Jr, 1997. Purification, identification, concentration and bioactivity of (Z)-7-dodecen-1-yl acetate: sex pheromone of the female Asian elephant, *Elephas maximus*. Chem. Senses 22:417-437.

## **Committee Members**

#### **ISCE Scientific Organizing Committee**

May Berenbaum, University of Illinois at Urbana-Champaign Ring Cardé, University of California, Riverside Larry Hanks, University of Illinois at Urbana-Champaign John Hildebrand, University of Arizona Monika Hilker, Freie Universität Berlin Stephen Foster, North Dakota State University Jocelyn Millar, University of California, Riverside Ann Ray, Xavier University Stefan Schulz, Technische Universität Braunschweig

#### **CSiV Scientific Organizing Committee**

Bruce Schulte, Western Kentucky University Thomas Goodwin, Hendrix College

#### **University of Illinois Local Arrangements Committee**

May Berenbaum, Chair Larry Hanks, Vice Chair Catherine Dana, Student Member Tania Jogesh, Student Member

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## **General Information**

### **Registration Desk Hours**

The registration desk is located at the Illini Union outside the Illini Rooms ABC, except as noted.

Tuesday, July 8:	5:00-9:00 PM (South Lounge)
Wednesday, July 9:	7:00 AM-4:30 PM
Thursday, July 10:	7:00 AM-4:30 PM
Friday, July 11:	7:00 AM-4:30 PM
Saturday, July 12:	7:00 AM-2:30 PM

Your registration includes the conference program, the opening reception on Tuesday, morning and afternoon refreshment breaks Wednesday-Saturday, lunch on Thursday and Friday, and the closing reception on Saturday. Tickets for the dinner banquet on Friday were available for purchase online with your conference registration. A very limited number of tickets may be available for purchase at the registration desk during the conference.

A message board will be available by the registration desk.

### **Meeting Venues**

**Illini Union** (primary location) 1401 W. Green St., Urbana, IL 61801 217-333-4666 www.union.illinois.edu

**Noyes Laboratory** (film festival) 505 S. Mathews Ave., Urbana, IL 61801

Alice Campbell Alumni Center (dinner banquet) 601 S. Lincoln Ave., Urbana, IL 61801 217-333-1471

## **Conference Hotels**

**Illini Union** (primary location of meeting) 1401 W. Green St., Urbana, IL 61801 217-333-1241

#### Illinois Street Residence Hall (ISR)

918 W. Illinois St., Urbana, IL 61801 217-333-1766

#### Hampton Inn

1200 W. University Ave., Urbana, IL 61801 217-337-1100

#### Urbana Landmark Hotel

210 S. Race St., Urbana, IL 61801 217-384-8800

#### **Comfort Suites**

2001 N. Lincoln Ave., Urbana, IL, 61801 217-328-3500

#### Holiday Inn

1001 W. Killarney St., Urbana, IL 61801 217-328-7900

#### **Hilton Garden Inn** 1501 S. Neil St., Champaign, IL, 61820 217-352-9970

#### Crowne Plaza Chicago O'Hare (for those coming

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in to Chicago before the meeting) 5440 N. River Rd., Rosemont, IL 60018 877-337-5793

### **Transportation**

Most of the conference hotels are either within walking distance of the Illini Union or have their own shuttle for trips to campus.

#### Tuesday, July 8

#### Peoria Charter Coach Bus to University of Illinois Campus from O'Hare International Airport (Chicago)

#### Departure Time: 3:00 PM

Departure Location: O'Hare Airport Bus/Shuttle Center, located on the ground level of the Main Parking Garage (Level 1 near Elevator Center 3)

Arrival Time: Approximately 6:00 PM

Drop-off Locations: Comfort Suites/Holiday Inn, Illini Union, ISR, Urbana Landmark Hotel, Hampton Inn, and Hilton Garden Inn

-or-

#### Peoria Charter Coach Bus to University of Illinois Campus from the Field Museum (Chicago)

Departure Time: 3:00 PM

Departure Location: Field Museum campus

Arrival Time: Approximately 6:00 PM

Drop-off Locations: Comfort Suites/Holiday Inn, Illini Union, ISR, Urbana Landmark Hotel, Hampton Inn, and Hilton Garden Inn

#### Sunday, July 13

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#### Peoria Charter Coach Bus to O'Hare Airport (Chicago) from University of Illinois Campus

Departure Locations and Times: Hilton Garden Inn (6:40 AM), Hampton Inn (6:50 AM), Urbana Landmark Hotel (7:00 AM), Comfort Suites/Holiday Inn (7:10 AM), ISR (7:20 AM), Illini Union (7:30 AM)

Arrival Time: Approximately 10:30 AM

Drop-off Location: O'Hare International Airport Bus/ Shuttle Center, located on the ground level of the Main Parking Garage (Level 1 near Elevator Center 3)

#### Champaign-Urbana MTD Bus System

Champaign-Urbana has a great public transportation system, with several stops on campus and near the conference hotels. The 27 Air Bus runs between Willard Airport in Savoy and the Illini Union several times a day. A standard ride costs \$1, one-way, with free transfers from route to route. Only coins and \$1 bills accepted. Drivers can make change for up to \$5 prior to 7:00 PM. Exact cash is required after that time. Visit www.cumtd.com for maps and schedules, or call 217-384-8188.

#### Other Bus and Train Services

Peoria Charter coach buses depart from campus and the Illinois Terminal in Champaign daily (800-448-0572 or www.peoriacharter.com). Greyhound buses also depart from the Illinois Terminal daily (217-352-4150 or www.greyhound.com). A few Amtrak trains serve Champaign-Urbana via the Illinois Terminal in Champaign (www.amtrak.com).

#### Taxi Service

Yellow Checker Cab, 217-355-3553

**Orange Taxi,** 217-363-1500

Green Taxi, 217-721-5533

Quality Limo & Taxi, 217-552-7400

#### Willard Airport

11 Airport Rd., Savoy, IL 61822 • 217-244-8618

## Parking

Parking at and around the meeting venues is limited. There are some meters that take quarters located in small lots or on the surrounding streets. If you are willing to walk a few blocks, you may be able to find free parking in the residential area east of the Illini Union, just past Lincoln Avenue. If you are staying at the Illini Union Hotel, you will be given a free parking permit. Parking is free and plentiful at the other conference hotels.

### Emergency Numbers and Procedures

#### Emergency (Police, Fire, or Ambulance): 911

#### Non-Emergency:

University Police: 217-333-1216 Urbana Police: 217-384-2320 Champaign Police: 217-351-4545

#### Medical Assistance:

Carle Foundation Hospital: 217-383-3311

Christie Clinic: 217-366-1200

Provena Covenant Medical Center: 217-337-2000

#### **Tornado Preparedness**

East-central Illinois is prone to summer thunderstorms; often conditions are conducive to tornadoes. If you hear the sirens go off, a tornado warning is in effect. Take cover immediately in the lowest floor of a building, and stay away from windows.

#### Fire Procedure

If a fire alarm goes off, exit the building in a calm and orderly manner. If you are on an upper level floor, exit by the nearest stairwell. Do not use elevators.

## **Checking E-mail**

The CLASS Computer Lab is located on the Lower Level of the Illini Union and is open Monday-Friday, 10:00 AM-Midnight and Saturday-Sunday, Noon-8:00 PM

### Moderators, Speakers, Poster Presenters, and Exhibitors

#### **Moderators**

If you are moderating a session, please arrive a few minutes early to ensure that audio-visual equipment is in place and functional. At each session, a student volunteer should be on hand to assist you. The speaker's allotted time includes time for questions. The moderator should alert speakers when 3 minutes remain and again indicate when one minute remains. After their allotted time, all speakers should be asked to leave the podium. If the speaker has used his/her entire allotted time, then the speaker cannot take questions. Moderators, please announce this format at the beginning of the session, and *stay on schedule*.

### Speakers

Please consult the program ahead of time to confirm the time and location of your talk. Arrive early at your session, and if you are planning to use slides, e-mail them in advance and also bring them on a USB stick. Find the session moderator and identify yourself so that he or she is aware that you are present. Please try to stay within your allotted time—it's a courtesy to your audience and fellow speakers not only in your session but in concurrent sessions as well.

#### **Poster Presenters**

The maximum size is the standard for scientific posters, 8-feet by 4-feet. Landscape is the typical orientation, but portrait is also acceptable as long as it isn't wider than 4-feet. The display boards will be numbered to indicate where to place your poster. Push pins will be provided to affix your poster to the board. Please stand by your poster during your designated poster session. Any posters remaining after the removal deadline will be discarded.

#### Poster Session I: P1-P34

Thursday, July 10 • 12:00-1:30 PM Illini Room C (Set up between 7:30 AM-12:00 PM, and remove by 6:30 PM)

#### Poster Session II: P35-P64

Friday, July 11 • 12:00-1:30 PM Illini Room C (Set up between 7:30-9:00 AM or 10:15-10:40 AM, and remove by 6:30 PM)

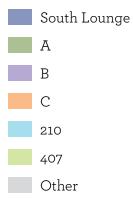
#### Exhibitors

Springer will be exhibiting in Illini Room C during both poster sessions.

## Schedule at a Glance

#### **Location Key**

All meeting events are in the Illini Union except as otherwise noted on the grid.



#### Plenaries & Open Session I: A

**Open Session II, Posters, & Exhibitors:** C

Concurrent Session I: 210

**Concurrent Session II: B** 

**CSiV:** 407

	Tuesday,	July 8			
7:00–8:00 AM					
8:00-8:30 AM	Bus from Crowne Plaz	za O'Hare Hotel			
8:30-9:00 AM	to Field Museum	(8:00-9:00)			
9:00-9:15 AM					
9:15-9:30 AM					
9:30-9:45 AM					
9:45–10:00 AM					
10:00-10:15 AM					
10:15–10:30 AM					
10:30-10:40 AM					
10:40–10:55 AM					
10:55-11:10 AM	Field Museum Tour				
11:10–11:25 AM	(9:00–11:00) & free time in Chicago				
11:25–11:40 AM					
11:40–11:55 AM					
12:00-12:30 PM					
12:30–1:00 PM					
1:00–1:30 PM					
1:30-2:00 PM					
2:00-2:20 PM					
2:30-2:45 PM					
2:45-3:00 PM					
3:00-3:15 PM					
3:15-3:30 PM					
3:30-3:45 PM		RNA-Seq Workshop (1:00–5:30)			
3:45-4:10 PM	Buses from Field Museum &	ACES Library,			
4:10-4:25 PM	O'Hare Airport to University	Room 029			
4:25-4:40 PM	of Illinois (3:00–6:00)				
4:40-4:55 PM					
4:55-5:10 PM	Registration in South Lounge (5:00–9:00)				
5:10–5:25 PM					
5:20-5:30 PM					
5:40-6:00 pm					
6:00-6:30 pm					
6:30-7:00 pm	Opening Reception	1 (6:00-9:00)			
7:00-7:30 PM					
7:30-8:00 pm	Silver Medal Lecture—1	viiiiar (7:00–7:50)			
8:00-8:30 pm					
8:30-9:00 pm	Opening Reception, cont'd				

Wednesday, July 9							
Registration (7:00–4:30) outside ABC							
			Plenary 1—Mein	· ·			
Applied chemical ecology: Attractants and repellents- -Riffell Applied chemical ecology: Attractants and repellents- -Riffell	Riffell Deletre Quero Quero Ginzel Pulido Cortesero Cha Q. Zhang Deng Il'ichev	Evolutionary ecology— Agrawal Evolutionary ecology— Agrawal	9:00 Agrawal 9:20 Coley 9:40 Forbey 10:00 Berenbaum (20 mins/ea) Refreshma 10:40 Burse 11:00 Dobler 11:20 Mohammadi 11:40 J. Santos (20 mins/ea)	Sustainable food production— Birkett/ Hillbur ent Break Sustainable food production— Birkett/ Hillbur	9:00 Pickett 9:20 Khan 9:40 Erb 10:00 Turlings (20 mins/ea) 10:40 Babikova 11:00 Tamiru 11:20 Legg <b>withdrawn</b> (20 mins/ea)	Bacteria in vertebrate signaling— Goodwin Bacteria in vertebrate signaling— Goodwin	9:00 Schulz         9:20 K. Theis         9:40 Buesching         10:00         Sperandio         (20 mins/ea)         10:40 Liberles         11:00 Vaelli         11:20 Whittaker         11:40 Goodwin         (20 mins/ea)
Lunch on your own (12:00–1:30) Plenary 4—Bassler (1:30–2:20)							
Forest insect chemical ecologyA. Ray	Allison Collignon Hughes A. Ray Imrei	Cuticular chemicals— Smith	2:30 Blomquist Blomquist 3:10 Etges 3:30 Liebig (20 mins/ea)	Quorum sensing and biofilms— Schulz	2:30 Bode Bode 3:10 Jensen 3:30 Sneed (20 mins/ea)	CSiV General Session IFerkin	Caspers Goldberg Apps Greene
			Refreshme	ent Break			
Forest insect chemical ecologyA. Ray	Paiva Hayes Keefover-Ring Rubert-Nason Jamieson	Cuticular chemicals— Smith	4:10 Wicker-Thomas 4:30 Chung 4:50 Bello 5:10 Bagnères	Quorum sensing and biofilms— Schulz	4:10 Helman Helman 4:50 Wagner- Doebler 5:10 Meijler	CSiV General Session IFerkin	Harris Goldberg Leclaire Muller CSiV open meeting
			(20 mins/ea)		(20 mins/ea)		
Silverstein-Simeone Lecture—Hansson (7:00–7:50) ISCE Committee Meeting (8:00–9:00)							
	<b>ly 8–12, 2014</b> ww.life.illinois.	University of	Illinois at Urba				

	Thursday, July 10					
7:00–8:00 AM	Registration (7:00–4:30) outside ABC					
8:00–8:30 AM		Plenary 2—Dulac (8:00–8:50)				
8:30-9:00 AM				Fieldary 2—Dula		
9:00-9:15 AM		Brooker		9:00 Robertson	Fungal super- highways—Morris	9:00 Egerton- Warburton
9:15-9:30 AM	ISCE Student	Baruffaldi	Chemical ecology of herbivore genomes—	Robertson		9:20 Zeng
9:30-9:45 AM	Travel Award	Balbuena		9:40 Tittiger		9:40 Morris
9:45-10:00 AM	Presentations	Dalla	Berenbaum	10:00 Heckel		10:00 Younginger
10:00–10:15 AM		Egan		(20 mins/ea)		(20 mins/ea)
10:15–10:30 AM				Refreshme	nt Prople	
10:30-10:40 AM				Kellesiille	III DIEak	
10:40-10:55 AM		Vidal		10:40 X. Li		10:40 Hale
10:55-11:10 AM	ISCE Student	Prado	Chemical ecology of herbivore	11:00 Briscoe	- Fungal super- highways—Morris	11:00 Mohney
11:10-11:25 AM	Travel Award Presentations	M. Mitchell	genomes— Berenbaum	11:20 Grbic		11:20 Alborn
11:25–11:40 AM		Wheeler		11:40 Simone		11:40 Godschalx
11:40–11:55 AM		Yan		(20 mins/ea)		(20 mins/ea)
12:00–12:30 PM			<u>.</u>	<u>.</u>	^	• •
12:30–1:00 PM	Lunch (provided) & Posters (12:00–1:30)					
1:00–1:30 PM						
1:30–2:00 PM						
2:00-2:20 PM		Plenary 5—Whittaker (1:30–2:20)				
2:30-2:45 PM		Yusuf		Dixson/		2:30 Hay
2:45-3:00 PM	Chemical ecology	Smith	Roundtable: Three generations of	Steppuhn	Chemical cues and signals structure	2:50 Nevitt
3:00-3:15 PM	of social behavior	Jirošová	chemical ecology—	Zarbin/Kah-Wei Hee	marine populations, communities, and ecosystems—Hay	3:10 Sotka
3:15-3:30 PM	Smith	Tian	Hilker/Kubanek (2:30–4:00)			3:30 Baker
3:30-3:45 рм		Sun	(2.30-4.00)	McNeil/Millar	ecosystems—Hay	(20 mins/ea)
3:45-4:10 PM				Refreshme	nt Break	
4:10-4:25 PM		Hofferberth				4:10 Smee
4:25-4:40 PM	Chemical signals—	Mateus			Chemical cues and signals structure	withdrawn
4:40-4:55 PM	Analysis and	Lapointe			marine populations,	4:50 Dixson
4:55-5:10 PM	synthesis Smith	Stoekl			communities, and	5:10 Kubanek
5:10-5:25 PM		Zacek			ecosystems—Hay	(20 mins/ea)
5:30-6:00 рм	Journal of Chemical Ecology Meeting—John Romeo (6:30) Timpone's Restaurant					
6:00-6:30 рм						
6:30-7:00 рм						
7:00-7:30 РМ						
7:30-8:00 рм	Film Festival (7:00–9:00 ) 100 Noyes Lab					
8:00-8:30 PM						
8:30-9:00 PM						

Thursday	y, July 10	Friday, July 11					
		Registration (7:00–4:30) outside ABC					
			Plenary 3—Anton (8:00–8:50)				
	Ghosal	Evolution,	Keeling		Ruzi		9:00 B. Schulte
CSiV General	Keller-Costa	genomics, and transcriptomics	Chiu	Factors mediating consumption	S. Ray	Zoo animals— Dehnhard	9:20 Pageat
Session IIB.	Sorensen	of chemical	Petschenka		Hervé		9:40 Dzięcioł
Schulte	L. Schulte	ecology and new directionsR. Mitchell	Bowers	Miresmailli	Röse		10:00 Santymire
	Waldman	Mitchell	Ayasse		Thaler		(20 mins/ea)
				Refreshme	ent Break		
	Ferkin	Evolution,	Lorenzo		Hermann	Zoo animals— Dehnhard	10:40 Dehnhard
CSiV General	Luzynski	genomics, and transcriptomics	Latorre Estivalis	Factors mediating	Crawley		11:00 Nordéus
Session IIB. Schulte	Pierson	directionsR.	R. Mitchell	consumption Miresmailli	Wiggins		11:20 Sorensen
	Thoß		Löfstedt		Lunt		11:40 Kester
	Voznessenskaya		Ding		Garvey		(20 mins/ea)
		Lunch (provided) & Posters (12:00–1:30)					
				Plenary 6—Ism	an (1:30–2:20)		
			Fürstenau		2:30 Boyd	Chemical methods—Millar	2:30 Yew
		Invasives and biological	T. Johnson	Pollution and insect chemical ecology— Trumble	2:50 Coudron		Yew
		control	Dekker		3:10 Lindroth		3:10 J. Miller
		Castells and T. Johnson	Hee		3:30 Jamieson		3:30 Schott
		001113011	Aldrich		(20 mins/ea)		(20 mins/ea)
				Refreshme	ent Break		
		Invasives and	Castells		4:10 Trumble		4:10 Schulz
		biological	Welzel	Pollution and insect chemical		Charriel	4:30 Bhandari
		control	Neff	insect chemical ecology—		Chemical methods—Millar	4:50 Zarbin
		Castells and T. Johnson	Vidal Gomez	Trumble			5:10 Levi-Zada
			Bagchi		(20 mins/ea)		(20 mins/ea)
		I	Dinner Banquet (	(6:00–9:00 ) Alice (	Campbell Alumni	i Center Ballroom	

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			Saturday	y, July 12		
7:00–8:00 AM	Registration (7:00–2:30) outside ABC					
8:00–8:30 AM						
8:30–9:00 am		Ea	arly Career Award	Lecture (8:00–8:5	50)	
9:00–9:15 AM		Konno		9:00 Dötterl	Evolution, genomics, and transcriptomics of chemical	withdrawn
9:15–9:30 AM	Plasticity of	Mutyambai	Chemical ecology and	Dötterl		Boevé
9:30–9:45 AM	plant defenses Pearse	Rowen	global decline	9:40 Manson		Röder
9:45–10:00 AM	Pearse	Gish	of pollinators— Lusebrink		ecology and new directions	Zavala
10:00-10:15 AM				(20 mins/ea)	Zavala	Lemmen
10:15–10:30 AM			Pofrochm	ient Break		
10:30–10:40 AM			Kenesiin			
10:40–10:55 AM		Jimenez- Aleman		10:40 LeConte	Mating and ovipositional cuesMcNeil	Pageat
10:55–11:10 AM	Plasticity of		ecology and	11:00 R. Johnson		Why
11:10–11:25 AM	plant defenses Pearse	Zeng	global decline of pollinators— Lusebrink	11:20 Trumble/ Hladun		Petit
11:25–11:40 AM		Steppuhn	puhn 11:40 Lusebrink	11:40 Lusebrink		Said
11:40–11:55 AM		Pearse		McNeil		
12:00–12:30 PM						
12:30–1:00 PM	Lunch on your own (12:00–1:30) ISCE Member Business Meeting					Meeting
1:00–1:30 PM						
1:30–2:00 PM	Plenary 7—Raguso (1:30–2:20)					
2:00–2:20 PM			Plenary /—Rag	Juso (1:30–2:20)		
2:30–2:45 PM						
2:45–3:00 PM						
3:00–3:15 PM	Music	Musical Interlude (2:40–3:40)				
3:15–3:30 PM						
3:30–3:45 PM	Local Tours (2:30–4:30)					
3:45-4:10 PM	Refreshment Break					
4:10-4:25 PM						
4:25-4:40 PM						
4:40-4:55 PM						
4:55–5:10 PM						
5:10–5:25 PM	Closing Reception (5:00–6:00) Illini Union Ballroom					
5:20–5:30 PM	Closing Reception (5.00-0:00) minit onion bailtoom					
5:40–6:00 pm						

## **Special Meetings**

## Tuesday, July 8

1:00-5:30 РМ **RNA-Seq Workshop** • ACES Library, Room 029 Instructor: Radhika Khetani (Must have pre-registered online)

### Wednesday, July 9

8:00-9:00 PM ISCE Committee Meeting • Illini Room B

## Thursday, July 10

2:30-4:00 PM ISCE 2014 Roundtable Discussion: Three Generations of Chemical Ecology • Room 210

Organizers: Monika Hilker and Julia Kubanek

The discussion will address issues of our Society itself, our roots, achievements and future perspectives; we will discuss how to improve the Society ´s life and organization, and how the Society can help promote the field of chemical ecology itself and careers of chemical ecologists. The discussion aims to shed some light on the methodological and scientific developments in the field of chemical ecology during the last decades and on the progress we have made when trying to bring together chemistry of natural products and ecology.

### Thursday, July 10

6:30 pm

Journal of Chemical Ecology Meeting • Timpone's Restaurant (710 S. Goodwin Ave., Urbana , 217-344-7619)

Led by: John Romeo

## Saturday, July 12

12:00-1:30 PM ISCE Business Meeting • Illini Room B

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## **Social Events**

## Tuesday, July 8

9:00–11:00 AM	<b>Behind the Scenes Tour of The Field Museum ·</b> <i>The Field Museum (1400 S. Lake Shore Dr., Chicago, 312-922-9410)</i>
	(Must have pre-registered online)
6:00-9:00 PM	<b>Opening Reception ·</b> Illini Union South Lounge

## Thursday, July 10

7:00-9:00 PM Pheromone Film Festival (open to the public) • 100 Noyes Lab

Although the term "pheromone" was coined in 1959, the word was remarkably slow to work its way into popular culture. Eventually, however, screenwriters discovered that using pheromones—chemicals released by one organism that could ostensibly cause an "automatic" response in another organism—was a great way to move a plot along without having to worry about logic, motivation, or continuity. Early on, behaviormodifying chemicals, not specifically identified as pheromones, were critical to the plot of The Deadly Bees (1967), in which a beekeeper purifies "the smell of fear" and uses the resulting liquid for nefarious purposes. As to when the term "pheromone" first appeared, that's a tough call, at least in part because the word was often mispronounced; in the 1978 feature The Bees, for example, in which unscrupulous cosmetics magnates hoping to exploit bees for their royal jelly smuggle killer bees into the U.S. with predictably disastrous results, a scientist named Dr. John Norman explains how "pheromes" can be used to stop the invasion by forcing drones to mate with each other instead of with the queen.

The Pheromone Fear Film Festival will provide a brief overview of 50 years of onscreen pheromones, featuring clips and trailers from film and television depicting chemical communication in taxa ranging from insects to snakes (on planes) to sheep to humans (most recently in Ocean's Thirteen). These will be followed by an outstanding representative of the genre—Empire of the Ants (1977), a feature film based (very) loosely on H.G. Well's novel "Food of the Gods." Directed by Bert I. Gordon (known in Hollywood as Mr. B.I.G.), Empire of the Ants showcases—SPOILER ALERT—interspecific (inter-phylum) use of pheromones by a colony of giant super-intelligent radioactivewaste-induced ants to enslave the local human population of a Florida town.

Pheromone-free popcorn and beverages will be provided.

## Friday, July 11

6:00-9:00 PM

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Dinner Banquet • Alice Campbell Alumni Center Ballroom

(Must have purchased ticket at registration)

### Saturday, July 12

2:30-4:30 PM Local Tours • All tours will depart from the Illini Union. Sign up at the registration table on July 8 or contact local organizers.

**Tour 1: Chemistry/entomology/ecology history tour of central UIUC campus** walking tour with May Berenbaum (includes visit to Morrow Plots, National Historical Landmark and longest-term continuous corn plot in the world)

**Tour 2: Art/architecture/artifact tour of central UIUC campus**—walking tour with Hugh Robertson and Christina Nordholm

**Tour 3: Tour of the UI Arboretum** (includes CU MTD transportation to Arboretum)—features the Miles C. Hartley Selections Garden (home of the UIUC American Selections Trial Gardens), the Idea Garden (highlighting new varieties of ornamentals and vegetables as well as a children's garden), the Hosta Garden (recipient of the 2014 American Hosta Society's National Display Garden Award), Japan House and its tea, dry and tranquil gardens, ponds with native wetland and prairie species, and, time permitting, the UI Pollinatarium and its associated reconstructed prairie.

**Tour 4: Visit to Prairie Fruits Farm**—local multi-faceted farm and creamery noted for award-winning nationally recognized goat cheese and gelato. Transportation provided (\$5 includes ride, tour, and samples)

5:00–6:00 PM Closing Reception • Illini Union Ballroom

Closing ceremony: award presentations, recognition of service, and preview of ISCE 2015

#### **GOING GREEN**

The pages that follow contain only a list of the talks and posters, and their presenters. The full conference program, including all abstracts and authors, is available on the USB stick attached to your name badge lanyard. It is also available to download from the conference website (www.life.illinois.edu/isce-csiv).

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## **Abstracts**

### **Plenary Presentations**

#### **Tuesday, July 8**

#### 7:00 PM

#### 1 Silver Medal Lecture • Travels with my ant: from macrolides to methyl **branches** • *Illini* Room C

Jocelyn Millar, University of California, Riverside

Presenter: Jocelyn Millar, University of California, Riverside

So much of chemical ecology is about details, and about getting the details right. For example, most semiochemical signals consist of blends rather than individual compounds, with minor and even trace components often being crucially important to the reconstruction of a bioactive blend. Conversely, even trace amounts of synthetic impurities can dramatically decrease the activity of a semiochemical blend. This is true not only for chemical purity, but also for enantiomeric purity: using a single pure stereoisomer, or in some cases the correct ratio of enantiomers, can be critically important to reproducing natural behaviors. But it does not end there, because numerous other factors also influence the success or failure of reconstructing a chemically mediated behavior, such as signal concentration, the context in which the semiochemical signal is presented, and the presence of other types of signals, such as visual or acoustic signals. I will use examples from our work to illustrate these points and show how lessons learned, sometimes painfully, have influenced the ongoing evolution of our research directions. Work from two of our current projects (chemical ecology of cerambycid beetles and deconstruction of insect cuticular lipids) will be used to illustrate how recent results have led to new hypotheses and new lines of enquiry.

#### Wednesday, July 9

8:00 AM

#### 2 **Chemical ecology's past, present, and possible future** • *Illini Room A*

Jerrold Meinwald, Cornell University

Presenter: Jerrold Meinwald, Cornell University

Cosmologists and astrophysicists have done a remarkable job of modeling the evolution of the chemical elements. While the origin of living organisms built from a small subset of these elements is not yet clear, the subsequent process of biological evolution is no longer a mystery. Living organisms gain information about their environment in a variety of ways, not the least of which involves the chemical senses. Some examples of the study of chemical interactions between organisms in nature which helped launch the discipline of chemical ecology will be presented. The possibility of elucidating the chemistry of most biotic interactions did not really exist until the mid-20th century, when chromatographic separation techniques, x-ray crystallography, mass spectrometry, and NMR spectroscopy made it possible to isolate and characterize messenger compounds quickly, in spite of the small quantities available. The founding of the Journal of Chemical Ecology in 1974 and the International Society of Chemical Ecology in 1984 played important roles in the recognition of chemical ecology as a significant scientific discipline. Although we are only a "teeny, tiny band" of researchers, we have begun to change the way

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that the communities of biologists and chemists interact. There is still enormous room for improvement of analytical techniques and for the deciphering of complex biological systems. With appropriate societal support, we can expect to understand life on earth at a much deeper level than we do now.

William Finn and James Lapine, Falsettos, 1992.

#### 1:30 PM 3 **Tiny conspiracies: cell-to-cell communication in bacteria** • *Illini Room A*

Bonnie L. Bassler, HHMI and Princeton University

Presenter: Bonnie L. Bassler, HHMI and Princeton University

Bacteria communicate with one another using small chemical molecules that they release into the environment. These molecules travel from cell to cell and the bacteria have receptors on their surfaces that allow them to detect and respond to the build up of the molecules. This process of cell-to-cell communication in bacteria is called "Quorum Sensing" and it allows bacteria to synchronize behavior on a population-wide scale. Bacterial behaviors controlled by quorum sensing are usually ones that are unproductive when undertaken by an individual bacterium acting alone but become effective when undertaken in unison by the group. For example, quorum sensing controls biofilm formation, virulence, sporulation, and the exchange of DNA. Quorum sensing studies are leading to an understanding of the evolution of and the requirements for robust collective behaviors. On the practical side, quorum-sensing studies are fostering development of therapies aimed at interfering with this process. Such therapies could be used to control bacterial pathogenicity. Likewise, with respect to beneficial bacteria or bacteria with industrial relevance, synthetic proquorum-sensing strategies are being explored to enhance bacterial processes of interest.

#### 7:00 PM 4 Silverstein-Simeone Lecture • Olfactory-based resource location and danger avoidance in *Drosophila* • *Illini Room C*

Bill Hansson, Max Planck Institute for Chemical Ecology

Presenter: Bill Hansson, Max Planck Institute for Chemical Ecology

Drosophila flies are heavily dependent on the sense of smell to locate optimal feeding, mating and oviposition sites. In a similar way they use olfaction to avoid danger in the form of toxic microbes and parasitoids. We dissect neural circuits involved in these contexts. Specific assemblies of olfactory sensory neurons on antenna and palp detect olfactory information. Some neurons are specifically involved in egg laying or mate location, some in detecting optimal food sources and others in sensing warning signs. Interestingly, detection of cues involved in attraction and oviposition behavior, respectively, seem to be uncoupled. Here I will present published as well as very recent data describing both peripheral and central events directly underlying behavioral sequences strongly correlated with fitness. Unexpectedly, many cues seem to be coded in a labeled line fashion.

#### Thursday, July 10

## 8:00 AM 5 **Molecular, genomic and neuronal bases of pheromonal signaling in mammals** • *Illini Room A*

Catherine Dulac, Harvard University

Presenter: Catherine Dulac, Harvard University

Our group is interested in the cellular and molecular architecture of neural circuits underlying instinctive social behaviors in the mouse. We will describe our recent advances in

#### Thursday, July 10

uncovering the identity of sensory neurons detecting social cues, and of command circuits associated with specific social responses in males and females.

1:30 PM

#### 6 Silent songs: content and context of avian chemical signals • Illini Room A

Danielle J. Whittaker, Michigan State University

Presenter: Danielle J. Whittaker, Michigan State University

In recent years, birds have finally joined the rest of the world's living creatures as a taxon of interest in chemical ecology. Behavioral, genomic, and neurobiological work has demonstrated that birds are able to detect and respond to odors from plants, predators, and other birds. Many studies have focused on volatile compounds present in preen oil, which is secreted from the avian uropygial gland and spread on the feathers for protection and maintenance. These compounds contain information about individual identity and quality, and may play an important role in kin recognition and mate choice. Birds only molt their feathers once or twice a year, and in most species an individual's song is learned and crystallized at a young age. Unlike these static visual or acoustic communication signals, chemical signals can change in a relatively short time span, allowing them to transmit more up-to-date information about an individual's health or quality. Our work with the dark-eyed junco (Junco hyemalis), a North American emberizid sparrow, has focused on how birds may use chemical communication when identifying and assessing potential mates. The abundance of several volatile compounds in junco preen oil track steroid hormone levels in both males and females throughout the season, indicating reproductive readiness. Odor also better predicts an individual's reproductive success within a breeding season than plumage ornaments. Chemical ecology has the potential to revolutionize our understanding of avian mate choice, speciation, and evolution-and avian studies that incorporate multimodal signaling could catalyze new directions in chemical ecology.

### Friday, July 11

8:00 AM

#### 7 Modulation of insect olfaction: from neurons to behavior • Illini Room A

Sylvia Anton, Angers University/INRA Angers Christophe Gadenne, Angers University/INRA Angers

Presenter: Sylvia Anton, Angers University/INRA Angers

Moths, as many other night-active insects, use olfactory cues to communicate and to orient toward different types of resources. The most well known example is sex pheromone communication, where females emit tiny amounts of a species-specific blend of compounds, which are detected by males over large distances. Male moths have a specialized, highly sensitive olfactory subsystem dedicated to sex pheromone detection and processing, leading ultimately to an oriented flight toward the conspecific pheromone signal. This behavior has long been thought to be innate and the associated sensory system to be hard-wired. There is now, however, strong evidence for plasticity within the sex pheromone system of male moths. In two noctuid moth models, we have shown that the physiological state, such as the hormonal or mating state, but also experience with different sensory signals have a strong impact on sex pheromone responses. In the black cutworm moth *Agrotis ipsilon*, increasing titers of juvenile hormone and ecdysone improve responses to the sex pheromone, whereas mating completely inhibits any pheromone responses. In the Egyptian cotton leafworm moth *Spodoptera littoralis*, brief exposure to behaviorally relevant sensory signals improves sex pheromone responses 24 h later. When searching

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#### Friday, July 11

for the neurobiological basis for the observed behavioral modifications, we found little change within the peripheral olfactory system but strong modulation of the sensitivity in the primary olfactory center, the antennal lobe. These mechanisms might help these insects to adapt to internal and environmental conditions in order to optimize reproduction success without a waste of energy.

1:30 PM

#### 8 Mixtures matter, and other things I've learned about insect-plant chemical interactions • Illini Room A

Murray B. Isman, University of British Columbia

Presenter: Murray B. Isman, University of British Columbia

Much of the scientific literature documenting the bioactivities of plant natural products follows from a reductionist strategy aimed at the bioassay-driven isolation of active principles from complex plant extracts. Plants rarely produce a single bioactive substance, but tend to produce suites of biogenically-related analogs, not all of which are equally active but that in certain plants can act synergistically to produce an effect greater than the sum of the individual constituents. In our original work on azadirachtin (aza) and neem insecticides we observed that, while limonoids other than azadirachtin were orders of magnitude less active than aza, natural mixtures of aza and other limonoids as they occur in neem extracts mitigate habituation to the feeding deterrent effect of aza and selection for resistance to aza. In our investigation of the role of individual constituents of rosemary oil in toxicity to spider mites, we found intriguing synergy between putative 'active' constituents and putative 'inactive' constituents. This phenomenon of internal synergy extends to the feeding deterrent effects of various plant essential oils to insectsnatural mixtures are more bioactive than the most active constituents alone. Our most recent research on the toxicity of essential oils to insects points to enhanced cuticular penetration as the likely mechanism underlying the synergy observed between specific binary mixtures of monoterpenes in selected plant essential oils.

#### Saturday, July 12

9

8:00 AM

## Early Career Award Lecture • Elevation gradients as optimal tools for studying variation in plant defense traits against herbivores • Illini Room A

Sergio Rasmann, University of California, Irvine

Presenter: Sergio Rasmann, University of California, Irvine

Over the last half-century, complementary theories and hypotheses have been developed to try to explain the extraordinary variation in plant defensive strategies against herbivores, in which syndromes of plant defenses are driven by the influence of community responses, inherited functional traits, abiotic conditions, and the geographical and historical contingencies affecting the community. Therefore, a better understanding of which factors affect plant defenses requires the use of holistic approaches. Specifically, I use elevation gradients as natural experiments to test classic hypotheses of plant defense theories across and within species. Recent work is showing two contrasting results. First, in accordance with the growth-defense trade-off hypothesis, I observed that high elevation plants grow smaller and are more defended than their low elevation congeners. In contrast, I then observed that high elevation plants that are less eaten by herbivores are also less defended. To reconcile these two contrasting results, I suggest that rare high elevation plants rely on higher toxicity to survive, whereas high elevation dominant plants rely primarily on tolerance and less on defensive chemistry to cope against herbivores. I

#### Saturday, July 12

thus argue that only a holistic approach such as this one will enable us to fully grasp the ecology and evolution of plant defenses against herbivores.

#### 1:30 PM 10 Landscapes of linalool: evolution of a volatile floral signal · Illini Room A

Robert A. Raguso, Cornell University Krissa Skogen, Chicago Botanic Garden Jeremie Fant, Chicago Botanic Garden

#### Presenter: Robert A. Raguso, Cornell University

Linalool, a volatile monoterpene alcohol, is broadly distributed among flowering plants as a component of essential oils, floral scents and herbivore-induced foliar emissions. Its many biological functions are mediated by the enantiomeric form and ecological context in which it occurs. My colleagues and I have long studied (S)-(+)-linalool as a dominant component of volatile blends emitted by night-blooming flowers, in the context of convergent evolution of hawkmoth pollination. Most recently, we have documented striking geographic variation in linalool emissions by flowers of Oenothera harringtonii (Onagraceae), a rare, self-incompatible annual endemic to the prairies of central Colorado, USA. Studies using microsatellite markers revealed no private alleles among 23 populations from Colorado Springs to Trinidad, suggesting that there is gene flow between them. Field observations identified hawkmoths as the primary pollinators (and agents of gene flow) in all populations, including Hyles lineata, which also uses Oenothera as a larval host. Despite gene flow, populations southeast of Walsburg completely lack linalool, which is a dominant component of scent in all other populations. In the absence of genetic drift, we are examining two alternative hypotheses for strong clinal variation in linalool; (a) that female Hyles are attracted by linalool, and utilize O. harringtonii plants as a preferred host in southeastern populations, or (b) that seed predatory Mompha moths are attracted by linalool and are most abundant in southeastern populations. We are addressing these hypotheses in multiple field populations in the context of the Geographic Mosaic Theory of Coevolution.

### **Symposium Presentations**

## Recent developments and new opportunities in chemical ecology for sustainable food production

#### Wednesday, July 9

11

#### 9:00 AM

#### New ways to exploit the chemical ecology of host stress for pest management • Illini Room B

John Pickett, Rothamsted Research

Presenter: John Pickett, Rothamsted Research

Attraction of pests and pathogen vectors can divert attack from crop plants, ourselves, and farmed animals. At the same time, we can exploit the growing understanding of sophisticated mechanisms that have evolved to allow such pests to avoid stressed or otherwise inappropriate but potential hosts. Where this involves signalling by small lipophilic molecules, such as stress-related pheromones and other semiochemicals, these can be deployed by agroecological approaches and a new generation of GM crops. New

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types of repellents against arthropod vectors of human pathogens also ensues, together with other behavioral stimuli and breeding programmes for reducing pest attractiveness to farmed animals.

9:20 AM

#### M 12 **Exploiting chemical ecology for sustainable crop protection in Africa** · *Illini Room B*

Zeyaur Khan, Charles Midega, Toby Bruce, Mike Birkett, Tony Hooper, and John Pickett, International Centre of Insect Physiology and Ecology

Presenter: Zeyaur Khan, International Centre of Insect Physiology and Ecology

We developed chemical ecology-based novel push-pull approaches for integrated pest management for smallholder cereal farmers in Africa. Appropriate plants were discovered that naturally emit signaling chemicals (semiochemicals) that influence insect-plant and plant-plant interactions. Plants highly attractive for egg-laying by stemborer pests were selected and employed as trap crops (pull), to draw pests away from the main crop. Of these, Napier grass, Pennisetum purpureum (Schumach), despite its attractiveness, supported minimal survival of the pests' immature stages. Plants that repelled stemborer pests, notably molasses grass, Melinis minutiflora P. Beauv., and forage legumes in the genus Desmodium, were selected as intercrops (push), which also attracted natural enemies of the pests. Desmodium intercrops suppressed the parasitic weed Striga hermonthica (Del.) Benth., through an allelopathic mechanism. Desmodium root exudates contain novel flavonoid compounds, which stimulate suicidal germination of S. hermonthica seeds and dramatically inhibit its attachment to host roots. Opportunities for semiochemical delivery by companion plants, including plant-plant signaling and early herbivory alert, are being explored for developing future smart IPM strategies for Africa. Recently, we adapted the push-pull system to drier and hotter conditions linked to climate change by identification and incorporation of drought tolerant trap and repellent intercrops. Currently over 75,000 smallholder farmers in Ethiopia, Kenya, Tanzania and Uganda have taken up the push-pull and have reported effective control of stemborers and striga weed resulting in significant increases in grain yields of both maize and sorghum. Other benefits mentioned included increases in fodder and milk production.

#### 9:40 AM 13 Plant secondary metabolite hijacking by a specialist root herbivore: From basic understanding to pest control implications • Illini Room B

Matthias Erb, University of Bern

Presenter: Matthias Erb, University of Bern

The ability to cope with toxic secondary metabolites is a key property of specialized herbivores. Understanding how monophagous pests deal with plant toxins can therefore help to identify new targets for pest control. We are studying the role of the major root secondary metabolites of maize, the toxic 1,3-benzoxazin-4-one derivatives (BXDs) in the interaction of the plant with one of its most damaging enemies, the western corn rootworm Diabrotica virgifera virgifera. Using a genetic approach, we found that *D. virgifera* is fully tolerant to BXDs. Furthermore, we discovered that the root-feeder uses differences in BXD blends to locate the most nutritious tissues [1]. As a result, BXD-guided foraging increases *D. v. virgifera* fitness and leads to crown root pruning, a typical *D. virgifera* associated damage phenotype. Based on these findings, we are now exploring which BXDs are used as foraging cues by *D. virgifera*. We have identified several enzymes that are responsible for the formation of individual BXDs in maize [2] and have created corresponding maize mutants. These genetic resources now allow us to identify behaviorally active BXDs in

vivo, which may eventually allow breeders to produce maize lines that confuse the insect and thereby guide it away from the most vital root tissues.

1. Robert C.A.M., et al. (2012). A specialist root herbivore exploits defensive metabolites to locate nutritious tissues. Ecology Letters, 15(1), 55-64.

2. Miehls L.N. et al. (2013). Natural variation in maize aphid resistance is associated with a DIMBOA-Glc methyltransferase. The Plant Cell, 25, 2341-2355.

#### 10:00 AM 14 **Protecting crops by exploring and exploiting plant-mediated tritrophic** interactions in the rhizosphere • *Illini Room B*

Ted C. J. Turlings, University of Neuchâtel

Presenter: Ted C. J. Turlings, University of Neuchâtel

Root-feeding larvae of the beetle *Diabrotica virgifera virgifera* (western corn rootworm) cause tremendous losses to maize growers in the USA and Europe. As a possible solution to fight this pest, we study entomopathogenic nematodes, tiny parasitic worms that kill the larvae within days. We have found that these nematodes are attracted to  $E(\beta)$ -caryophyllene, a sesquiterpene that is specifically emitted from maize roots after rootworm attack. American maize lines have lost the ability to emit this signal and using genetic transformation we restored caryophyllene emission in one such line. The renewed release of caryophyllene resulted in enhanced protection against rootworm damage by the nematodes, but it was also found that the pest itself is attracted to caryophyllene. Using our knowledge of the system, we are currently developing new strategies for the application of entomopathogenic nematodes to control rootworms and other soil pests. These strategies involved: 1) the selection for highly effective nematode strains, 2) the application of nematodes in beads that can be planted with maize seeds, and 3) increasing the "shelf life" of nematodes with the use of a plant-derived compound that puts them in a state of quiescence.

#### 10:40 AM

15

## **Mycorrhizal fungal networks communicate pest defense between plants** · *Illini Room B*

Zdenka Babikova, University of Aberdeen Lucy Gilbert, James Hutton Institute Toby J. A. Bruce, Rothamsted Research Michael Birkett, Rothamsted Research John C. Caulfield, Rothamsted Research Christine Woodcock, Rothamsted Research John A. Pickett, Rothamsted Research

Presenter: Zdenka Babikova, Institute of Biological and Environmental Sciences

The roots of most land plants are colonized by arbuscular mycorrhizal fungi, which can form shared mycelial networks that connect individual plants. We tested the hypothesis that shared fungal networks facilitate communication between plants and that such signalling could be induced as a response to insect herbivory. Plants emit volatile organic compounds that act both as cues for herbivorous insects locating plant hosts and also as a defense response following attack, after which the composition of volatiles changes to become repellent for insect herbivores but attractive for their natural enemies such as parasitoid wasps. We demonstrate that shared fungal networks facilitate plant-toplant communication in response to insect herbivory that induces systemic changes in

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production of plant volatiles, making them repellent to aphids and attractive to parasitoids. For plants not themselves infested with aphids, these effects occur only if the plants are connected to an infested plant via mycorrhizal fungal networks. This underground messaging system allows neighboring plants to invoke herbivore defenses before they come into direct contact with aphids. This is first experimental evidence demonstrating that shared fungal networks shape multitrophic interactions by communicating information on aphid attack from plant-to-plant, and influence the behavior of aphids and their parasitoids. Hence, mycorrhizal fungi are more important drivers of above-ground ecological interactions than ever considered. We now need to determine what is the ecological significance of the signalling, how is it regulated in nature and in agroecosystems, and how can we manipulate mycorrhizal fungi to provide sustainable solutions to insect pest management.

11:00 AM

#### 16 Indirect defense signaling in maize induced by stemborer oviposition • *Illini Room B*

Amanuel Tamiru, International Centre of Insect Physiology and Ecology (ICIPE), Kenya Toby Bruce, Rothamsted Research, UK

Charles Midega, International Centre of Insect Physiology and Ecology (ICIPE), Kenya John Pickett, Rothamsted Research, UK

Zeyaur Khan, International Centre of Insect Physiology and Ecology (ICIPE), Kenya

Presenter: Amanuel Tamiru, International Centre for Insect Physiology and Ecology (ICIPE), Kenya

Feeding by insects causes maize to emit herbivore induced plant volatiles (HIPVs) attractive to natural enemies. However, little was known about the reaction of maize to oviposition by insects and genotypic variation in indirect plant defense traits elicited by eggs. Results from our study showed emission of HIPVs attractive to egg and larval parasitoids Trichogramma bournieri and Cotesia sesamiae by certain maize landraces in response to oviposition by stemborer Chilo partellus, a major insect pest of maize in Africa. GC-EAG recording with attractive samples revealed the parasitoids were responsive to ten volatile organic compounds, namely, (E)-ocimene, (R)-linalool, (E)-4,8-dimethyl-1,3,7-nonatriene, methyl salicylate, decanal, methyleugenol, (E)-caryophyllene,  $\alpha$ -bergamotene, (E)- $\beta$ farnesene and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene. Volatile analysis by GC and GC-MS revealed marked increases in emission of these compounds in maize landraces exposed to stemborer oviposition compared to unexposed. The effect was systemic and caused by an elicitor extracted from egg materials associated with attachment to leaves. In contrast, volatiles from two standard commercial varieties exposed to oviposition were not attractive to either parasitoid, implying the ability to produce the defense volatile signals might have been lost during breeding processes. The current research findings pave the way for developing novel and ecologically sound approaches to control destructive stemborer pests by introgressing these traits into mainstream maize varieties.

#### References

Tamiru A., et al. (2011). Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. Ecology Letters 14:1075-1083

Tamiru A., et al. (2012). Oviposition induced volatile emissions from African smallholder farmers' maize varieties. J.Chem.Ecol. 38:231-234

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11:20 AM

#### 17 Bemisa whiteflies: a globally destructive pest and virus vector, and important potential target for chemical ecology research • Illini Room B

James P. Legg, International Institute of Tropical Agriculture, Dar es Salaam, Tanzania Marcus Stensmyr, Lund University, Lund, Sweden

Ylva Hillbur, International Institute of Tropical Agriculture, Ibadan, Nigeria

Presenter: James Legg, International Institute of Tropical Agriculture, Dar es Salaam, Tanzania

The whitefly Bemisia tabaci is recognized as one of the most globally damaging insect pests. Although it has been the subject of much taxonomic debate in recent years, there is now a growing consensus that *B. tabaci* is a cryptic species complex comprising more than 30 distinct species. Many of these putative species cause major economic losses either through direct physical damage or through the vectoring of plant pathogenic viruses. Several putative species have invasive characteristics and have spread globally, with notable examples including the MEAM1 (= biotype B) and Mediterranean (= biotype O) putative species. In Africa, populations of the sub-Saharan Africa 1 (SSA1) putative species are associated with the emergence and spread of the twin pandemics of cassava mosaic virus disease (CMD) and cassava brown streak virus disease (CBSD). These pandemics have affected large parts of the cassava-growing regions of East and Central Africa, continue to spread, and are responsible for crop losses of > US\$ 1 billion annually. Although pandemic management efforts have focused on virus control through host plant resistance, there is an increasing awareness of the importance of controlling the whitefly vector. It is recognized that interference with host finding might offer promise as a potential control tactic. Visual cues are known to be important for whitefly host recognition, and this has been exploited both in field situations, where yellow mulch has been shown to reduce whitefly populations in vegetable crops, as well as in protected agriculture, where yellow sticky traps are widely used to provide partial whitefly control. Chemical cues for whitefly host recognition have received little research attention. Several recent studies, however, have illustrated the semiochemical activity of sesquiterpene volatiles of tomato, showing that 7-epizingiberene and R-curcumene have a repellent activity on nearby B. tabaci whiteflies. These results highlight the need for similar research in other crop systems. In this paper we review the potential for effective management of B. tabaci whiteflies and consider possible opportunities for the contribution of chemical ecology research to this goal.

#### 11:40 AM

18

African smallholder agriculture and the diverse pathways towards Sustainable Intensification • Illini Room B

Bernard Vanlauwe, International Institute of Tropical Agriculture (IITA)

Presenter: Bernard Vanlauwe, International Institute of Tropical Agriculture (IITA)

In sub-Saharan Africa, intensification of smallholder agriculture is a must in the densely populated areas in order to feed the rapidly growing and urbanizing population while for areas that contain valuable natural ecosystems, such as, e.g., the primary forest in the Congo basin, intensification of forest margins is one of the conditions for conserving such ecosystems. Sustainable Intensification denotes a commonly accepted framework for intensification and most of its definitions are phrased around three principles: (i) production of more food, feed, fuel and/or fiber per unit of land, labor, and/or capital used, (ii) preservation of important ecosystem services, including those governed by healthy soils, and (iii) resilience to shocks and stresses, including climate change. Sustainable intensification is operationalized at landscape scale since certain ecosystem services

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operate at that level. African farming systems exhibit a high degree of heterogeneity in crops and crop arrangements, access to markets, effectiveness of institutions, and agroecological conditions. Within farms, a long-term interplay of geological and landscape conditions and plot-specific management have generated within-farm soil fertility gradients, with some soils no longer responding to standard fertilizer (often referred to as 'non-responsive soils'). Above diversity at different scales suggests that various pathways exist toward the sustainable intensification of smallholder farming in sub-Saharan Africa, thereby recognizing that short-term benefits from farming (e.g., crop produce) will be a requirement for any viable pathway.

Micro and macro-evolution of inducible defense in the milkweeds • Room 210

#### Evolutionary ecology of chemically mediated interactions

9:00 AM

19

Anurag Agrawal, Cornell University

Presenter: Anurag Agrawal, Cornell University

Based in plant defense theory, constitutive and inducible resistance have long been predicted to show a pattern of negative correlation within and across species. The basis for this hypothesis is that plants investing in high levels of constitutive defense (i.e., traits that are always expressed) will experience minimal attack and need not be inducible following herbivory. Conversely, where the probability of attack is unpredictable, plants may be expected to invest relatively little in constitutive defense but to show high levels of inducibility following attack. The predicted negative correlation between constitutive and induced resistance could be adaptive if the traits are each beneficial, redundant, and costly. We have been studying the relationship between constitutive and induced cardenolide production in milkweeds. In both above- and below-ground tissues, we have repeatedly found the striking pattern of a negative genetic correlation between constitutive and induced cardenolides within species and a positive association between constitutive cardenolides and induction across species. This pattern suggests that species variation in production of cardenolides is greater than variation in allocation patterns to constitutive and induced resistance. The countervailing micro- and macroevolutionary patterns reflect constraints within species and major shifts in total plant investment in defense across species.

#### 9:20 AM 20 **Evolution of anti-herbivore defenses in expanding leaves of Inga and host** choice by herbivores. • *Room 210*

Thomas A. Kursar, University of Utah Phyllis D. Coley, University of Utah M. J. Endara, University of Utah Ryan Bixenmann, American Association for the Advancement of Science K. G. Dexter, University of Edinburgh Catherine Kidner, Royal Botanic Garden Edinburgh James. A. Nicholls, University of Edinburgh R. Toby Pennington, Royal Botanic Garden Edinburgh Graham N. Stone, University of Edinburgh *Presenter: Phyllis D. Coley, University of Utah* 

*Inga* (Fabaceae) has >300 species in the Neotropics and is the most abundant and speciose tree genus in most lowland forests. We suggest that both high local diversity and rapid speciation of *Inga* are driven by interactions with herbivores. Secondary metabolites in young leaves are not induced by herbivory but are constitutively high, as is predicted by

the consistently high levels of herbivore pressure. The arms race appears to be driving rapid evolution of defenses: there is no phylogenetic signal for defensive traits (secondary metabolites, ant defense, leaf development). Host choice by leaf-chewing herbivores depends significantly on defensive traits. Sawfly spp. (Order Hymenoptera) are very specialized. Moreover, in some cases, related species feed on the same Inga species at different sites across the Amazon. In contrast, phloem-feeding Coreidae (Order Hemiptera) feed broadly within the genus, perhaps avoiding secondary metabolites in the leaves. Species of the order Lepidoptera, the most abundant herbivores, show intermediate specialization. There is no correlation between host selection by Lepidoptera and host genetic distance, showing that Lepidoptera do not choose hosts based on phylogenetic similarity. Instead, results demonstrate that herbivores choose hosts based on similarity of secondary metabolites and the community of ants visiting extra-floral nectaries. Individual plants growing as neighbors are more dissimilar in secondary metabolites than predicted, suggesting that herbivore attack is minimized by being chemically different from neighbors, and that this may be the main driver of the high alpha diversity of tropical forests.

#### Molecular mechanisms and ecological consequences of plant chemical 9:40 AM 21 defenses in vertebrate herbivores · Room 210

Jennifer Sorensen Forbey, Boise State University

Presenter: Jennifer Sorensen Forbey, Boise State University

A central focus in the field of foraging ecology is to understand how diet quality influences the behavior, distribution and evolution of animals. For vertebrate herbivores, there is strong evidence that the quality and quantity of "toxins" (i.e. secondary metabolites) in plants can significantly alter the foraging behavior and physiology of captive individuals. However, there remains a general lack of ability to use in vivo studies focused on individual herbivores to generate a priori predictions of how herbivores respond to the spatial and temporal heterogeneity of toxins in nature. My aim is to demonstrate how understanding molecular mechanisms of toxins and the development of biomolecular and remote sensing tools can be used to scale up our understanding of co-evolutionary interactions between plants and herbivores from cells, to individual organisms, to landscapes. Specifically, I use in vitro pharmacological assays to investigate dose-responses between naturally consumed toxins and proteins that have been isolated from wild herbivores. In addition, I use biomarkers of toxins and herbivore responses detected in the field to predict dosedependent, ecological consequences of plant-herbivore interactions in natural systems.

#### 10:00 AM

22

Real-time evolution of tolerance in the invasive weed Pastinaca sativa after reassociation with its specialist herbivore Depressaria pastinacella  $\cdot$  Room 210

Tania Jogesh, University of Illinois at Urbana-Champaign Margaret Stanley, University of Auckland May R. Berenbaum, University of Illinois at Urbana-Champaign

#### Presenter: May R. Berenbaum, University of Illinois at Urbana-Champaign

The interaction between the European wild parsnip Pastinaca sativa and its coevolved florivore the parsnip webworm Depressaria pastinacella, established in North America for over 150 years, has resulted in evolution of local chemical phenotype matching. The recent invasion of New Zealand by webworms, exposing parsnips there to florivore selection for the first time, provided an opportunity to assess rates of adaptive response in a real-time experiment. We planted reciprocal common gardens in the US and NZ with seeds from: 1) US populations with a long history of webworm association; 2) NZ populations that

had never been infested and 3) NZ populations infested for 3 years (since 2007) or 6 years (since 2004). We measured impacts of florivory on realized fitness, reproductive effort, and pollination success, and measured phenotypic changes in infested NZ populations relative to uninfested NZ populations to determine if rapid adaptive evolution in response to florivory occurred. Irrespective of country of origin or location, webworms significantly reduced plant fitness. Webworms reduced pollination success in small plants but not in larger plants. While defense chemistry remained unchanged, plants in infested populations were larger after three to six years of webworm florivory. As plant size is a strong predictor of realized fitness, evolution of large size as a component of florivore tolerance may occur more rapidly than evolution of enhanced chemical defense.

#### Defensive secretions of leaf beetles: A small step from deterrent de novo 10:40 AM 23 synthesis to sequestration in Chrysomelina evolution? • Room 210

Antje Burse, Peter Rahfeld, Anja Straus, Ding Wang, and Wilhelm Boland, Max Planck Institute for Chemical Ecology

Presenter: Antje Burse, Max Plack Institute for Chemical Ecology

Beetles have emerged successfully on the planet because of the development of diverse and often astonishing defensive strategies against their enemies. The larvae of the leaf beetle subtribe Chrysomelina, for example, secrete deterrents from specialized defensive glands on their backs. Some species evolved the de novo synthesis of iridoids (cyclopentanoid monoterpenoids) for their protection, which is considered the ancestral strategy and pre-dates the sequestration of phytochemicals for deterrent production, such as the salicin sequestration from salicacaeous food plants for further conversion into the repellent salicylaldehyde in the glands. From our recent studies, it became clear that not only enzymatic reactions, e.g. catalyzed by non-selective glucosidases or selective oxidoreductases, found in the secretions of sequestering species, but also transport processes essential for sequestration are already developed in the ancestral species. Comparative studies on de novo iridoid-producing and sequestering larvae have indicated the existence of a complex influx-efflux transport network quiding deterrent precursors through barriers of different selectivity as a general principle found in Chrysomelina. This network includes particularly broad-spectrum ATP binding cassette transporters acting in excretion mechanisms by Malpighian tubules and defensive glands which provide a flexible base to build on a plant-dependent chemical defense [1]. Hence, although the use of phytochemicals for anti-predator defenses appears to be a spectacular evolutionary innovation, it requires only a few modifications from ancestral processes.

[1] Strauss A.S., Peters S., Boland W., Burse A. 2013 ABC transporter functions as a pacemaker for sequestration of plant glucosides in leaf beetles. eLife 2

#### 24 Trapped between plants and predators—convergent evolution of adaptations to cardenolides in leaf beetles • Room 210

Susanne Dobler, Vera Wagschal, Michael Baum, and Samuel Waldron, Hamburg University

Presenter: Susanne Dobler, Hamburg University

Cardenolide-containing host plants impose a serious hurdle to phytophagous insects as these toxins inhibit the Na,K-ATPase which is essential for the establishment of membrane potentials and the conduction of action potentials. Nevertheless, leaf beetles in four unrelated lineages have colonized cardenolide-containing plants while a fifth lineage is producing cardenolides autogenously as highly effective anti-predator defenses. A closer look at the underlying physiological adaptations that prevent a blocking of the Na,K-ATPase

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11:00 AM

reveals striking similarities in four of these lineages. Here, identical and obviously convergently evolved amino acid substitutions at decisive residues of the cardenolide binding pocket dramatically decrease the sensitivity of the Na,K-ATPase to cardenolides. This striking convergence is, however, not the only solution to the challenge of feeding on cardenolide-containing plants. Alternatively or in addition, compartmentalization that prevents the toxins from reaching their target site is achieved in three leaf beetle lineages: in one case cardenolides are apparently unable to pass the gut membrane and reach the hemolymph while in two others efflux carriers in the neural sheath, the equivalent to the mammalian blood-brain barrier, may prevent access of cardenolides to Na,K-ATPase in the nervous tissue.

#### 11:20 AM

#### AM 25 Molecular and physiological mechanisms of bufadienolide resistance in toadeating snakes. • *Room 210*

Shabnam Mohammadi, Utah State University Alan H. Savitzky, Utah State University Georg Petschenka, Cornell University

Presenter: Shabnam Mohammadi, Utah State University

Toads are chemically defended by bufadienolides, a class of cardiotonic steroids lethal to most predators, including many snakes. Bufadienolides typically bind to Na+,K+-ATPase (NKA), inhibiting the enzyme's ability to transport ions. This inhibition leads to a series of physiological events that cause cardiac arrhythmia and increased cardiac contractility which, if prolonged, are lethal. However, a number of snake species are resistant to bufadienolides and consume toads with no apparent ill effects. The molecular and physiological mechanisms underlying resistance in those species have not yet been fully elucidated. Resistance to cardiotonic steroids, such as cardenolides and bufadienolides, has been studied in other animals, and their resistance has been linked to mutations in NKA that limit the binding of such toxins to the target enzyme. However, a full understanding of the complex physiology of resistance remains to be determined, even for relatively well-studied species. We present results from comparisons of molecular structures and physiological responses to bufadienolides in various North American toad-eating and nontoad-eating snakes. We have found that, in addition to toad specialists, a number of natricine snakes that do not specialize on toads also exhibit resistance. We compared the coding sequences of the M1-M2 extracellular loop of NKA, a region identified as a primary binding site for cardiotonic steroids, in these species. We also compared organ-specific NKA sensitivity to bufadienolides and changes in NKA expression in response to bufadienolide dosing. Stress responses to bufadienolides were determined using measures of circulating corticosterone levels before and after dosing.

#### 11:40 AM 26 **Chemical ecology of poison frogs: evolution of alkaloid sequestration and autoimmunity** • *Room 210*

Juan C. Santos, University of British Columbia-Biodiversity Research Centre Rebecca Tarvin, The University of Texas at Austin Lauren O'Connell, Harvard University

Presenter: Juan C. Santos, University of British Columbia-Biodiversity Research Centre

Aposematism is an anti-predator mechanism usually defined by the joint presence of warning signals and defense. Among vertebrates, poison frogs are a well-known example; this clade is characterized by recurrent origins of an association between conspicuous coloration and diet-derived chemical defense (toxic alkaloids). The study of aposematism

in poison frogs has shown that the poison frog aposematic phenotype integrates with other aspects of their ecology including dietary specialization, metabolic rates, and alkaloid autoimmunity. This last aspect is especially important because some predators evolve resistance to prey alkaloids, and poison frogs must be resistant to their own alkaloids in order to accumulate them. In this presentation, I will review our current understanding of aposematism and autoimmunity in poison frogs and summarize how aposematism evolved as a complex phenotype, facilitating the sequestration of alkaloids from the environment. Then, I will discuss our progress toward understanding how poison frogs have become resistant to their own chemical defenses.

#### Wednesday, July 9

#### The role of bacteria in vertebrate chemical signaling: the scents of symbiosis

9:00 AM 27 The chemical space of microbial volatiles • Room 407

Stefan Schulz, Technische Universität Braunschweig

Presenter: Stefan Schulz, Technische Universität Braunschweig

Bacteria are well known to be important players shaping our environment and influencing interactions of organisms. The exact role they play in such interactions is only starting to emerge. One aspect of bacterial physiology that has been almost neglected for a long time is their ability to release volatile compounds. Bacteria contribute importantly to the odor space on Earth, which is also shaped by plants and animals as well as geological processes. Like plants, bacteria use major biochemical pathways to generate certain volatiles. Major groups of volatiles comprise compounds derived from the fatty acid pathway, terpenoids (mostly sesquiterpenes), aromatic compounds, sulfur compounds, or pyrazines/acetoins. While there seems to be a group of compounds which are repeatedly produced by many bacteria, adding to a still to define typical bacterial odor space, certain bacteria produce singular unique compounds. The functions of the bacterial volatiles are currently not well understood, although recently encouraging progress has been made. In this contribution, the different compounds produced by bacteria will be discussed and new results reported.

9:20 AM

#### Modeling the development of the hyena scent pouch microbiome • Room 407

Kevin Theis, Michigan State University Arvind Venkataraman, University of Michigan Thomas Schmidt, University of Michigan Kay Holekamp, Michigan State University

Presenter: Kevin Theis, Michigan State University

Animal-microbial collaborations are ubiquitous, and microbes are known to contribute to animal nutrition, immune health and behavior. We recently provided empirical evidence suggesting symbiotic bacteria underlie species, sex and reproductive chemical signaling among hyenas. Here, we model the ontogenetic development of the spotted hyena scent pouch microbiome and begin evaluating its behavioral relevance for juvenile hyenas. Specifically, we use contemporary microbial survey tools and analyses to show that bacterial communities in the scent pouches of juvenile hyenas are structured differently and are more variable than those in the pouches of adults. In general, juvenile scent pouch bacterial communities are diverse yet incomplete versions of the sex-specific phenotypes of adults. Nevertheless, the scent pouch bacterial communities of juvenile hyenas do vary from communities inhabiting juveniles' other body sites, and ecological models based

on the neutral theory of community assembly indicate that prominent bacterial types in juvenile scent pouch communities are being selected for in that particular environment. We show that these and other juvenile scent pouch bacteria are from clades whose characterized members are known to produce odorants that are typically present in hyena scent secretions. Collectively, these findings suggest that the spotted hyena juvenile scent pouch microbiome is, at least in part, under host control and that it functions in chemical signaling among juveniles. We conclude by comparing the development of the spotted hyena scent pouch microbiome with that of the striped hyena and discuss observed differences in the context of the two species' disparate social structures.

9:40 AM

#### The olfactory dilemma of badgers: Fitting in while standing out • Room 407 29

Christina D. Buesching, University of Oxford H. Veronica Tinnesand, Telemark University Simon Yungwa Sin, University of Oxford Kevin R. Theis, Michigan State University David W. Macdonald, University of Oxford

#### Presenter: Christina D. Buesching, University of Oxford

Social mammals often face an olfactory dilemma: maintaining a common group-odor without losing the means of self-advertisement. Consistent with the fermentation hypothesis, mammalian odors are not only dependent on the primary gland products secreted by the animal, but also on microbial metabolites of these gland products. Thus, this dilemma is exacerbated by the animals' need to manage and maintain the microbiota inhabiting their scent glands.

The subcaudal gland secretion of European badgers Meles meles represents a suitable model to study how animals can solve this dilemma. The secretion encodes individualspecific as well as group-related information through analogue coding, and symbiotic microbial communities in the subcaudal pouch, characterized through T-RFLP analyses, relate to some of the chemical components found in the secretion. Here, I will present some of the behavioral and physiological mechanisms badgers employ to maintain their individual- and group-specific microbial communities, before discussing future directions of this research.

#### E. coli sings: pour some sugar on me · Room 407 10:00 AM 30

#### Vanessa Sperandio, UT Southwestern Medical Center

Presenter: Vanessa Sperandio, UT Southwestern Medical Center

Gastrointestinal (GI) bacteria sense diverse environmental signals, including host hormones and nutrients, as cues for differential gene regulation and niche adaptation. Although the impact of carbon nutrition on the colonization of the gut by the microbiota has been extensively studied, the extent to which carbon sources affect the regulation of virulence factors by invading pathogens has not been fully defined. The enteric pathogen enterohemorrhagic Escherichia coli (EHEC) gages sugar sources as an important cue to regulate expression of its virulence genes. Specifically, this sugar-dependent regulation fine-tunes the expression of the locus of enterocyte effacement (LEE) pathogenicity island, which encodes for a type three secretion system, effectors, and an adhesin necessary for the formation of attaching and effacing (AE) lesions on enterocytes. Glycolytic environments inhibit the expression of the LEE genes. Conversely, growth within a gluconeogenic environment activates expression of these genes. Part of this sugar-dependent regulation

is achieved through two transcription factors: KdpE and Cra. Cra and KdpE interact to optimally directly activate expression of the LEE genes in a metabolite-dependent fashion. This sugar-dependent regulation is key during infection of the mammalian host, given that a kdpE mutant is attenuated in vivo. Additionally, a novel two-component signal transduction system, named FusKR (where FusK is a membrane bound histidine sensor kinase, and FusR a response regulator) that senses fucose, controls expression of the LEE genes. This fucose-sensing system is required for robust EHEC intestinal colonization. During growth in mucus, the glycophagic prominent member of the GI microbiota, *Bacteroides thetaiotaomicron*, supplies fucose to EHEC, modulating its virulence gene expression. Our findings suggest that EHEC uses fucose, a host-derived signal made available by the microbiota, to modulate EHEC virulence and metabolism, and suggest a new layer of complexity in the inter-kingdom signaling that underlies EHEC pathogenicity.

#### 10:40 AM 31 Olfaction and sex-specific catabolism of a bacterial odor in mice • Room 407

Stephen Liberles, Harvard Medical School

Presenter: Stephen Liberles, Harvard Medical School

How aversive and attractive odors are differentially processed by olfactory circuits to produce specific responses remains poorly defined. We recently identified a family of olfactory receptors termed trace amine-associated receptors (TAARs), and one of these receptors, TAAR5, mediates behavioral attraction to the sexually dimorphic mouse odor trimethylamine<sup>1</sup>. Trimethylamine is produced by commensal microflora in the gut, and evolution of the trimethylamine catabolism pathway in *Mus* enabled abundant and sex-dependent release. Trimethylamine is an aversive odor to rats and humans but is attractive to mice, who release >1,000-fold higher levels in scent depositions. Knockout of Taar5 eliminates attraction to trimethylamine, while TAAR4- encoded by an immediately adjacent gene- detects an aversive predator odor<sup>2</sup>. Based on these findings, TAARs provide a particularly powerful model system for studying odor aversion and attraction, and a framework for mechanistic dissection of how specific sensory receptor inputs are channeled in the brain to orchestrate appropriate behavioral outcomes.

#### References

<sup>1</sup> Li, Q. et al. Synchronous evolution of an odor biosynthesis pathway and behavioral response. Curr Biol 23, 11-20, (2013).

<sup>2</sup> Ferrero, D. M. et al. Detection and avoidance of a carnivore odor by prey. Proc Natl Acad Sci U S A 108, 11235-11240, (2011).

## 11:00 AM 32 A lethal neurotoxin as a chemical cue in the rough-skinned newt (*Taricha granulosa*) • Room 407

Patric M. Vaelli, Michigan State University Kevin R. Theis, Michigan State University Heather L. Eisthen, Michigan State University

Presenter: Patric M. Vaelli, Michigan State University

Tetrodotoxin (TTX) is a potent neurotoxin present in many species of amphibians and puffer fishes that inhibits the generation of action potentials and neural signaling in animal nervous systems. Consequently, TTX has been implicated primarily in predator defense. However, some animals such as the grass puffer (*Fugu niphobles*) and the rough-skinned newt (*Taricha granulosa*) are behaviorally attracted to TTX and can smell it at nanomolar concentrations. Our lab is combining behavioral studies and electroolfactogram (EOG)

recordings of newt nasal epithelia to elucidate the mechanisms by which newts detect TTX. Through manipulation of extracellular bath solution and application of selective ion channel blockers, our results reveal that TTX transduction does not involve the canonical odorant transduction pathway but instead involves a sodium channel, suggesting the evolution of a novel transduction mechanism. Furthermore, TTX production in many marine animals has been attributed to symbiotic bacteria inhabiting host tissues, but a TTX-producing symbiont has not been identified in amphibians, nor in any terrestrial or freshwater animal. We are characterizing the newt epithelial microbiome through 16S rRNA molecular surveys and employing ecologically-guided cultivation techniques to investigate TTX production by newt symbionts. To date, we have cultured 24 distinct bacterial types, 6 of which are from genera with identified TTX-producing species. Our research will describe the mechanism by which newts have evolved the ability to detect TTX, an otherwise deadly neurotoxin, as well as the role of symbiotic bacteria in producing this compound with profound effects on the ecology and physiology of the newt host.

#### Keeping it in the family: Social environment drives avian preen gland 33 microbiome structure · Room 407

Danielle J. Whittaker, Michigan State University Kevin Theis, Michigan State University

Presenter: Danielle J. Whittaker, Michigan State University

Birds produce species-specific volatile compounds that may be important in mate choice, as they convey information about individual identity and quality. These odors are present in preen oil, a substance secreted by the uropygial or "preen" gland that functions in maintaining feather health and, recent research suggests, chemical signaling. The preen gland harbors diverse microbial communities which we hypothesize produce many of the oil's volatile compounds. We examined patterns of preen gland microbiome similarity among adults and their offspring in a songbird, the dark-eyed junco (Junco hyemalis). Juncos are socially but not genetically monogamous–nearly 1/3 of all nestlings are sired by a male other than their social father. Both social parents feed the offspring, but only females incubate the eggs and brood the nestlings, thus spending much more time in physical contact with them. We used Illumina sequencing to target the 16S rRNA phylogenetic marker gene in bacteria swabbed from nestlings and adults at 13 nests during a single summer. Preen gland microbiomes contain many known odor-producers. There was a strong effect of nest on preen gland microbiome similarity in all individuals. Nestlings' microbiomes were markedly more similar to those of their mothers than fathers. Genetic relatedness of social fathers did not affect their microbiome similarity to the nestlings, nor did nests containing full- and half-siblings show different levels of similarity. Our data suggest that social environment and behavior are a primary driver of an individual's microbiome, with important implications for the evolution and development of animal chemical communication.

#### Microbe-mediated chemical signals of elephant musth · Room 407 34

Thomas E. Goodwin, Hendrix College

Presenter: Thomas E. Goodwin, Hendrix College

Mature male African and Asian elephants periodically experience a rut-like state called "musth", involving elevated serum testosterone, swollen and draining temporal glands, urine dribbling, lowered appetite, increased aggression, and enhanced reproductive success. Alkan-2-ones and alkane-2-ols are more abundant in secretions and excretions

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from musth males than in those from non-musth males. Using solid phase dynamic extraction (SPDE)/GC-MS, we have shown that these compounds continue to increase in concentration exogenously in expelled urine, apparently due to microbial metabolism (J. Chem. Ecol. 2012, 38, 81-87). It is reasonable to hypothesize that these ketones and alcohols are formed by metabolism of urinary fatty acids by a variant of the beta oxidation pathway. Shoemaker and Elliott developed an automated GC-MS analysis of urine samples for carbohydrates, organic and amino acids (J. Chromatogr. 1991, 562, 125-138). This technique has been used to identify and quantitate a series of long-chain fatty acids in musth and non-musth elephant urine. We hypothesize that just as albumin transports these hydrophobic fatty acids in blood plasma, it also transports them into the urine. We have isolated this urinary albumin by SDS-PAGE and sequenced it. We propose that microbes may play an important role in the temporal release of semiochemicals via metabolism of urinary fatty acids during elephant musth.

#### Quorum sensing and biofilms

2:30 PM

35 **Small talk and big effects: chemical signals from entomopathogenic bacteria** • *Illini Room B* 

Helge B. Bode, Goethe University, Frankfurt, Germany

Presenter: Helge B. Bode, Goethe University, Frankfurt, Germany

Entomopathogenic bacteria of the genera *Photorhabdus* and *Xenorhabdus* live in symbiosis with nematodes of the genera Heterorhabditis and Steinernema, respectively, and have been shown to be prolific producers of natural products. A detailed analysis of 300 different strains of these bacteria grown without nematodes led to the identification of several natural products that are novel and often conserved in specific phylogenetic clades of these bacteria, thus indicating also specific conserved functions of these compounds. Current efforts in our group deal with the activation and optimization of secondary metabolite production via the manipulation of specific or global regulators, the exchange of natural promoters against strong or inducible ones, and the production of compounds which are specifically produced exclusively in insects. Among the novel compound classes identified during our work are several new nonribosomally made peptides or peptide derivatives, widespread but overlooked dialkylresorcinols and cyclohexanediones, pyrones acting as a novel bacterial signalling system, and structurally unique polyamine natural products named fabclavines. The major goal of our work is to elucidate the natural function of these compounds as well as the regulatory mechanisms that enable their production.

3:10 PM

36 Competitive strategies differentiate closely related species of marine bacteria.
 Illini Room B

Paul R. Jensen, Katherine Duncan, and Natsassia Patin, Scripps Institution of Oceanography, UCSD

#### Presenter: Paul R. Jensen, Scripps Institution of Oceanography, UCSD

The application of molecular phylogeny to studies of microbial diversity has revealed that bacteria can be readily grouped into well-supported clades whose members share greater levels of sequence identity with each other than with the members of neighboring clades. While it has been suggested that these sequence clusters represent distinct ecotypes, creating links between fine-scale phylogeny and ecological function remains a major challenge for the field of microbial ecology. Here we show that two closely related species within the marine actinomycete genus *Salinispora* are characterized by distinct

competitive strategies. Using a direct competition assay to investigate antagonistic interactions with co-occurring members of the bacterial community, we observed temporal differences in the onset of allelopathy in the two species. The majority of inhibition observed *from S. arenicola* occurred early in the growth cycle and could be linked to antibiotic production. In contrast, inhibition from *S. tropica* occurred later in the growth cycle and could be linked to nutrient depletion. Growth curves further showed that *S. arenicola* has significantly slower doubling times than *S. tropica*, suggesting that *S. arenicola* uses interference competition. These patterns were consistent at the species level, yet the targets of the antibiotic activity were highly variable among strains providing experimental support for recent bioinformatic evidence of extensive plasticity in the *Salinispora* secondary metabolome. These results provide the first experimental evidence for the ecological divergence of two co-occurring and closely related species of marine sediment inhabiting bacteria.

3:30 PM

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#### Macroalgae may interrupt important cues for coral larval settlement • *Illini* Room B

Jennifer M. Sneed, Sarah J. Harrison, Lawrence J. Houk, and Valerie J. Paul, Smithsonian Marine Station at Fort Pierce

#### Presenter: Jennifer M. Sneed, Smithsonian Marine Station at Fort Pierce

Coral reefs are becoming increasingly dominated by fleshy macroalgae. Recovery of reefs is dependent on the recruitment of new corals and the presence of certain macroalgae impedes the settlement of coral larvae directly through physical and chemical competition. However, there may be a third impediment to the successful recruitment of coral larvae on algal-dominated reefs, namely the interruption of bacterially produced settlement cues. For some corals, the settlement process appears to be dependent on the presence of chemical cues produced by biofilm bacteria. To determine if macroalgae impact bacterial communities on settlement substrata, we examined the effects of two species of macroalgae commonly found on Caribbean reefs (Halimeda opuntia and Dictyota sp.) on the bacterial communities associated with the crustose coralline alga (CCA) Hydrolithon boergesenii. We attached either a live clump of algae or a fake aquarium plant to CCA pieces and placed them in individual flow-through chambers (n = 5) on the reef. After 48 hours, we sampled the biofilm communities on the CCA and the algae and analyzed them using next-generation sequencing. Both algae caused a shift in the bacterial community found on the surface of the CCA; however *H. opuntia* had the greatest impact. Organic extracts of H. opuntia affected growth of bacterial strains isolated from the surface of H. boergesenii in laboratory assays and the compound halimedatetraacetate demonstrated antibiotic activity against several strains. This study demonstrates that macroalgae can alter biofilm bacterial communities, some of which may provide chemical cues necessary for coral larval settlement.

4:10 PM

## 38 A volatile mediated, hitchhiking strategy between bacterial species, promotes spreading on solid surfaces • Illini Room B

Efrat Hagai, The Hebrew University of Jerusalem Reut Dvora, The Hebrew University of Jerusalem Tal Havkin-Blank, The Hebrew University of Jerusalem Einat Zelinger, The Hebrew University of Jerusalem Ziv Porat, Weizmann Institute, Israel Stefan Schulz, Technische Universität Braunschweig Yael Helman, The Hebrew University of Jerusalem

## Presenter: Yael Helman, The Hebrew University of Jerusalem

The ability to move on solid surfaces provides ecological advantages for bacteria, yet many bacterial species lack this trait. We found that *Xanthomonas* spp. overcome this limitation by making use of the swarming ability of proficient bacterial swarmers in their vicinity. Using *X. perforans* and *Paenibacillus vortex* as models, we show that *X. perforans* attracts proficient swarmers and uses them as a "ride" for dispersal. Interestingly, *X. perforans* not only attract the swarmers, they also increase their spreading zone by up to fourfold, without affecting their growth rate. Examination of the interaction in bi-partite Petri plates indicated that the active attractant produced by *X. perforans* is airborne. Using fluorescent stained *X. perforans* cells, we show that this hitchhiking strategy also occurs on tomato leaves, implicating an important role for epiphytic survival, colonization and host infection. The described interaction was observed between several Xanthomonads and swarming bacterial species, thus suggesting that this swarming induction and hitchhiking strategy might be widespread and ecologically important. This study provides an example as to how bacteria can rely on the skills of their neighboring species for their own benefit, signifying the importance of a communal organization for fitness.

## 4:50 PM 39 Pathogens talking to each other in dental biofilms • Illini Room B

Irene Wagner-Doebler, Helena Sztajer, Szymon P. Szafranski, Jürgen Tomasch, Michael Reck, Manfred Nimtz, and Manfred Rohde, Helmholtz-Centre for Infection Research

## Presenter: Irene Wagner-Doebler, Helmholtz-Centre for Infection Research

Cell-cell communication is suspected to play an important role in polymicrobial biofilms due to the close physical contact between species, but very few such interactions have actually been observed. Here we studied two human pathogens co-occurring in the oral cavity, the opportunistic fungus Candida albicans and the caries-promoting bacterium Streptococcus mutans. Dual-species biofilms reached higher biomass and cell numbers than mono-species biofilms. The production of extracellular polymeric substance (EPS) by S. mutans was strongly suppressed in co-culture with C. albicans, and thus its cariogenic potential was impaired. To detect interkingdom communication, we used a strain of S. mutans carrying a transcriptional fusion between a green fluorescent protein-encoding gene and the promoter for SigX, the alternative sigma factor of S. mutans which is induced by quorum sensing signals. Strong induction of sigX was observed in dual-species biofilms. Transcriptome analysis of S. mutans confirmed increased expression of all structural components of the quorum sensing system as well as the downstream genes for genetic competence. We showed for the first time the stimulation of the complete quorum sensing system of *S. mutans* by a species from another kingdom, namely the fungus *C. albicans*. Cell-cell communication and cross-feeding resulted in fundamentally changed virulence properties of the caries pathogen in dual species biofilms with C. albicans.

5:10 PM

## M 40 **Chemical crosstalk within and between species** • *Illini Room B*

Aviad Mandabi, Niva Levy, Rachel Gregor, Josep Rayo, and Michael M. Meijler, Ben-Gurion University of the Negev

Presenter: Michael M. Meijler, Ben-Gurion University of the Negev

Quorum sensing enables unicellular organisms to coordinate their behavior and function in such a way that they can adapt to changing environments and compete, as well as coexist, with multicellular organisms. *Pseudomonas aeruginosa* is an opportunistic pathogen that causes disease in immunocompromised patients. Quorum sensing in this pathogen is mediated by binding of the transcriptional activator, LasR, to its ligand 3-oxo-C12-HSL, leading to biofilm formation and secretion of virulence factors. We are targeting QS in *P. aeruginosa* and other bacteria with various chemical tools, such as a set of electrophilic probes that are designed to bind LasR covalently, leading to inhibition of QS-regulated gene expression. These probes can be used as molecular tools to obtain new insights into the mechanisms of activation and deactivation of bacterial quorum sensing. Furthermore, we recently found that certain QS molecules and other natural products can also directly affect the behavior of other bacterial species as well as that of eukaryotes. Diverse eukaryotes have been found to react strongly to the presence of these compounds (often initiating counter-warfare to jam bacterial communication); however, to date no eukaryotic protein has been identified that binds bacterial QS molecules. We have synthesized and evaluated a set of 'tag-free' probes to isolate and identify such receptors, in order to unravel mechanisms that govern these important interkingdom signaling events. We have also discovered several previously unknown signaling molecules from plants that interfere with bacterial communication.

## Insect communication through cuticular chemicals

2:30 PM

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Cuticular hydrocarbons and chemical communication—How are unique blends of hydrocarbons achieved? • Room 210

Gary J. Blomquist, University of Nevada, Reno Sharon Young, University of Nevada, Reno Marina MacLean, University of Nevada, Reno Rene Feyereisen, Institut Sophia Agrobiotech, Universite de Nice Sophia Antipolis Claus Tittiger, University of Nevada, Reno

## Presenter: Gary J. Blomquist, University of Nevada Reno

Long-chain hydrocarbons of insects play central roles in the waterproofing of the insect cuticle and function extensively in chemical communication where relatively non-volatile chemicals are required. The appreciation of the critical roles that hydrocarbons serve as sex pheromones, kairomones, species and gender recognition cues, nestmate recognition, dominance and fertility cues, chemical mimicry, primer pheromones and task-specific cues has resulted in an explosion of new information in the past several decades. This presentation will highlight some of the early discoveries on the role of hydrocarbons in insect communication and focus on the production of the complex mixtures of n-, terminally branched, internally branched and unsaturated components. A combination of enzyme-catalyzed reactions, including fatty acid synthase, desaturation, elongation, reduction to aldehyde and oxidative decarbonylation, produce the final products. The oxidative decarbonylation of very long chain aldehydes is catalyzed by a novel cytochrome P450, CYP4G1/2 in *Drosophila* and *Musca*. Preliminary evidence suggests that the methyl-branched hydrocarbons arise from a novel microsomal fatty acid synthase.

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## 3:10 PM 43 Ecological divergence and speciation • Room 210

William J. Etges, University of Arkansas

## Presenter: William J. Etges, University of Arkansas

Divergence in chemical communication in species of cactophilic Drosophila includes epicuticular hydrocarbon (CHC) and triacylglyceride (TAG) variation that influences mating success. Expression of CHCs and TAGs have a significant phylogenetic component with TAG producing species restricted to the subgenus Drosophila and types of CHCs, but not their abundance, conserved across species clades. In D. mojavensis from the Sonoran Desert that use different host cacti, covarying groups of C29 to C40 branched alkanes, alkenes, alkadienes, trienes, and tetraenes influence mate choice behavior in a cactus-specific manner. Higher resolution analysis of intact cuticles has revealed a larger number of CHCs (up to C50) and TAGs localized in the anogenital region of D. mojavensis and relatives. Male TAGs are synthesized in the ejaculatory bulb, transferred to females during mating, and inhibit female remating. Sexual isolation between allopatric D. mojavensis populations indicative of incipient speciation is caused by CHC and courtship song divergence. Behavioral isolation and CHC divergence are genetically correlated with variation in a component of fitness, egg to adult development time, associated with use of different host cacti. Genetic analysis revealed a number of common QTL influencing covariation in development time and amounts of CHCs associated with male mating success, suggesting ecologically driven reproductive divergence. Further genetic fine mapping and genome sequencing will be discussed in efforts to understand the causes of CHC divergence and reproductive isolation in this system.

## 3:30 PM 44 **Detection and discrimination of cuticular hydrocarbons in social insects** • *Room 210*

Majid Ghaninia, Arizona State University Brittany Enzmann, Arizona State University Dani Moore, Arizona State University Shelley L. Berger, University of Pennsylvania Claude Desplan, New York University Danny Reinberg, New York University School of Medicine and Howard Hughes Medical Institute Laurence J. Zwiebel, Vanderbilt University

Anandasankar Ray, University of California, Riverside

Presenter: Juergen Liebig, Arizona State University

The integrity of ant and other insect societies heavily relies on communication among colony members often through the olfactory channel. Mixtures of cuticular hydrocarbons are used in different contexts such as nestmate recognition and fertility signaling. Despite their important role in chemical communication, very little is known about their detection and discrimination by colony members. We performed single sensillum recordings to investigate how broadly hydrocarbons and other compounds can be detected by antennal basiconic sensilla of workers of the ant *Harpegnathos saltator*. We found that members of a panel with 31 hydrocarbons and 14 general odorants reliably induced a response in sensory neurons associated with basiconic sensilla of *H. saltator* workers. In this species, workers can become reproductive, which is associated with an on average five time increase in longevity. We were interested in whether this status change affects neuronal detection ability. Our findings indicate that the shift to reproduction is associated with weaker sensillar responses to hydrocarbon compounds which became more

prominent with increasing age. Interestingly, no age-dependent change in responsiveness was found in non-reproductive workers. We investigated the limits of discrimination of pairs of hydrocarbons with similar molecular structure in the ant species *Camponotus floridanus*, where workers can be conditioned using a sugar reward. In these bioassays, worker ants were able to discriminate between long-chained hydrocarbons that differ in chain-length by one carbon and between methyl-branched enantiomers. Our results provide insights into the neurophysiology of the remarkable good ability of ants to detect and discriminate cuticular hydrocarbons.

4:10 PM

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45

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## Drosophila oenocytes : role in hydrocarbon synthesis and in resistance to desiccation • Room 210

Claude Wicker-Thomas, Gwénaëlle Bontonou, Jean-Philippe Parvy, and Jacques Montagne, CNRS, LEGS

## Presenter: Claude Wicker-Thomas, CNRS, LEGS

*Drosophila* hydrocarbons are synthesized from fatty acid precursors that are desaturated by acyl-CoA desaturases, chain lengthened by elongases, and then subjected to oxidative decarbonylation to generate hydrocarbons. Most of the synthesis takes place in the oenocytes. Laboratory experiments have focused on the role of cuticular hydrocarbons as pheromones and in sex isolation. However, our understanding on the role of oenocytes in fatty acid metabolism is far from complete. We investigated the role of several genes transcribed in the oenocytes. RNAi-knock down of acetyl-CoA-carboxylase in the oenocytes led to an accumulation of lipid droplets in the oenocytes, as previously shown in larvae. No hydrocarbons were produced and the adults showed poor resistance to desiccation. The effect of other genes was diverse: RNAi-knock down of the LPR receptors prevented the entry of lipid droplets into the oenocytes but had no effect on hydrocarbon synthesis and resistance to desiccation. Other genes seemed to act on hydrocarbons only, on resistance to desiccation only, or on both. The results are presented and discussed.

4:30 PM

## A single gene affects both ecological divergence and mate choice in *Drosophila* • Room 210

Henry Chung, University of Wisconsin–Madison David W. Loehlin, University of Wisconsin–Madison Heloise D. Dufour, University of Wisconsin–Madison Kathy Vaccaro, University of Wisconsin–Madison Jocelyn G. Millar, University of California, Riverside Sean B. Carroll, University of Wisconsin–Madison

Presenter: Henry Chung, University of Wisconsin-Madison

Evolutionary changes in traits involved in both ecological divergence and mate choice may produce reproductive isolation and speciation. However, there are few examples of such dual traits, and the genetic and molecular bases of their evolution have not been identified. We show that methyl-branched cuticular hydrocarbons (mbCHCs) are a dual trait that affects both desiccation resistance and mate choice in *Drosophila serrata*. We identify a fatty acid synthase mFAS (CG3524) responsible for mbCHC production in Drosophila and find that expression of mFAS is undetectable in oenocytes (cells that produce CHCs) of a closely related, desiccation-sensitive species, *D. birchii*, due in part to multiple changes in cis-regulatory sequences of mFAS. We suggest that ecologically

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influenced changes in the production of mbCHCs have contributed to reproductive isolation between the two species.

### 4:50 PM

## 47 Removing the mystery from chiral methyl-branched hydrocarbons as contact pheromones. • *Room 210*

Jan Edgar Bello, University of California, Riverside Stephan Kuhbandner, University of Regensburg Gabriel P. Hughes, Purdue University Matthew Ginzel, Purdue University Joachim Ruther, University of Regensburg Jocelyn G. Millar, University of California, Riverside

Presenter: Jan Edgar Bello, University of California, Riverside

Methyl-branched hydrocarbons (MBCHs) are ubiquitous components of insect cuticular lipids. Several have been shown to function as contact pheromones, and it is likely that many more remain to be discovered. The majority of insect-produced MBCHs are chiral, but there have been no studies to determine whether they are biosynthesized enantiospecifically. In fact, there have been only a handful of studies on the effects of chirality on the biological activities of MBCH contact pheromones. This is primarily a result of the small to vanishingly small specific rotations of MBCHs (~3° to a tiny fraction of a degree), which in the past made enantiomeric analysis through polarimetry impractical, particularly in light of the small amounts of hydrocarbons (ng to  $\mu$ g) that can be obtained from many insects. The problem was compounded by difficulties in isolation of individual MBCHs from the crude mixture, and the time-consuming synthesis of chiral MBCH standards, both of which have hindered research on MBCH chirality. We will describe the isolation of insect MBCHs from crude extracts, using a combination of simple fractionating techniques and reverse phase HPLC with nonaqueous solvent systems, detecting all compounds with an evaporative light-scattering detector. Stereochemical analysis of the isolated MBCHs with a digital polarimeter revealed that the absolute stereochemistry of these insect natural products is conserved through at least nine orders of Insecta. We also present an efficient asymmetric synthesis of chiral MBCH standards. The resulting enantiopure compounds were used to test the effects of chirality of the bioactivity of the contact pheromones of a parasitic wasp, Lariophagus distinguendus, and the red-headed ash borer, Neoclytus acuminatus (Cerambycidae).

## 5:10 PM 152 Parasites may promote complexity and population divergence in recognition cues in social insects • *Room 210*

Anne-Genevieve Bagnères, CNRS and University of Tours Azzani Laura, University of Turin Bonelli Mariaelena, University of Tours and University of Turin Lorenzi Maria Cristina, University of Turin

## Presenter: Anne-Genevieve Bagnères, CNRS and University of Tours

Chemical cues used by social insects for nestmate recognition show remarkable complexity, but how this complexity has arisen is unclear. These cues mainly consist of tens of different hydrocarbons that cover their cuticle and function both for protection and communication. The paper wasp Polistes biglumis has a patchy distribution in mountain meadows of the Alps at elevation above 1200 m. Due to the strict environmental conditions, the colony cycle is reduced and colonies are always founded by a single foundress that is responsible for colony odor. Their social parasite *P. atrimandibularis* perfectly mimics host colonial signature and may exert strong seletion pressure on these cues. We found

that in this species cuticular hydrocarbon blends vary between geographically isolated populations. This divergence could be favored either by local temperature and humidity and/or local pressures on communication, i.e., the presence of social parasites that hijack host cues. We tested these two hypotheses in three populations of *P. biglumis* that differed in terms of concentration of cuticular hydrocarbons, proportions of branched hydrocarbons and overall variation in chemical profiles. To avoid potential short-term effects of parasite presence on the host signatures, we analyzed the cuticular hydrocarbons of non-parasitized nests. In two parasitized populations, host cuticular hydrocarbons had larger quantitative variation, higher concentration and larger proportion of branched hydrocarbons than in a parasite-free population, whereas temperature and humidity had relatively less effect on these traits. Therefore, obligate social parasites may act as 'engines of diversity' on host chemical signatures and operate in favor of diverging communication codes.

## Thursday, July 10

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## Fungal superhighways: common mycorrhizal networks mediating plant communication

### 9:00 AM

Hydraulic redistribution by common mycorrhizal networks • Illini Room B

Louise Egerton-Warburton, Chicago Botanic Garden Benjamin Morgan, Northwestern University José Ignacio Querejeta, CBAS-CSIC, Murcia, Spain Michael Allen, University of California, Riverside

Presenter: Louise Egerton-Warburton, Chicago Botanic Garden

In environments that experience seasonal or extended drought, plant productivity is limited by the availability of water. One mechanism that is important to whole plant water balance in these environments is hydraulic lift (HL), a passive process driven by gradients in water potential among soils layers. HL water also sustains mycorrhizal fungi, a root symbiosis that enhances the acquisition of mineral nutrients in terrestrial plants. Plant roots that are linked by shared or common mycorrhizal networks (CMNs) may also constitute pathways for the transfer of resources. We asked: could water supplied by HL be transferred among plants by CMNs? We examined this possibility using genetic, isotopic, and tracer dye studies of HL and water transfer by ectomycorrhizal (EM) and arbuscular mycorrhizal fungi (AMF) in Quercus (oak) seedlings following imposed drought, and AMF trees in dry seasonal tropical forests during the dry season. In oak seedlings, water transfer between donor and receiver plants was mediated by CMNs and occurred at night in concert with HL. The HL water was transferred between oak seedlings by EM networks, and to a lesser extent from oaks to AMF Salvia by AMF networks. In tropical forests, different tree species hosted similar AMF communities thereby increasing the potential for water transfer during the dry season. However, the extent of water transfer was limited. These results suggest that movement of water by CMNs is important to plant survival during drought, and that the functional ecophysiological traits of different mycorrhizal types might control the limits of this mechanism.

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#### Hijacking underground common mycorrhizal networks for interplant defense 9:20 AM 50 **communication** • *Illini* Room B

Rensen Zeng, Fujian Agriculture and Forestry University

## Presenter: Rensen Zeng, Fujian Agriculture and Forestry University

Mycorrhizas are ubiquitous plant-fungus symbiosis in terrestrial ecosystems. They play a vital role in plant nutrition and stress resistance. Common mycorrhizal networks (CMNs) link multiple plants together in ecosystems. Our study shows that CMNs mediate plantplant communication between healthy plants and enemy-challenged tomato plants. After establishment of CMNs with the arbuscular mycorrhizal fungus Glomus mosseae between tomato plants, inoculation of 'donor' plants with a pathogen or herbivorous insect led to increases in resistance and activities of the putative defensive enzymes, as well as induction of defense-related genes in healthy neighboring 'receiver' plants, suggesting that CMNs may function as a plant-plant underground talking conduit for systemic defense. However, use of a JA biosynthesis- defective mutant spr2 as 'donor' plants resulted in no induction of defense responses and no change in insect resistance in 'receiver' plants, suggesting that JA signaling is required for CMNs-mediated interplant communication. Our results indicate that plants are able to hijack CMNs for herbivore-induced defense signal transfer and interplant defense communication.

#### **Targeted defense signaling along common mycorrhizal networks** • Illini Room B 9:40 AM 51

Don Cipollini, Wright State University Rachel Fletcher, Xavier University E Kathryn Morris, Xavier University

## Presenter: Kathryn Morris, Xavier University

When under attack by insect and microbial pathogens, plants produce signaling compounds to induce defenses, and these signaling compounds appear to travel along common mycorrhizal networks (CMNs) linking plants belowground. We explored the possibility that CMNs provide a route for targeted defense signaling between conspecific individuals, while leaving unrelated plants out of the network. Although arbuscular mycorrhizal fungi (AMF) are not host-specific, most AMF demonstrate clear host preferences, making it likely that, in mixed species communities, congeneric plants may well be more connected to each other than to unrelated individuals. We grew corn and tomato plants together in both halves of pots divided by a mesh barrier to control CMN connectivity. After the CMN established, we induced defenses in the corn or tomato plant in one-half of each pot and measured defense induction in the corn and tomato plant in the other pot half. Greater defense induction in congeneric individuals supports the hypothesis that congeneric plants can communicate preferentially with each other. This has interesting implications for any plant interaction involving transfer of infochemicals via fungal networks.

#### Aboveground fungal pathogens reduce belowground microbial mutualists • 10:00 AM 52

## Illini Room B

Daniel J. Ballhorn, Portland State University Brett Younginger, Portland State University Stefanie Kautz, Field Museum of Natural History

Presenter: Brett Younginger, Portland State University

Induced aboveground plant defenses against pathogens can have negative effects on belowground microbial symbionts. Several studies have utilized chemical elicitors to induce such defenses, but there is little evidence that actual aboveground pathogens affect

root-associated microbes. We report here that an aboveground fungal pathogen induces a defense response that inhibits the colonization of roots by rhizobia and arbuscular mycorrhizal fungi (AMF). Our data suggest that a systemic increase in the activity of polyphenol oxidases (PPOs) is the causative agent for the observed belowground effects. Foliage of plants inoculated with either rhizobia or AMF were treated with live Colletotrichum gloeosporioides and C. gloeosporioides extract. PPO, chitinase and  $\beta$ -1,3-glucanase activity in leaves and roots, as well as the extent of rhizobia and AMF colonization, were measured after pathogen treatments over an experimental period of 21 days. Both live pathogen and pathogen extract significantly increased PPO, chitinase and  $\beta$ -1,3-glucanase activity in leaves, but only PPO-activity increased in roots. Treatments with live C. gloeosporioides and C. gloeosporioides extract had 126% and 54% greater PPO-activity in the roots, respectively, when compared to control plants. Rhizobia colonization was reduced by 154% and 149% in live pathogen and pathogen extract treatments. Similarly, AMF colonization was reduced by 168% and 159% in both treatments. We demonstrate that aboveground fungal pathogens can affect very different types of belowground microbial symbionts. We predict that the top-down effects we show here can drastically impact plant performance in soils with limited nutrients and water, with stress conditions usually mitigated by belowground microbial mutualists.

## 4 53 Allelopathic disruption of the mycorrhizal-plant mutualism reduces carbon acquisition, allocation and growth in a native forest herb • Illini Room B

Alison N. Hale, University of Pittsburgh Susan Kalisz, University of Pittsburgh

## Presenter: Alison N. Hale, University of Pittsburgh

Mutualism theory focuses on understanding the persistence of mutualisms over evolutionary time scales. However, the unprecedented pace of global change has introduced novel selection pressures over compressed, ecological time frames. Understanding the ability of mutualistic species to rapidly respond is critical for protecting these key species interactions. Among land plants, ~90% of species form associations with obligately mutualistic arbuscular mycorrhizal fungi (AMF). In this interaction, plants provide carbon to AMF and receive soil nutrients and water in exchange. External forces that reduce the function of the AMF mutualism are expected to compromise the plant's physiological status and, when chronic, result in carbon starvation and ultimately population decline. In this study, we provide physiological evidence for the disruption of AMF mutualism by an invasive allelopathic plant (Alliaria petiolata). Results from a greenhouse experiment with Maianthemum racemosum, a common, highly mycorrhizal native forest herb, demonstrate that prolonged exposure to allelochemicals results in lower photosynthetic rates, 17% less carbon storage, reduced root growth and asexual reproduction, and reduced soil fungal hyphae relative to control plants. We observed similar trends in fungicide-treated plants, indicating that these declines arise from AMF mutualism disruption. Leaf gas exchange data and nutrient analyses indicate that the declines in physiological performance result from water stress and altered source-sink dynamics. Our data indicate that AMF mutualism disruption can reduce native plant vigor and could ultimately diminish their population stability. When impacts on mutualistic species are dramatic and swift, management efforts may be required to protect the interaction from extinction.

10:40 AM

## 11:00 AM 54 Silicone Tubing Microextraction (STME) for in situ sampling of lipophilic allelochemicals in soil and water • Illini Room B

Brian K. Mohney, Ashland University Kathryn Morris, Xavier University Jeffrey D. Weidenhamer, Ashland University

## Presenter: Brian K. Mohney, Ashland University

The lack of methods to monitor allelochemical dynamics in soil has hindered research on allelopathy. Polydimethylsiloxane (PDMS) microtubing can monitor the release of lipophilic allelochemicals in soil, providing measurements of soil allelochemicals which are proportional to soil fluxes. STME is a new sampling technique in which PDMS microtubing is placed directly in the sample. Lipophilic analytes diffuse and absorb into the silicone and are recovered by passing solvent through the tubing lumen. The silicone acts as a diffusive sink, analytes are recovered directly for chromatographic analysis, and solvent never directly contacts the sample matrix. The technique is superior in cost and ease of coupling to HPLC over solid phase microextraction and stir bar sorptive extraction because it facilitates repeated, non-destructive sampling of soil and biological matrices and the extract can be directly injected for analysis. Because PDMS selectively sorbs nonpolar compounds, marigolds are an excellent model system for method development due to the lipophilic character of their thiophenes. A key advantage of STME is the ability to repeatedly sample soil without disturbance, allowing the analysis of allelochemical concentrations at particular locations beneath a plant. STME has been used to demonstrate that thiophene release by marigold roots exhibits tremendous spatial and temporal heterogeneity and that these compounds move through the soil's common mycorrhizal network, extending bioactive zones around plant roots. Our results demonstrate that STME can be used as tool to provide a much more finely-resolved picture of the dynamics of allelochemicals in the root zone than has previously been available.

## 11:20 AM 55 **Root zone chemical ecology; sampling of below-ground volatile semiochemicals** • *Illini Room B*

Hans T. Alborn, USDA ARS CMAVE Denis S. Willett, University of Florida CREC

## Presenter: Hans T. Alborn, USDA ARS CMAVE

Studies of plant- and insect- produced semiochemicals released above-ground have resulted in successful techniques to control and monitor insect pests. It is now also well established that plant roots can release herbivore-induced volatile organic compounds (VOCs) that attract beneficial organisms such as entomopathogenic nematodes. However, more basic study of below-ground multitrophic interactions lags because of the complexity of the system. In addition to plants roots, VOCs can be produced by insects, microorganisms and nematodes and, contrary to the above-ground environment, in soil these VOCs are released into a virtually static airspace where they disperse solely by diffusion. To bypass this complexity, root-related VOCs have been sampled from uprooted and washed plants where air surrounding the exposed roots is drawn through an adsorption filter that trap VOCs, or by maceration and solvent extraction. These techniques create VOC profiles with little relevance to the system intended to be studied. Probes were designed for direct in-soil sampling of VOCs that when used in combination with thermal desorption GC/ MS analyses allow for sensitive and less intrusive in vivo sampling. This technique makes

it possible to continuously monitor and follow the dynamics of root zone VOC release in response to insect or nematode infestations. This technique was evaluated for reproducibility and qualitative and quantitative limitations in relation to environmental factors such as soil type and water content.

## 11:40 AM 56 Rhizobia decrease indirect defense of lima bean (*Phaseolus lunatus*): less extrafloral nectar and fewer ants • *Illini Room B*

Adrienne L. Godschalx, Portland State University

Presenter: Adrienne L. Godschalx, Portland State University

Many plants maintain symbiotic relationships with multiple partners that do not interact directly but are connected through their common host. Understanding the functional interplay of symbionts associated with the same host remains an important challenge in biology. Here we show nitrogen-fixing rhizobia alter the plant chemistry and defensive strategy of lima bean (Phaseolus lunatus) by differentially affecting direct and indirect defenses against herbivores. We inoculated lima bean plants (R+) with a natural rhizobium strain and measured nutritive and defensive plant traits for young, intermediate, and mature leaves in comparison to rhizobia-free (R-) controls. Furthermore, we experimentally induced indirect defense (extrafloral nectar; EFN) and subsequently counted ants attracted to each plant. Rhizobia increased cyanogenesis, a constitutive direct chemical defense against herbivores, but decreased inducible EFN production to 0.5 mg sugar g-1 dw in plants with rhizobia, relative to 1.6 mg sugar g-1dw in rhizobia-free controls. R+ plants attracted significantly fewer ants (mean= 0.9 ants) than R- plants (mean= 2.6 ants). The fundamentally different rhizobia-mediated effects on simultaneously expressed defensive plant traits indicate rhizobia can have significant bottom-up effects on higher trophic levels. Lower ant recruitment in R+ plants likely resulted from decreased EFN, which may be the side-effect of a carbon tradeoff within the plant between EFN and rhizobia. Our results show belowground symbionts can play a critical and underestimated role in determining complex aboveground interactions.

## Chemical ecology of insect herbivore genomes

9:00 AM

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57 **Molecular evolution of the arthropod odorant and gustatory receptor families** • *Room 210* 

Hugh Robertson, University of Illinois at Urbana-Champaign

Presenter: Hugh Robertson, University of Illinois at Urbana-Champaign

The odorant (OR) and gustatory (GR) families form a superfamily of ligand-gated ion channels that serve as chemoreceptors mediating most of the specificity and sensitivity of olfaction and taste in arthropods, along with the more recently described but unrelated ionotropic receptor (IR) family. We have characterized this gene superfamily from most publicly available arthropod genome sequences. I will describe three aspects of the molecular evolution of this superfamily. First, the repertoire sizes of the two families are largely congruent with the known complexity of the chemical ecology and sensory physiology of these arthropod species. Second, studies of recently expanded subfamilies in particular species reveal that positively selected amino acids are primarily in the three extracellular loops or the outside edges of the seven-transmembrane protein domains of these ligand-gated ion channels, supporting the notion from experimental studies that the ligand-binding pocket is a complex structure comprising these regions. Third, we have found representatives of the GR family in the publicly available genomes of various other

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animal phyla, including Nematoda, Mollusca, Annelida, Cnidaria, and Placozoa, as well as basal lineages of the Chordata, but not any vertebrates. The superfamily is therefore ancient in animals, but has apparently been lost completely from some lineages, e.g. Ctenophora and Porifera in addition to Vertebrata. It is also not present in the two sequenced choanoflagellates. In contrast, the OR family is only present in insects, representing an expansion of a single GR lineage, perhaps as part of adaptation to terrestriality.

#### 9:40 AM 58 Pheromone production and resin metabolism in pine bark beetles • Room 210

Claus Tittiger, University of Nevada, Reno Minmin Song, University of Nevada, Reno Sharon Young, University of Nevada, Reno Jeff Nadeau, University of Nevada, Reno Christopher I. Keeling, University of British Columbia Joerg Bohlmann, University of British Columbia Gary J. Blomquist, University of Nevada, Reno

## Presenter: Claus Tittiger, University of Nevada, Reno

Pine bark beetles are obligate parasites of their hosts. Their strategy to overcome host defensive resin and successfully reproduce includes a pheromone-mediated "mass attack" that rapidly attenuates the tree's ability to produce toxins, as well as metabolic conversion of resin components. These processes are windows into bark beetle-host tree co-evolution and are also attractive targets to develop potential new management strategies. The aggregation pheromone system in the mountain pine beetle (Dendroctonus ponderosae) consists of monoterpenoid (frontalin, (-)-trans-verbenol) and fatty acid-derived (exobrevicomin) components. Functional genomics resources were used to tentatively identify genes involved in pheromone component biosynthesis, including dehydrogenases, cytochromes P450, and terpene synthases. Their roles in pheromone biosynthesis were confirmed through functional assays of their translation products. Some related P450 enzymes likely function in resin detoxification, supporting the hypothesized evolutionary relationship between pheromone biosynthesis and resin metabolism and providing new insight regarding bark beetle interactions with host trees.

#### Genomic insights into herbivore strategies for dealing with secondary plant 59 compouns and chemical and biological insecticides • Room 210

David Heckel, Max Planck Institute for Chemical Ecology

Presenter: David Heckel, Max Planck Institute for Chemical Ecology

The genomes of several herbivorous insects have now been sequenced, and comparative annotation of gene families can provide an overview of the potential tools each species can bring to bear in dealing with challenges posed by toxic secondary plant compounds and insecticides. However, this information does not tell us how effective these tools are, nor how they are deployed in specific contexts. Dynamic analyses of transcript abundances, whether by microarrays or by deep sequencing approaches, reveal several different requlatory modules. Some of these respond to specific chemical insults and others to more general stresses, while still others regulate metabolism to counterbalance the effects of stress. Comparing these responses across a broad range of conditions addresses the issue of how finely responses are tailored to particular detoxicative needs, and whether herbivorous insects can upregulate countermeasures in anticipation of their host plants' own induced antiherbivory defenses.

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10:00 AM

## 10:40 AM 60 Insights into herbivory and detoxification through the *Plutella xylostella* genome • *Room 210*

Xianchun Li, University of Arizona Weiyi He, Fujian Agriculture and Forestry University Guang Yang, Fujian Agriculture and Forestry University Jun Wang, BGI-Shenzhen Minsheng You, Fujian Agriculture and Forestry University

Presenter: Xianchun Li, University of Arizona

How an insect evolves to become a successful herbivore is of profound biological and practical importance. Herbivores are often adapted to feed on a specific group of evolutionarily and biochemically related host plants, but the genetic and molecular bases for adaptation to plant defense compounds remain poorly understood. We report the first whole-genome sequence of an important lepidopteran pest of cruciferous plants, *Plutella xylostella*, which contains 18,071 protein-coding and 1,412 unique genes with an expansion of gene families associated with perception and detoxification of plant defense compounds. A recent expansion of retrotransposons near detoxification-related genes and a wider system used in the metabolism of plant defense compounds are also involved in the development of insecticide resistance. This work shows the genetic and molecular bases for the evolutionary success of this worldwide herbivore and offers wider insights into insect adaptation to plant feeding, as well as opening avenues for more sustainable pest management.

## 11:00 AM 61 **Female behavior drives expression and evolution of gustatory receptors in butterflies** • *Room 210*

Adriana D. Briscoe, University of California, Irvine

Presenter: Adriana D. Briscoe, University of California, Irvine

Secondary plant compounds are strong deterrents of insect oviposition and feeding but may also be attractants for specialist herbivores. These insect-plant interactions are mediated by insect gustatory receptors (Grs) and olfactory receptors (Ors). An analysis of the reference genome of the butterfly Heliconius melpomene, which feeds on passion-flower vines (Passiflora spp.), together with whole-genome sequencing within the species and across the Heliconius phylogeny has permitted an unprecedented opportunity to study the patterns of gene duplication and copy-number variation (CNV) among these key sensory genes. We report in silico gene predictions of 73 Gr genes in the H. melpomene reference genome, including putative CO2, sugar, sugar alcohol, fructose, and bitter receptors. The majority of these Grs are the result of gene duplications since Heliconius shared a common ancestor with the monarch butterfly or the silkmoth. Among Grs but not Ors, CNVs are more common within species in those gene lineages that have also duplicated over this evolutionary time-scale, suggesting ongoing rapid gene family evolution. Deep sequencing (,1 billion reads) of transcriptomes from proboscis and labial palps, antennae, and legs of adult *H. melpomene* males and females indicates that 67 of the predicted 73 Gr genes and 67 of the 70 predicted Or genes are expressed in these three tissues. Intriguingly, we find that one-third of all Grs show female-biased gene expression (n = 26) and nearly all of these (n = 21) are Heliconius-specific Grs. In fact, a significant excess of Grs that are expressed in female

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legs but not male legs are the result of recent gene duplication. This difference in Gr gene expression diversity between the sexes is accompanied by a striking sexual dimorphism in the abundance of gustatory sensilla on the forelegs of *H. melpomene*, suggesting that female oviposition behavior drives the evolution of new gustatory receptors in butterfly genomes.

## 11:20 AM 62 **The genome signature of polyphagy and monophagy: spider mite comparative genomics** • *Room 210*

Michael Grbic, University of Western Ontario

## Presenter: Michael Grbic, University of Western Ontario

The two spotted spider mite *Tetranychus urticae* Koch is a cosmopolitan agricultural pest with an extensive host plant range and an extreme record of pesticide resistance. *T. urticae* represents one of the most polyphagous arthropod herbivores, feeding on more than 1100 plant species belonging to more than 140 different plant families including species known to produce toxic compounds. It is a major pest in greenhouse production and field crops, destroying annual and perennial crops such as tomatoes, peppers, cucumbers, strawberries, maize, soy, apples, grapes, and citrus. This genus contains also oligophagous and stenophagous species. To understand the evolution of polyphagy and monophagy our international consortium sequenced and annotated spider mite genomes including polyphagous *T. urticae*, oligophagous *T. evansi* (feeding on solanace) and stenophagous T. *lintearius* genome searching for genome signatures of different feeding modes and genome evolution. All 3 genomes are similar in size and displaying remarkable instances of gene gains and losses. The completion of the 3 spider mite genomes opens new avenues for understanding the fundamentals of plant-herbivore interactions, developing novel pest management strategies and producing new bionanomaterials.

## 11:40 AM 63 Impacts of antimicrobial plant resins on honey bee gene expression • Room 210

Michael Simone-Finstrom, North Carolina State University

Presenter: Michael Simone-Finstrom, North Carolina State University

Honey bees have evolved many traits and behaviors to combat parasites and disease. Once such behavior is the harvesting of complex antimicrobial plant resins and incorporation of these volatile rich materials in the nest architecture. While this amalgamation of plant resins, known as propolis in the apiculture world, may serve many roles in hive maintenance, current research has focused on it as a mechanism of social immunity whereby the presence of propolis confers colony-level disease resistance benefits. Gene expression of bees in resin-rich environments is altered, likely due to subsequent changes in the hive microbial environment, which can affect individual lifespan and colony productivity. Exposure to components of propolis can also alter expression of detoxification-related genes and increase survival after treatment with toxins. Further research on identifying the important biologically active components in these diverse mixtures using modern techniques, such as metabolomics, allow us to better identify plant sources of these resins. Experiments examining how honey bees utilize difference sources both temporally and after exposure to pathogens can then use these methods to more accurately address this question.

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## Chemical cues and signals structure marine populations, communities, and ecosystems

2:30 PM

## Chemical ecology as a Rosetta stone for understanding the structure, function, and resilience of coral reefs · *Illini Room B*

Mark E. Hay, Georgia Institute of Technology

Presenter: Mark E. Hay, Georgia Institute of Technology

Coral reefs are in precipitous global decline. In the last 3-4 decades, coral cover has declined by 80% throughout the Caribbean and 50% throughout the tropical Pacific, with seaweeds commonly replacing corals. Much of the decline and lack of recovery can be attributed to alterations in fundamental biotic interactions that are mediated via bioactive secondary metabolites. Experiments on Fijian reefs demonstrate that herbivory by specific mixes of herbivorous fishes (those resistant to different seaweed defenses) is critical for suppressing chemically-rich seaweeds that damage corals on contact via allelopathic lipids. Interactions are dynamic, with seaweeds inducing greater allelopathy when contacted by corals and coral chemically cueing fish to consume the seaweeds. Of equal importance is how coral and fish larvae respond to chemical cues from overfished areas dominated by seaweeds versus no-take marine protected areas (MPAs) dominated by corals. Recruiting fishes and corals chemically sense and are attracted to coral-dominated areas protected from fishing while being chemically repulsed by seaweed-dominated areas that are overfished. Attraction and repulsion are cued by odors from specific corals and seaweeds that best predict reef quality. Both recruiting fishes and coral larvae refused to settle in overfished, seaweed dominated areas but recruited readily to immediately adjacent reefs where fishing was banned and corals dominated. These chemically-cued behaviors can close the open nature of marine populations, suppress larval export from coral dominated marine protected areas to degraded reefs, and prevent recovery of coral and fish populations once reefs degrade and become dominated by seaweeds.

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## Evidence that dimethyl sulfide facilitates a tritrophic mutalism between primary producers and marine top predators in the Southern Ocean · Illini Room B

Gabrielle Nevitt, University of California, Davis Matthew Savoca, University of California, Davis

## Presenter: Gabrielle Nevitt, University of California, Davis

Tritrophic mutualisms have been best studied in plant-insect systems. During these interactions, plants release volatiles in response to herbivore damage, which, in turn, facilitates predation on primary consumers or benefits the primary producer by providing nutrients. Here, we explore a similar interaction in the Southern Ocean food web, where soluble iron limits primary productivity. Dimethyl sulfide (DMS) has been studied in the context of global climate regulation and is an established foraging cue for marine top predators. We present evidence that procellariiform seabird species that use DMS as a foraging cue selectively forage on phytoplankton grazers. Their contribution of beneficial iron recycled to marine phytoplankton via excretion suggests that DMS mediates a link between marine top predators and oceanic primary production. Taken together, our results imply that marine top predators play a critical role in maintaining both ocean health and global climate via this chemically mediated mutualistic interaction with marine phytoplankton.

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#### Diffuse coevolutionary arms races among plants and herbivores in the sea · 3:10 PM 65 Illini Room B

Erik Sotka, College of Charleston

Presenter: Erik Sotka, College of Charleston

Seaweeds commonly produce chemical defenses that minimize the impact of herbivorous fishes and urchins, who must evolve physiological mechanisms to tolerate these metabolites. Recent evidence indicates that diffuse coevolutionary arms races between seaweeds and herbivores are rampant in marine ecosystems. At higher latitudes, the coevolutionary dynamics are mediated by tannin-based defenses while terpene-based defenses mediate coevolution at lower latitudes. These results also reinforce the important roles that microevolution, phylogenetic lineage and geographic origin have in the feeding habits of highly generalist herbivores.

#### Chemical mediation of macroalgal-herbivore interactions on the western 3:30 PM 70 **Antarctic peninsula** • *Illini Room B*

Charles D. Amsler, University of Alabama at Birmingham James B. McClintock, University of Alabama at Birmingham Bill J. Baker, University of South Florida

Presenter: Bill J. Baker, University of South Florida

The structure of the hard bottom community of the western Antarctic Peninsula includes a robust diversity of invertebrates and a dominant macroalgal canopy. Attending these macroalgal forests are high densities of mesograzers that include both amphipods and gastropods, as well as an assemblage of omnivorous fish. Neither amphipods nor fish include the macroalgae as a significant component of their diet and most macroalgal species are deterrent to fish. The amphipods take advantage of the fish-deterrent algae as refuge, gaining protection from foraging fish. Macroalgae benefit from acting as host due to epiphyte removal by amphipod grazing. Because amphipods and macroalgae comprise major components of this community, their relationship can be regarded as a communitywide mutualism. While neither hosting nor grazing services are unique to Antarctica, this presentation will consider the scale and scope of contrasts with lower latitudes.

#### The world is my oyster: Chemical signals govern settlement and predatory 71 interactions on oyster reefs • Illini Room B

Lee Smee, Texas A&M-Corpus Christi

## Presenter: Lee Smee, Texas A&M-Corpus Christi

Chemical cues mediate many important processes life history processes in marine communities including settlement, foraging, predator deterrence, and mating. We investigated the role of chemical signals in mediating settlement choices and predation responses of Eastern Oysters (Crassostrea virginica). Oysters are broadcast spawners that have planktonic larvae. Once oysters select a location for settlement, they are unable to move, making their settlement choice of paramount importance. We investigated how chemical cues mediate oyster settlement in the field. Our results indicated that oyster larvae detect exudates from living adult oysters and settle preferentially near them. Further, by increasing the genetic diversity on oyster reefs, more oyster larvae were induced to settle. Oysters are the first sexually reproducing ecosystem-engineer in which genetic diversity is known to affect an important ecological function. This finding suggests that reduction

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4:10 PM

of genetic diversity on oyster reefs can have severe negative effects on oyster recruitment and hasten reef decline. After settlement, oysters are vulnerable to a variety of fish, crab, and gastropod consumers, and we investigated potential defensive responses of oysters to two common predators: mud crabs (*Panopeus herbstii*) and blue crabs (*Callinectes sapidus*). When reared in laboratory mesocoms, oysters grew heavier shells that required more force to break in response to predator exudates; the change in shell morphology increased survival in subsequent feeding assays. Although the heavier shell was an effective predator deterrent, it was costly and resulted in oysters producing less soft tissue and potentially having lower fecundity. Future studies are planned to ascertain the nature of chemical signals that modulate these interactions.

## 4:30 PM 67 Talk withdrawn

4:50 PM

## 66 Behavioral impairments in reef fishes caused by ocean acidification at CO2 seeps • Illini Room B

Katharina E Fabricius, Australian Institute of Marine Science Philip L Munday, James Cook University Alistair J Cheal, Australian Institute of Marine Science Jodie L Rummer, James Cook University

Presenter: Danielle L. Dixson, Georgia Institute of Technology

Experiments have shown that the behavior of reef fishes can be seriously affected by projected future carbon dioxide (CO2) concentrations in the ocean. However, whether fish can acclimate to elevated CO2 over the longer term, and the consequences of altered behavior on the structure of fish communities, are unknown. We used marine CO2 seeps in Papua New Guinea as a natural laboratory to test these questions. Here we show that juvenile reef fishes at CO2 seeps exhibit behavioral abnormalities similar to those seen in laboratory experiments. Fish from CO2 seeps were attracted to predator odor, did not distinguish between odors of different habitats, and exhibited bolder behavior than fish from control reefs. High CO2 did not, however, have any effect on metabolic rate of aerobic performance. Contrary to expectations, fish diversity and community structure differed little between CO2 seeps and nearby control reefs. Differences in abundances of some fishes could be driven by the different coral community at CO2 seeps rather than by direct effects of high CO2. Our results suggest that recruitment of juvenile reef fish from outside the seeps, along with fewer predators within the seeps, is currently sufficient to offset any negative effects of high CO2 within the seeps. However, continuous exposure does not reduce the effect of high CO2 on the behavior in natural reef habitats, and this could be a serious problem for fish communities in the future when ocean acidification becomes widespread as a result of continued uptake of anthropogenic CO2 emissions.

5:10 PM

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## Metabolomics and proteomics reveal metabolic impacts of allelopathy in the marine plankton • Illini Room B

Kelsey L. Poulson-Ellestad, Georgia Institute of Technology Christina M. Jones, Georgia Institute of Technology Jessie Roy, Georgia Institute of Technology Mark R. Viant, University of Birmingham Facundo M. Fernandez, Georgia Institute of Technology

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Julia Kubanek, Georgia Institute of Technology Brook L. Nunn, University of Washington

## Presenter: Julia Kubanek, Georgia Institute of Technology

Competition is a major force structuring marine planktonic communities. The release of compounds that inhibit competitors, a process known as allelopathy, may play a role in the maintenance of large blooms of the red tide dinoflagellate Karenia brevis, which produces potent neurotoxins that negatively impact coastal marine ecosystems. K. brevis is variably allelopathic to multiple competitors, typically causing sublethal suppression of growth. We employed metabolomic and proteomic analyses to investigate the role of chemically mediated ecological interactions between K. brevis and two diatom competitors, Asterionellopsis glacialis and Thalassiosira pseudonana. The impact of K. brevis allelopathy on competitor physiology was reflected in the metabolomes and expressed proteomes of both diatoms, although the diatom that co-occurs with K. brevis blooms (A. glacialis) exhibited more robust metabolism in response to K. brevis. The observed partial resistance of A. glacialis to allelopathy may be a result of its frequent exposure to K. brevis blooms in the Gulf of Mexico. For the more sensitive diatom, T. pseudonana, which may not have had opportunity to evolve resistance to K. brevis, allelopathy disrupted energy metabolism and impeded cellular protection mechanisms including altered cell membrane components, inhibited osmoregulation, and increased oxidative stress. Allelopathic compounds appear to target multiple physiological pathways in sensitive competitors, demonstrating that chemical cues in the plankton have the potential to alter large scale ecosystem processes including primary production and nutrient cycling.

2:30–4:00 pm Roundtable Discussion: Three generations of chemical ecology • Room 210 Organizers: Monika Hilker, Freie Universität Berlin, and Julia Kubanek, Georgia Tech Generation 3: Danielle Dixson, USA, and Anke Steppuhn, Germany Generation 2: Paulo Zarbin, Brazil, and Alvin Kah-Wei Hee, Malaysia Generation 1: Jeremy McNeil, Canada, and Jocelyn Millar, USA

## Friday, July 11

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## The use/application of semiochemicals to manage farm, zoo and wild animals

9:00 AM

Learning and applications of chemical signals in vertebrates for conservation and management  $\cdot$  Room 407

Bruce A. Schulte, Western Kentucky University

Presenter: Bruce A. Schulte, Western Kentucky University

At its most unfettered, conservation seeks to increase or decrease the abundance of a biotic or abiotic resource to enhance and protect biodiversity. Whether saving an endangered species or eradicating an invasive one, methodologies have ranged from the landscape to the individual level. In trying to enhance the stability of beneficial animal populations or reduce the presence of animals that create conflict with humans, a conservation behavior approach advocates an understanding of individual needs, constraints and sensory mechanisms. Despite being the oldest form of communication, humans put little reliance on taste and smell in their daily activities, which may contribute to the relatively rudimentary application of natural chemical signals in conservation. The use of semiochemicals is now a standard component to integrated pest management in

agriculture, but natural chemical signals have not had the universal application or success as perhaps once hoped. The application of chemical signals for vertebrate conservation, while also promising, has been fraught with obstacles. Compared to invertebrates, few vertebrate pheromones have been discovered. The multimodal evaluation of signals by many vertebrates and the potential use of social odors in lieu of specific pheromones may limit the use of chemical signals as a singular management tool. Nevertheless, odors for humans are known to enhance memory and evoke strong recollections. Many vertebrate species have a capacity for extensive learning and meaningful or unique odors in a learning protocol may provide a heretofore underutilized instrument in the conservation and management of vertebrate species.

### 9:20 AM

### M 73 Using feline pheromones: from pet cats to wild felids. • Room 407

Manuel Mengoli, IRSEA Alessandro Cozzi, IRSEA

### Presenter: Patrick Pageat, IRSEA

Twenty years ago, we identified the compounds of the scent marks released by cats, from their jugal sebaceous glands, during facial marking. From this complex, a secretion involved in territorial marking (F3) was synthetized and its efficacy in the management of various behavior problems, was validated through different published studies. In domestic cats, urine marking, novelty-related stress, hospitalization-related stress, and idiopathic cystitis, are now some of the most common uses of this secretion. Later on, the efficacy of other facial secretions, such as the allomarking pheromone (F4), or the maternal pheromone released by the inter-mammary chains' skin (CAP), has been assessed thanks to blinded multicentric studies. The first attempt to use domestic feline pheromones in wild felids was undertaken in lions and tigers and was targeted to the control of urine spraying. This study, achieved in the Edinburgh Zoo, showed a significant efficacy of the F3 pheromone in tigers, whereas there was no significant effect in lions. Other studies confirmed the efficacy of F3 and F4 on improving social interactions in tigers. More recent studies in the same species were targeted to the management of inter-individual aggression and to the facilitation of exploratory behavior, thanks to F3 or CAP, in both lions and tigers. This paper is a review of the studies dedicated to the validation of such approaches.

### 9:40 AM 74 **The sex pheromones in dogs** · *Room* 407

## Michał Dzięcioł, Antoni Szumny, and Wojciech Niżański, Wroclaw University of Environmental and Life Sciences

## Presenter: Michał Dzięcioł, Wroclaw University of Environmental and Life Sciences

Methyl paraben is speculated to stimulate sexual reflexes in stud dogs but there are conflicting literature reports. In our study, the question of the presence of methyl paraben in bitches´ secretions (GC/MS analysis) and the ability of this substance to stimulate sexual reflexes in stud dogs (behavioral tests) were evaluated. The hypothesis suggesting the ability of some dogs to detect the most fertile period during heat was verified and we looked for an objective method for the evaluation of the influence of sex pheromones on the male dog's physiology. The results obtained showed that methyl paraben is not present in the bitches' secretions and we did not confirm its effect on male dog sexual stimulation, while the ability of dogs to identify the most fertile period in bitches was confirmed. Three different methods for the evaluation of the influence of the sex pheromones on male dog physiology were assessed. Electroencephalography was not effective tool for that kind of evaluation. Electrocardiography was a useful indicator but it was less specific. Evaluation of the blood flow (color Doppler) in ramification of the penile artery

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in response to specific semiochemical signals has been found tp be a useful and specific method for detection of pheromonal stimulation in males.

Dzięcioł et al. 2012a, Observation on possibility to identify by the stud dogs the signs of the fertile period in bitches. JAVA 11, 962-967.

Dzięcioł et al. 2012b. Influence of the bitches sex pheromones on the heart rate and chosen parameters of the blood flow of the stud dogs (*Canis familiaris*). Res Vet Sci 93, 1241-1247

10:00 AM

## 75 Hormonal and behavioral responses to odor cues in zoo-housed African wild dogs • Room 407

Rachel Santymire, Lincoln Park Zoo Matthew Heintz, Lincoln Park Zoo Michelle Rafacz, Columbia College

Presenter: Rachel Santymire, Lincoln Park Zoo

Environmental enrichment can elicit natural physiological and behavioral responses, which can enhance zoo-housed animal welfare. Objectives were to: 1) monitor activity level, behavior and stress physiology in response to fecal samples from Lion, Grant's gazelle and Cattle to determine if these odor cues would function as environmental enrichment in four 3-year-old female African wild dogs (Lycaon pictus) housed at the Lincoln Park Zoo. Each odor cue was presented for 3 days with 2-week intervals. Behavior and feces were collected at least twice weekly. Fecal glucocorticoid metabolites (FGMs) were analyzed using corticosterone enzyme immunoassay. Data were analyzed by social rank, individual and cue. Mean (± sem) FGMs were higher (P=0.036) in dominant (304.6 ± 38.2 ng/g) than subordinate (201.4 ± 36.3 ng/g) females for Lion cue, but higher (P=0.026) in subordinate  $(354.4 \pm 56.9 \text{ ng/g})$  than dominant females  $(248.8 \pm 27.7 \text{ ng/g})$  for Cattle cue. Subordinate female with the lowest (P=0.005) overall FGM values had the highest (P=0.033) FGMs during Cattle (354.7 ± 100.8 ng/g) compared to Gazelle (137.7 ± 29.3 ng/g) and Lion (145.3  $\pm$  28.3 ng/g) cues. Females were more active (P=0.023) during cues (29.9  $\pm$  4.3%) than pre-(19.9 ± 3.4%) and post-cues (13.9 ± 3.1%). Overall, subordinates (25.7 ± 3.8%) were more active (P=0.054) than dominant individuals (16.8 ± 2.3%). In summary, all cues increased activity levels while Lion cue increased FGMs in dominant individuals and Cattle cue increased FGMs in subordinates. Therefore, odor cues may be effective environmental enrichment; however, responses may be affected by social rank.

## 10:40 AM 76 Chemical signals in giant panda urine (Ailuropoda melanoleuca) · Room 407

Martin Dehnhard, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany Thomas B. Hildebrandt, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany Cathleen Meerheim, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany Iain Valentine, Royal Zoological Society of Scotland, Edinburgh Zoo, Edinburgh, Scotland Frank Göritz, Leibniz Institute for Zoo and Wildlife Research, Berlin, Germany

## Presenter: Martin Dehnhard, Leipniz Institute for Zoo and Wildlife Research, Berlin, Germany

The endangered giant panda, *Ailuropoda melanoleuca*, inhabits fragmented mountainous areas in China. Poor in eyesight and hearing ability, pandas mainly use anogenital gland secretions and urine to mediate their social interactions. Male pandas have shown intensive vocalization and marking behavior in response to urine from estrous females; thus, we hypothesised that females' urine may possess estrous-related signals that can be used to predict the 2-3 day period for fertilization. One of the main issues after successful copulation is the prevalence of pseudo-pregnancies. However, if a true pregnancy occurs,

a placenta will be formed which has its own endocrine properties. We hypothesize that the placenta's hormone production may be detected as urinary volatiles. An Agilent GCMS with a thermal desorption unit was used to compare techniques to analyze urinary volatiles such as solid-phase microextraction (SPME), GERSTEL-twister, static and dynamic headspace. Using SPME we showed in 2003 that urinary free fatty acids increased dramatically during estrous. During analytic continuation in 2013 we diluted urine samples to 0.5 mg/ml creatinine due to their extreme variation in creatinine levels (up to 10.7 mg/ml) during the estrous period. Fatty acid analysis revealed a reasonable increase during estrous. Urine samples collected during pregnancy also revealed an unknown substance (mw 120) that was related to luteal activity. Additionally, methyl salicylate appeared at the end of pregnancy. Unfortunately, the panda aborted and no cub was born. The analyses will be continued this year, including samples from a hopefully pregnant panda following artificial insemination on April 13th.

11:00 AM

## 77 Searching for bovine pheromones—biological potential of oestrus-related substances in intra- and inter-species chemical communication • Room 407

Kristina Nordéus, Department of Clinical Sciences, Swedish University of Agricultural Sciences Renée Båge, Department of Clinical Sciences, Swedish University of Agricultural Sciences Robert Glinwood, Department of Ecology, Swedish University of Agricultural Sciences Hans Gustafsson, Växa Sverige

Lennart Söderquist, Department of Clinical Sciences, Swedish University of Agricultural Sciences

## Presenter: Kristina Nordéus, Department of Clinical Sciences, Swedish University of Agricultural Sciences

Declining dairy cow fertility makes it a priority to find new tools for reproductive management. Identification of bioactive compounds associated with bovine cyclicity might provide such tools. Reproductive parameters were monitored in ten dairy heifers during five oestrous cycles. Animals were exposed to distilled water or oestrous urine and vaginal mucus. Four animals were also subjected to intensive blood sampling to study the LH pulsatility pattern (LHP) preceding the preovulatory LH surge (LHS). The treatment had significant effects on when the different signs of oestrus occurred, on the intensity of oestrus expression as well as on LHP. To find a quick bioassay for bioactive substances, two heifers and two bulls were exposed to different body fluids while heart rates were registered. The significant effects seen were not of sufficient magnitude to be useful. Subsequently an attempt was made to identify oestrus-specific compounds using the face fly (Musca autumnalis) as a biological detector. Female chose between either oestrous (OU) or luteal urine (LU) and distilled water (control) in a Y-tube olfactometer. Flies were significantly repelled by OU, but not by LU. One-hexadecanol was present in higher amounts in the headspace of OU than of LU. In a dose-response test, the lowest dose of 1-hexadecanol attracted flies, while a higher dose was repellent. The findings support the existence of bovine pheromones. Further investigation is needed to map the effects of

exposure to body fluids and to determine the role of 1-hexadecanol in the endocrinology of the bovine oestrous cycle.

11:20 AM

#### Invasive silver carp detect unique sex steroids and F prostaglandins that 78 function as sex pheromones and could be used in their control · Room 407

Peter Sorensen, Joe Leese, Hangkyo Lim, and Elizabeth Fox, University of Minnesota Presenter: Peter Sorensen, University of Minnesota

Although it is well established that the goldfish and its close relative the common carp employ hormonal products as pheromones to mediate spawning, little is known about sex pheromone function in the other 2500 species of cyprinids, the world's largest family of freshwater fish. Especially poorly understood are members of the genus Hypophthalmichthys, known commonly as the bigheaded or Asian carps. Two species in this genus, the bighead carp H. nobilis and the silver carp H. molitrix, have recently gained special interest as a highly invasive nuisance species in large river systems. These fish have a well-developed olfactory system, spawn en masse in turbid rivers and lack sexually dimorphic features, suggesting they rely heavily on sex pheromones to mediate reproductive activities. In this study, we examined the sensitivity of silver carp to 216 steroid hormones and several prostaglandins (PGs) suspected of having pheromonal activity using electro-olfactogram (EOG) recording. Nine steroids and three F-type prostaglandins elicited notable responses at nanomolar concentrations, consistent with expectations for pheromonal detection. Further, when artificially masculinized with androgens, silver carp sensitivity to PGFs increased, while response to steroids remained constant. Furthermore, we found that silver carp implanted with prostaglandin  $F2\alpha$  metabolize and release two downstream byproducts (15-keto-prostaglandinF2 $\alpha$  and 13,14-dihydro-15-keto-prostaglandinF2 $\alpha$ ), which are attractive to masculinized silver carp in mazes. Together, these data suggest that PGFs act as sex pheromones and have potential for use as part of a management plan to control this invasive species. (Funded by the Minnesota Environmental and Natural Resources Trust Fund and USGS)

#### Urinary volatile compounds differ by season and sex in the maned wolf 79 (Chrysocyon brachyurus) · Room 407

Marieke Kester, Smithsonian Conservation Biology Institute Zuzy Abdala, George Mason University Nucharin Songsasen, Smithsonian Conservation Biology Institute Tom Huff, George Mason University

## Presenter: Marieke Kester, Smithsonian Conservation Biology Institute

The maned wolf (Chrysocyon brachyurus) is a unique canid that has developed induced ovulation as an adaptation to its solitary lifestyle. There is evidence to suggest that olfactory signals from the male prime the female for estrus and / or ovulation. The present study identifies compounds that differ by sex and by season in the urine of eight maned wolves (four male, four female) collected weekly throughout 2013. Volatile compounds in the headspace above urine were sampled using solid-phase microextraction followed by gas chromatography-mass spectrometry. Several compounds were identified as uniquely produced in the male and several others were unique to females. Typical compounds found include compounds known to be bioactive such as 1-(methylthio)-2-methylbut-2-ene and fragrance compounds such as 2,5-dimethyl pyrazine and 4-heptanone. Results represent a significant step toward the identification of male urinary semiochemicals responsible for estrus and / or ovulation induction in this species.

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11:40 AM

## **Chemical methods**

## 80 **DART and UV-LDI mass spectrometry analyses of insect cuticular lipids** • *Illini Room B*

Joanne Yew, Temasek Life Sciences Lab

Presenter: Joanne Yew, Temasek Life Sciences Lab

I will describe the use of alternative mass spectrometry (MS) methods for direct pheromone analysis from single, intact insects and dissected body parts. In contrast to standard gas chromatography methods, UV-laser desorption/ ionization (UV-LDI) MS and Direct Analysis in Real Time (DART) MS have greater sensitivity for higher molecular weight polar compounds and offer spatially resolved chemical profiling of intact insects. Whole insects or dissected body parts are analyzed directly in the mass spectrometer, allowing rapid chemical profiles to be obtained with little sample preparation and no chemical matrix. I will show examples from our work where the analysis of cuticular lipid profiles by UV-LDI and DART MS resulted in the discovery of unexpected chemical classes of pheromones. In addition, the fine spatial resolution of UV-LDI MS (100-200  $\mu$ m) was used to pinpoint the location of male secretory glands in Sepsidae flies and transfer of the secretions to female wings during mating. Recent experiments using MS imaging have allowed in-situ visualization of a pheromone production organ in Drosophila. Lastly, new lipid-related genes involved in thebiochemistry of pheromone synthesis have been identified using an MS-based screen.

## 3:10 PM 81 Modeling of possible mechanisms in mating disruption • Illini Room B

Jim Miller, Michigan State University

Presenter: Jim Miller, Michigan State University

Past research attempting to explain insect mating disruption has tended to emphasize the possible involvement of many mechanisms rather than seeking specifically to identify the first cause for communicational disruption of the given pest species. By drawing parallels between enzyme kinetics and its associated graphical techniques, we have been able to demonstrate that mating disruption mechanisms also fall into two main categories – competitive (e.g., false-plume following) and non-competitive (e.g., camouflage; desensitization). This talk will present a novel dichotomous key for all known mechanisms of mating disruption and demonstrate graphical and other techniques for proving which mechanisms are leading vs. following causes. The practical advantages and disadvantages of these respective disruption families will be compared.

## 3:30 PM 82 In-needle extractions and/or portable GCMS-Ead · Illini Room B

Mattias Schott, Justus Liebig University Giessen

Presenter: Mattias Schott, Justus Liebig University Giessen

The insight in the qualitative and quantitative composition of semiochemicals is important in all fields of chemical ecology. In particular when dealing with small insects that emit minor amounts of substances, a powerful and reliable enrichment method is of great value and indispensable. The development of solid phase micro extraction (SPME) has already enabled chemical ecologists all over the world to examine odor compositions in small micro habitats. Compared to closed loop striping analysis (CLSA) a very important advantage of this method is the solvent-free enrichment and the possibility of automation. A new method, also based on a small needle, turns the SPME concept inside out. The small amount of trapping material is inside the needle and the outside air is actively sucked

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<sup>2:30</sup> PM

through the needle. In combination with a mass flow controller, for monitoring volume and air flow, the method simplifies quantification. The small amount of trapping material allows a fast and reliable desorption. This dynamic headspace method fits perfectly in the toolbox of chemical ecologists. In this paper I aim to show the application possibilities of this method especially in field by means of actual examples from studies at our laboratories. This enrichment method was utilized by field measurements of Lobesia botrana pheromone concentrations in vineyards. Even the examination of varying concentrations in headspace in behavioral studies of Forficula auricularia was possible. Our results will hopefully encourage the wider usage of this robust method in chemical ecology.

### 4:10 PM

#### 83 **Analysis of microbial volatiles by orthogonal methods** • *Illini Room B*

Stefan Schulz, Ulrike Groenhagen, Michael Maczka, and Lisa Ziesche, Technische Universität Braunschweig

## Presenter: Stefan Schulz, Technische Universität Braunschweig

The volatiles released by bacteria can be analyzed by the same methods used for other organisms. An important method is Closed-loop-stripping-analysis (CLSA) that results in concentrated extracts in a solvent ready for GC/MS analysis. Another possibility is the use of SPME. Both methods have different compound class preferences depending on the adsorbent or coating used. SPME can be also used to monitor variation in compound production, especially when coupled to online GC/MS. Another factor greatly influencing the odor pattern is the medium. Different odor profiles are furthermore obtained when bacteria on agar plates or liquid cultures are analyzed. Nevertheless, both methods cannot give a total picture of the released small compounds. Solvent extraction of the culture filtrate and GC/MS sometimes shows the presence of other components not detected by SPME or CLSA, e. g. amines or certain amides. Several aspects of the analysis of volatiles will be discussed using results from our lab.

[1] S. Schulz, J. S. Dickschat, Nat. Prod. Rep. 2007, 24, 814-842.

Metabolite localization in insect body sections and individual organs using 84 4:30 PM high-resolution mass spectrometry imaging • Illini Room B

> Dhaka Bhandari, Matthias Schott, Andreas Römpp, Andreas Vilcinskas, and Bernhard Spengler, Justus Liebig University Giessen

## Presenter: Dhaka Bhandari, Justus Liebig University Giessen

In mass spectrometry imaging (MSI) a thin tissue section is scanned spot by spot in two dimensions and mass spectra are recorded for each spot. Signal intensities of selected components are extracted from each mass spectrum for every laser spot and are transformed into color scale values in a pixel image. The generated images then exhibit the distribution and indicate relative (non-calibrated) concentrations of substances throughout the tissue and organs. MSI provides the ability of non-targeted, label-free chemical imaging. Additionally, the image generation is user-independent as every image is based on numerical values. In this study, a high-resolution atmospheric-pressure scanning microprobe matrix assisted laser desorption/ionization ion source (AP-SMALDI10, TransMIT GmbH, Giessen, Germany) was combined with an orbital trapping mass spectrometer (Exactive / Q Exactive, Thermo Scientific GmbH, Bremen) to understand the distribution of defensive compounds in tissue from the rove beetle, *Paederus riparius* (Coleoptera: Staphylinidae) and the lady beetle Harmonia axyridis (Coleoptera: Coccinellidae). In case of Paederus riparius, whole body sagittal tissue sections were obtained. In case of Harmonia, a transverse section from the abdomen was obtained. The tissue sections were

scanned with 10 to 20  $\mu$ m step size. Highly mass-resolved MS data (50,000 to 100,000 @ m/z 200) were obtained. In *Paederus*, the defensive compound pederin was found in the reservoir of the sternal defensive gland and also in other organs such as ovaries or accessory glands. In case of *Harmonia*, the defensive compound harmonine was found to be distributed uniformly in the hemolymph. Additionally, organ-specific signals were identified for brain, nerves, eggs, gut, ovaries and Malpighian tubules. We assigned key lipids for each organ to clearly identify organ positions and to identify structures without chemical labelling.

#### 4:50 PM

## 85 **GC/FTIR applications in chemical ecology** • *Illini Room B*

## Paulo Zarbin, Federal University of Parana

## Presenter: Paulo Zarbin, Federal University of Parana

Infrared spectroscopy (IR) is one of the most complete and important techniques in the structural elucidation of organic molecules. IR spectra provide information about functional groups and double bond configuration, among others, acting as a complementary technique to mass spectrometry. The coupling of this technique to gas chromatography led to a powerful tool for identification of compounds, including those present in organic mixtures in small quantities, such as the semiochemicals. In our research group, GC/FTIR has been a key technique for the correct elucidation of different classes of semiochemicals. Some recent examples on identification of insect pheromones will be discussed in this presentation.

## 86 Identification of pheromones with "Automated Sequential SPME/GCMS Analysis" • Illini Room B

Anat Levi-Zada, Maayan David, Daniela Fefer, and Ezra Dunkelblum, Agricultural Research Organization, Volcani Center

Presenter: Anat Levi-Zada, Agricultural Research Organization, Volcani center

The classical methods of identification of sex pheromones are based either on solvent extraction of sex pheromone glands or whole insects, or on airborne collection of the released pheromones on appropriate adsorbents and subsequent solvent extraction of the pheromone. Both methods have the disadvantage of collecting non-relevant compounds, loss of pheromone components due to solvent extraction and subsequent concentration of the solutions for GC (Gas Chromatography), GCMS (GC Mass Spectrometer) and GCEAD (GC Electroantennogram Detection) analyses. The procedures are time- consuming and require relatively large numbers of insects. Recently we developed the sequential SPME (Solid Phase MicroExtraction)/GCMS analysis coupled with an automatic sampling system enabling continuous collection of volatiles released by only a few living insects<sup>1</sup>. The method has been applied for the identification of the sex pheromone of the lesser date moth, Batrachedra amydraula, using single females, that was extremely difficult to achieve by conventional methods. In another study, we showed that the male of the greater date moth, Aphomia sabella, releases a mixture of putative pheromonal compounds in a circadian rhythm<sup>2</sup>. The circadian release rhythm enables analyzing directly and specifically potential pheromonal components and differentiates them from non-relevant

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compounds. The advantages and achievements of using sequential SPME/GCMS analysis for pheromones of various insects will be discussed.

1. Levi-Zada et al (2013) Chemoecology 23:13-20

2. Levi-Zada et al (2014) J. Chem. Ecol. 40:236-243

## Effects of pollution on plant defenses, insect behavior and evolution

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## Heavy metal effects on plant chemical defenses, and heavy metals as plant chemical defenses $\cdot$ Room 210

Robert S. Boyd, Auburn University

Presenter: Robert S. Boyd, Auburn University

Heavy metals are important environmental contaminants yet also are relatively abundant in some natural settings. In this talk I consider effects of heavy metals on plant chemical defenses in both situations. As a pollutant, heavy metals are an environmental stressor with mixed effects: they can increase or decrease effectiveness of plant chemical defenses depending on the particular situation. In some natural settings, such as serpentine soils, high levels of certain heavy metals are common. Some plants adapted to these environments take up unusually large amounts of one or more metals into their tissues. These hyperaccumulator plants may use those metals for herbivore defense (defense hypothesis) or the metals may have other functions (elemental allelopathy, drought resistance, and the like). High metal levels can deter herbivores, increase plant resistance to herbivory, and increase plant tolerance of herbivory. Depending on effective metal concentration, herbivore defense may provide a selective benefit that has driven the evolution of hyperaccumulation. Joint effects of metals and organic chemical defenses may lower the defensively effective concentration of a metal and thus may have contributed to hyperaccumulation evolution. Finally, some insect herbivores have apparently evolved metal tolerance and can feed on hyperaccumulators with impunity. Some of these metal-tolerant herbivores have relatively high whole body metal concentrations that may protect them from predators/ pathogens. Tests of this hypothesis, using Melanotrichus boydi (Heteroptera: Miridae), have reported mixed results but provide a basis for future investigations.

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## 88 The lingering effects of trace element pollution on biological control • Room 210

Thomas Coudron, USDA Agricultural Research Service Holly Popham, USDA Agricultural Research Service Kent Shelby, USDA Agricultural Research Service

Presenter: Tom Coudron, USDA Agricultural Research Service

Rearing insects on diets other than their natural food is an old craft. Studies to determine the role of trace elements in insect nutrition have been going on for centuries. Yet, until the advent of advanced mass spectrometry early in the 21st century, the quantification of trace elements in insect tissues and food samples was rare. Only recently have we acquired detailed information on the dynamics between the levels of trace elements in food and the levels retained within insects. Now we know that levels in the food affect levels in the insect. The concentrations in insect tissues differ from the food and differ with insect developmental stage. However, a homeostatic effect of trace elements in eggs appears to be independent of the food source. Recently we have begun to understand a tritrophic role played by trace elements. It is becoming more apparent the role goes beyond essential dietary nutrients. Increased plasma levels of trace elements have been correlated with elevated resistance in insects to pathogenic viruses, but that viral infection alters

the tissue levels of trace elements. Hence, now we know that insects mediate the uptake and retention of trace elements. Seemingly, the intention is to maximize nutritional and protectant attributes yet not to accumulate toxic levels. These recent findings provide the impetus and information for further studies on the role of trace elements with transgenerational effects as well.

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### 89 Signaling by plant volatiles in polluted atmospheres • Room 210

Jarmo K. Holopainen, Tao Li, James Blande, Pasi Yli-Pirilä, and Jorma Joutsensaari, University of Eastern Finland

### Presenter: Jarmo K. Holopainen, University of Eastern Finland

When damaged by herbivores, plants start to emit inducible volatiles, which transmit information to neighboring plants, other herbivores and natural enemies of herbivores. These signaling molecules induce/prime anti-herbivore defenses in neighboring plants and attract predators and parasitoid of herbivores. Many of the herbivore-induced volatiles are known to be reactive with O3 (ozone) and OH and NO3 radicals. We predicted that herbivore-induced volatiles are transformed to condensable semivolatiles or solid aerosols particles in atmospheric reactions and some signaling value is lost. Our second hypothesis is that these reaction products can be deposited on surfaces of plants and they may directly affect herbivore behavior. We followed atmospheric behavior of constitutive and autumnal moth (Epirrita autumnata) -induced volatiles of mountain birch in reaction chambers and tested with Brassica volatiles and diamondback moth, DBM (Plutella xylostella) how the larval response to herbivore-induced volatiles differ in filtered air and in ozone-enriched air. We found that herbivore damage on mountain birch substantially increased volatile emissions and formation rate of secondary organic aerosol particles in ozone-enriched air. Also in Brassica plants, herbivore damage induced emissions of several terpenes and GLVs. DBM larvae preferred the volatiles from plants damaged earlier by DBM larvae, but this attraction was lost in ozone-enriched atmosphere. Our results show that the herbivore-induced volatile compounds emitted after feeding damage are transformed in the ozone-enriched atmospheres to other less volatile compounds. These compounds do not induce similar responses in herbivores as the compounds emitted from herbivore-damaged plants.

### 3:30 PM 90 Plant-insect interactions in a carbonated-ozonated world • Room 210

Richard L. Lindroth, University of Wisconsin–Madison John J. Couture, University of Wisconsin–Madison Michael Hillstrom, Wisconsin Department of Natural Resources Timothy Meehan, University of Wisconsin–Madison Eric Kruger, University of Wisconsin–Madison

Presenter: Richard L. Lindroth, University of Wisconsin-Madison

Atmospheric carbon dioxide  $(CO_2)$  and ozone  $(O_3)$  are pollutants with profound and extensive impacts on plant life. Central to understanding the effects of atmospheric change on Earth's biota are the facts that plant secondary metabolites respond to global environmental change; perpetuate, via interaction networks, the consequences of global change; and feed back to influence future global change. Here I report results from over a decade of research at the Aspen Free Air CO<sub>2</sub> Enrichment (FACE) facility on the effects of enriched CO<sub>2</sub> and O<sub>3</sub> on tree chemical quality and tree-insect interactions. I will

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summarize the effects of fumigation, genotype, and plant age on foliar chemistry, and the consequences thereof for individual insect species, tritrophic interactions, community composition, canopy-level damage, and feedbacks to primary production. Results at the whole (FACE) plot level reveal that the "fertilization effect" of enriched  $CO_2$  atmospheres can be substantially reduced by increased insect herbivory under high  $CO_2$ . Results from this work parallel those of other studies, in that the impacts of atmospheric change on plant-insect interactions are variable and species-specific. Due to the specificity of insect-host relationships, the complex of multiple direct and indirect factors affecting insect fitness, and the dynamic nature of insect populations over space and time, accurate predictions of the impacts of atmospheric change for individual species remain elusive.

### 4:10 PM

## 91 Effects of nitrogen deposition on plant defenses and plant-insect interactions •

## Room 210

## Mary A. Jamieson, University of Wisconsin-Madison

## Presenter: Mary A. Jamieson, University of Wisconsin-Madison

Human nitrogen inputs in terrestrial ecosystems have increased substantially in recent decades. These inputs may alter plant allocation patterns, plant defense, and plantherbivore interactions. Although numerous studies have investigated the influence of nutrient availability on growth and defense, far fewer studies address the effects of nitrogen enrichment alone and within a range of predicted increase. My talk will review research examining the effects of nitrogen deposition on plant allocation to growth, reproduction, and allelochemistry as well as plant-mediated effects on insect herbivores. Specifically, I will highlight results of studies investigating the invasive plant Dalmatian toadflax (Linaria dalmatica; Plantaginaceae) and one of its biological control agents, a lepidopteran herbivore (Calophasia lunula; Noctuidae) that has the ability to sequester host plant defense compounds. Toadflax produces iridoid glycosides, which are a group of terpenoid compounds that can act as defenses against generalist herbivores and pathogens as well as attractants or feeding stimulants for specialist herbivores. Results from my research indicate nitrogen enrichment positively affects plant growth and reproduction and can reduce costs of defense; however, the effects of increased soil nitrogen on allelochemistry are more complex and depend on various abiotic and biotic factors. Furthermore, nitrogen enrichment can lead to indirect, plant-mediated effects on insect herbivores and potentially higher trophic levels. In this talk, I will discuss the importance of examining intra-plant allelochemical variation, seasonal and ontogenetic shifts in phytochemical concentrations, and interactive effects of global change factors when characterizing and predicting plant and/or insect response to nitrogen deposition and other environmental changes.

4:50 PM

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## Overview of pollution effects on insect behaviors in aquatic systems and movement of arsenic between aquatic and terrestrial systems • Room 210

John Trumble, University of California, Riverside Christina Mogren, North Central Agricultural Research Laboratory, USDA-ARS

## Presenter: John Trumble, University of California, Riverside

Although insects are critical components of most ecosystems, and pollution levels continue to increase both nationally and internationally, surprisingly little is known about the effects of metal and metalloid pollution on aquatic systems. Short term assays are still the norm, with 95% of papers reporting 24h or 48h toxicity of a single compound. This presentation focuses initially on unexpected joint effects of pollutants, particularly selenium and mercury. We then describe changes in behaviors known to occur with

exposure (ingestion, taxis, and reproduction). Interestingly, a large number of species cannot detect, or at least do not respond to fitness-reducing pollutants. Finally we conclude with a series of studies on arsenic, a metalloid of increasing importance as an aquatic contaminant. Specifically we describe the roles that insect feeding strategy can have on transfer of arsenic from aquatic to terrestrial environments.

### References

Mogren, C.L., W. E. Walton, D. Parker, and J. T. Trumble. 2013. Trophic transfer as a route of arsenic movement from aquatic to terrestrial environments. PLOS ONE 8(6) e67817. doi:10.1371/journal.pone.0067817.

Mogren, C.L. and Trumble, J.T. 2010. The Impacts of metals and metalloids on insect behavior. Entomologia Experimentalis et Applicata 135: 1-17.

## Saturday, July 12

9:00 AM

## Chemical ecology and global decline of pollinators

## 93 Various strategies of basal angiosperms to attract different groups of beetle pollinators by olfactory cues • *Room 210*

Stefan Dötterl, University of Salzburg

Presenter: Stefan Dötterl, University of Salzburg

Many basal angiosperms (e.g., Annonaceae, Araceae, Magnoliaceae) are pollinated by beetles, which they attract at night. Several of these plants are well known for their strong floral scents, but still little is known about the chemistry and the importance of volatile compounds in pollinator attraction. By analyzing scents in plants pollinated by sap beetles (Nitidulidae) and cyclocephaline scarabs (Scarabaeidae), and identifying the compounds responsible for luring them, we asked: (1) do plants pollinated by the different groups of beetles differ in their floral scent composition and are plants pollinated by the same group of beetles characterized by similar scents?, and (2) do cyclocephaline scarab species exhibit similar olfactory preferences and are they attracted by biosynthetically related compounds? Our chemical analytical studies revealed that the investigated plant taxa release compounds from diverse biosynthetic origins, several of which are uncommon (even new) among documented floral scent constituents. The scent spectra strongly differed among plants pollinated by the different groups of beetles and also among plants pollinated by the same group of beetles. Compounds of various biosynthetic origins were involved in luring the pollinator cyclocephaline scarabs, different species of which responded to different compounds. Our studies introduce an olfactory-guided pollination system well-suited for answering questions about the importance of scent chemistry in the evolution of angiosperms and their highly specialized beetle pollinators.

### 9:40 AM

95

## Ecological consequences of nectar secondary metabolites: balancing costs vs. benefits for native pollinators • *Room 210*

Jessamyn Manson, University of Alberta

### Presenter: Jessamyn Manson, University of Alberta

The relationship between plants and their pollinators is rooted in the exchange of floral resources for pollination services. However, floral nectar produced primarily to reward pollinators can also contain secondary metabolites, compounds that are often unpalatable, deterrent or toxic. The function of nectar secondary metabolites for plants and the

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consequences of these compounds for nectar-feeding animals are poorly understood, but recent studies suggest that there can be both costs and benefits for pollinators. As generalists, bumble bees collect and consume a wide range of nectar secondary metabolites and demonstrate variation in their behavioral and physiological responses to different compounds. Bumble bees can be deterred by nectar alkaloids under certain circumstances and actively forage on alkaloid-rich nectar under others, or nectar alkaloids may have no effect on worker behavior or pollination services whatsoever. Consuming nectar alkaloids can lead to significant reductions in worker mobility and protein assimilation, but effects are detected at concentrations that are often unnaturally high. In contrast, the consumption of some nectar secondary metabolites can significantly reduce the infection intensity of a common bumble bee gut pathogen, which could benefit worker performance and colony fitness. Given that bumble bees are globally in decline and growing concerns that pathogens may be driving this decline, nectar secondary metabolites may play a critical but undervalued role in pollinator-pathogen dynamics and disease suppression strategies. Moreover, understanding the relationship between nectar chemistry and pollinator performance may help to predict how changes in plant community composition will exacerbate or mitigate pollinator population declines.

#### 10:40 AM

96

## Stresses interactions and chemical communication in honey bee losses • Room 210

Yves Le Conte, INRA Claudia Dussaubat, INRA Cédric Alaux, INRA

Presenter: Yves Le Conte, INRA

Massive honey bee losses have been reported in many places in the world, and the specific causes are usually unknown. Single factors have not yet explained this global decline, leading to the hypothesis of multifactorial syndromes. Consequently, testing the integrative effects of more than one stress is an interesting approach to understand colony losses. The honey bee colony is an example of social organization based on communication. Chemical communication is particularly important in honey bee colony social regulation and one hypothesis would be that stresses related to honey bee losses act on chemical communication processes, modulating production or reception of the pheromonal compounds.

We tested the effects of an infectious organism and an insecticide on honey bee health. We demonstrated that a synergistic effect between both agents significantly weakened honey bees. *Nosema* in combination with imidacloprid caused in the short term a higher rate of mortality. We demonstrated an effect of *Nosema* on worker pheromone production, which shows that a pheromonal disruption related to different stresses could be involved in weakening colonies. We did not yet find an effect of imidacloprid on worker pheromone production. Data will also be presented on queen pheromonal production in presence of those stresses. *Varroa destructor* can also disrupt chemical communication between individuals of the colony, but we will present evidence of chemical communication and molecular level. We thus provide evidence for integrative effects of different agents and stresses on honeybee health modulating chemical communication in the honeybee.

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## 11:00 AM 97 Pollinator P450s: A lack of "blooms" increases pesticide risks? • Room 210

Reed Johnson, The Ohio State University

Presenter: Reed Johnson, The Ohio State University

Since the sequencing and annotation of the honey bee (*Apis mellifera*) genome in 2006 there have been four additional bees for which the genome sequence has been made available (*Apis florea, Bombus impatiens, B. terrestris*, and *Megachile rotundata*). A notable finding from the honey bee genome was the reduced set of cytochrome P450 monooxygenases (P450s) genes relative to other insects. It was hypothesized that this reduction could be related to the sensitivity honey bees show toward pesticides and pesticide combinations through inhibition of this important detoxicative enzyme system. The availability of additional bee genomes makes a comparative toxicogenomic approach possible. Bycomparing detoxication-related gene sequences and their expression between bee species it will be possible to gain an improved understanding of the patterns of evolution in the P450 family. Comparative bioassays of pesticide toxicity using different bee species, in combination with P450 enzyme inhibitors, will in shed light on how similar or different the diversity of bees are in their susceptibility to pesticides. Comparative genomics and comparative toxicology together may lead to a better understanding of the toxicity of pesticides to this group of highly beneficial insects.

### 11**:**20 AM

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## Metal pollutants and their impact on pollinators: Examining behavior, survival, and ecology • Room 210

Kristen R. Hladun and John T. Trumble, University of California, Riverside

Presenter: John Trumble, University of California, Riverside

Rapid industrialization and urbanization worldwide has continued to release more and more anthropogenic contaminants into the environment. Pollutants such as selenium, cadmium, copper, and lead have appeared in honey bee (*Apis mellifera* L.) hives throughout the world, yet there is very little research on their toxic effects. Our work focuses on the toxicity of various pollutants to behavior, survival, and ecology of the common pollinator, the honey bee. We conducted laboratory bioassays involving proboscis extension reflex (PER) and survival. Foragers dosed with certain pollutants exhibited reduced learning, and larvae experienced high mortality even at low doses (Hladun et al. 2013a)1. In a cage study examining the honey bee colony as a whole, caste differences in selenium distribution reduced brood exposure, but even modest transfers of selenium can cause developmental defects. In greenhouse and semi-field studies, certain plant species accumulated pollutants in their flowers, yet still remained attractive to foraging honey bees (Hladun et al. 2013b)1. Knowing which pollutants pose the most risk to pollinators is crucial information for farmers and beekeepers alike. Minimizing exposure to environmental stressors is essential for maintaining healthy honey bee colonies for optimal pollinating services.

<sup>1</sup>Hladun KR, Kaftanoglu O, Parker DR, Tran KD, and Trumble JT (2013a) Effects of selenium on development, survival, and accumulation in the honeybee (*Apis mellifera* L.). Environmental Toxicology and Chemistry 32: 2584-2592

<sup>2</sup>Hladun KR, Parker DR, Tran KD, and Trumble JT (2013b) Effects of selenium accumulation on phytotoxicity, herbivory, and pollination ecology in radish (*Raphanus sativus* L.). Environmental Pollution 172: 70-75

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## Saturday, July 12

## 11:40 AM 99 Does diesel exhaust affect foraging efficiency of honey bees? • Room 210

Inka Lusebrink, University of Southampton Presenter: Inka Lusebrink, University of Southampton

In recent years the numbers of honey bees, *Apis mellifera*, and other pollinators have declined. The likely causes are combinations of multiple stressors including diseases, poor nutrition, habitat loss, pesticides and environmental pollution. The objective of our study is to investigate the role of diesel pollution as another contributor to this decline. In order to investigate if diesel exhaust negatively affects the foraging efficiency of honey bees we conducted a series of experiments with the following objectives: (1) Does diesel exhaust alter floral scent leading to an interruption of honey bee-plant interactions? and (2) Does diesel exhaust alter learning and memory formation in honey bees? The exposure to diesel exhaust altered 30% of our tested flower scent compounds, which are used by honey bees for flower recognition. The chemically reactive nitrogen oxides fraction of diesel exhaust gas was identified as capable of causing the degradation of floral volatiles by diesel exhaust. Preliminary results suggest that the exposure of honey bees to diesel exhaust. Preliminary results suggest that the exposure of honey bees to diesel exhaust impairs their long-term memory formation. Future experiments with free-flying honey bees in the field are planned to determine if diesel exhaust pollution has the potential to be another factor contributing to pollinator decline.

## **Concurrent & Open Presentations**

## Wednesday, July 9

## Applied chemical ecology: Attractants and repellents

9:00 AM	94	<b>Flower discrimination by pollinators in a dynamic chemical environment</b> · <i>Illini Room A</i>
		Jeff Riffell, University of Washington
		Presenter: Jeff Riffell, University of Washington
		Pollinators use their sense of smell to locate flowers from long distances, but little is known about how they are able to discriminate their target odor from a mélange of other natural and anthropogenic odors. Here, we measured the plume from <i>Datura wrightii</i> flowers, a nectar resource for <i>Manduca sexta</i> moths, and show that the scent was dynamic and rapidly embedded among background odors. The moth's ability to track the odor was dependent on the background and odor frequency. By influencing the balance of excitation and inhibition in the antennal lobe, background odors altered the neuronal representation of the target odor and the ability of the moth to track the plume. These results show that the mix of odors present in the environment influence the pollinator's olfactory ability.
9:15 AM	102	<b>Naturally occurring bioactive compounds against the whitefly Bemisia tabaci</b> · <i>Illini Room A</i>
		Emilie Deletre, Cirad Martin Thibaud, Cirad
		Presenter: Emilie Deletre, Cirad
		In tropical countries, the use of netting to protect horticultural crops is an effective sustain- able tool against Lepidoptera but not against small pests such as <i>Bemisia tabaci</i> , unlike

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in Northern regions, where smaller mesh can be used. A possible solution is to combine an insect-proof net with a naturally occurring repellent. Our study investigates in the laboratory the efficiency of nets dipped in natural plant extracts. Our results showed that from the 20 plant extracts tested, the most repellent against *B. tabaci* were aframomum (*Aframomum pruinosum*), lemongrass (*Cymbopogon citratus*), cinnamon (*Cinnamomum zeylanicum*), geranium (*Pelargonium graveolens*) and savory (*Satureja montana*). The most irritant and toxic were the same, except lemongrass. We have also identified the bioactive compounds among two essential oils, cinnamon and lemongrass. Their repellency, irritancy, or toxicity varied with the product and the concentration and were independent of one another. It can therefore be presumed that the repellent mechanism and the irritant/toxic mechanism are not the same. The effects of these different compounds mixed together add up the bioactivity of the mixture, suggesting positive interactions between the compounds. The use of repellent compounds in combination with netting as a sustainable strategy for protecting vegetable crops against whiteflies is discussed but also the use of companion plants which could produce such bioactive compounds.

103 Semiochemical approaches to control the tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) • *Illini Room A* 

> Carmen Quero, IQAC (CSIC), Barcelona, Spain Antonio Ortiz, University of Jaén, Spain Marc Puigmartí, IQAC (CSIC), Barcelona, Spain Ana Bernabé, IQAC (CSIC), Barcelona, Spain Pilar Bosch, IQAC (CSIC), Barcelona, Spain Gloria Rosell, IQAC (CSIC), Barcelona, Spain Angel Guerrero, IQAC (CSIC), Barcelona, Spain

Presenter: Carmen Quero, IQAC (CSIC), Barcelona, Spain

The tomato leafminer *Tuta absoluta* is a major pest of great economic importance throughout South America and Europe. Its primary host is tomato (*Solanum lycopersicum*), although potato (*Solanum tuberosum*), eggplant (*Solanum melongena*), and various wild solanaceous plants are also suitable hosts. As result of the high reproduction potential of *T. absoluta* (females may lay up to 300 eggs with 10-12 generations per year), and because of the mining behavior of the larvae, control of this species is extremely difficult. Therefore, development of environmentally-friendly control strategies is highly desirable. Previous studies on *T. absoluta* show the essential role of plant volatiles not only on attraction, but also in female egg-laying (Proffit et al. 2011). In the present study, we will present the activity of different compounds in tomato and potato plants, and the importance of the presence of tomato plants on female pheromone production. In addition, we will also report the activity in the field of dienic pheromone analogues as putative antagonists of the sex pheromone as a new approach to control the pest.

M. Proffit, G. Birgersson, M. Bengtsson, R. Reiss Jr, P. Witzgall and E. Lima (2011). J. Chem. Ecol. 37, 565-574.

## 9:45 AM 104 Capacity of conophthorin to enhance the attraction of two Xylosandrus species (Coleoptera: Curculionidae: Scolytinae) to ethanol and the efficacy of verbenone as a deterrent · Illini Room A

Nicole R. VanDerLaan and Matthew D. Ginzel, Purdue University

Presenter: Matthew D. Ginzel, Purdue University

Wood-boring beetles in the subfamily Scolytinae (Curculionidae) are among the most commonly intercepted insects at ports-of-entry worldwide, and their establishment has

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9:30 AM

severe economic and ecological consequences. Current detection efforts for these pests, including exotic ambrosia beetles, generally rely on ethanol as a general attractant. However, other semiochemicals may hold promise as management tools for Xylosandrus ambrosia beetles. For example, conophthorin, a common bark volatile of deciduous trees, acts as an attractant for the exotic black stem borer, X. germanus (Blandford). Nevertheless, the extent to which a congener, X. crassiusculus (Motschulsky), and other ambrosia beetles are attracted to conophthorin remains unclear. The capacity of conophthorin to enhance the attraction of Xylosandrus beetles to traps baited with ethanol is also unknown. In this study we evaluate the response of two invasive ambrosia beetles, X. crassiusculus and X. germanus, to conophthorin in mixed-hardwood forests in north central Indiana, and determine the extent to which conophthorin enhances the attraction of these beetles to ethanol-baited traps. We also tested the capacity of verbenone, an anti-aggregation pheromone component of several coniferophagous bark beetles, to deter both species. More X. crassiusculus were captured in traps baited with both conophthorin and ethanol than in those containing either compound alone, suggesting that conophthorin enhances the response of X. crassiusculus to ethanol. Furthermore, verbenone deterred both X. germanus and X. crassiusculus, suggesting that the use of conophthorin and ethanol as an attractant and verbenone as a deterrent can be incorporated into effective management programs.

# 10:00 AM105Beneficial rhizobacteria mixtures and a pathogenic plant virus differentially<br/>alter herbivore-induced soybean volatiles and modify the behavior of<br/>parasitoid natural enemies • Illini Room A

Hannier Pulido, The Pennsylvania State University Kerry Mauck, Department of Environmental Systems Science, Zürich, Switzerland Mark Mescher, Department of Environmental Systems Science, Zürich, Switzerland Consuelo De Moraes, Department of Environmental Systems Science, Zürich, Switzerland

## Presenter: Hannier Pulido, The Pennsylvania State University

Soil-borne microorganisms can have a significant effects on aboveground interactions between plants and other organisms, including pathogens and insect herbivores. For example, non-pathogenic soil microbes can stimulate Induced Systemic Resistance (ISR) by altering leaf metabolites and volatile emissions that enhance plant defense capabilities. In a multi-factorial experiment, we characterized the emission of volatile organic compounds (VOC) by soybean plants in the presence/absence of two species of beneficial rhizobacteria and with or without infection by bean pod mottle virus (BPMV) and herbivory by a beetle (Epilachna varivestis) that vectors this pathogen. In a separate experiment, we assessed the attraction of *Pediobius foveolatus*, a parasitic wasp that attacks *E. varivestis*, to soybean plants given similar treatments. We found that specific combinations of rhizobacteria enhanced herbivore-induced VOC emissions, partially compensating for the observed suppression of VOC emissions by BPMY. This pattern was reflected in our behavioral assays, where parasitoid attraction was reduced by BPMV infection but partially restored in the presence of some rhizobacteria combinations. Furthermore, parasitoids were most attracted to plants whose rhizobia combinations provided the greatest enhancement of herbivore-induced VOC emissions. These findings indicate that both virus infection and root colonization by rhizobacteria can alter the concentration and composition of VOC emissions in soybean, with potential implications for herbivore-natural enemy interactions and the spread of BPMV by *E. varivestis*.

10:40 AM

## 106 Characterizing volatiles and attractiveness of five brassicaceous plants with potential for a 'push-pull' strategy toward the cabbage root fly *Delia radicum* · *Illini Room A*

Alan Kergunteuil, Institute for Genetics, Environment and Plant Protection Sébastien Dugravot, Institute for Genetics, Environment and Plant Protection Holger Danner, Institute for Water and Wetland Research Nicole van Dam, Institute for Water and Wetland Research Anne-Marie Cortesero, Institute for Genetics, Environment and Plant Protection

## Presenter: Anne Marie Cortesero, Institute for Genetics, Environment and Plant Protection

Volatile organic compounds (VOCs) released by plants are involved in different orientation processes of herbivorous insects and consequently play a crucial role in their reproductive success. In the context of developing new strategies for crop protection, several studies have previously demonstrated the possibility to limit insect density on crops using either host or non host-plants releasing attractive or repellent VOCs respectively. The cabbage root fly, Delia radicum, is an important pest of brassicaceous crops for which control methods have to be implemented. Several studies have shown that plant odors influence cabbage root fly behavior but only a few VOCs have been identified so far. The present study aims at selecting both plants and olfactory stimuli that could be used in the development of a "push-pull" strategy against the cabbage root fly. Our olfactometer results revealed that plants belonging to the same family exhibited different levels of attractiveness toward D. radicum. Combining behavioral observations with results from gas chromatographic analyses of volatile profiles indicated higher total VOC emission rates from attractive plants. Their volatile blends were also characterized by the presence of terpenes such as linalool,  $\alpha$ -farnesene or  $\beta$ -caryophyllene. This study represents a first step to identify both attractive plants of agronomic interest and additional volatiles that could be used as trap crops to protect broccoli fields against the cabbage root fly.

## 107 Quantity dependent synergism between acetic acid and acetoin increases chemical attraction to a 4-component synthetic blend in spotted wing Drosophila • Illini Room A

Dong H. Cha, Yakima Agricultural Research Laboratory, USDA-ARS Todd B. Adams, Oregon Department of Agriculture Helmuth W. Rogg, Oregon Department of Agriculture Peter J. Landolt, Yakima Agricultural Research Laboratory, USDA-ARS

Presenter: Dong H. Cha, Yakima Agricultural Research Laboratory, USDA-ARS

We investigated the role that the component amounts, and thus the resultant blend ratio, of a 4-component fermentation volatile blend play in providing greater attractiveness for the invasive *Drosophila suzukii*, the spotted wing drosophila (SWD). Using a previously identified attractive blend of acetic acid, ethanol, acetoin and methionol, we found that within the tested ranges the numbers of SWD trapped increased logarithmically with increases in the release of acetoin (from 0.5 to 34 mg/d) and the concentrations of acetic acid (from 0.25 to 4%) or ethanol (from 0.08 to 2%) in the trap drowning solution. However, we saw no such increase in SWD trapped with increases over the methionol release rates tested (from 0.4 to 4.9 mg/d or from 0.19 to 0.8 mg/d). A new blend formulation based on the optimized amounts of these four chemicals yielded two times more SWD trapped than the original formulation. Further field testing confirmed that simultaneous increases in the release of acetoin and the concentration of acetic acid were necessary for the increased in the increased of acetoin and the concentration of acetic acid were necessary for the increased in the increase of acetoin and the concentration of acetic acid were necessary for the increased in the increase of acetoin and the concentration of acetic acid were necessary for the increased in the increase of acetoin and the concentration of acetic acid were necessary for the increased in the increase in the increase in the increase of acetoin and the concentration of acetic acid were necessary for the increased i

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10**:**55 AM

fly trap response to the new formulation, providing evidence for a positive interaction between the increased amounts of acetic acid and acetoin released from the trap. Taken together, these results demonstrate that the differential attraction of SWD to the two blend formulations that differed only in the ratios of the blend components was due, in part, to a quantity-dependent synergism between acetic acid and acetoin. The practical implications of our findings on efforts to detect and monitor SWD are discussed.

## 11:10 AM 108 **RESCUE® TRAPS, pheromone attractants and repellents for brown marmorated stink bugs** • *Illini Room A*

Qing-He Zhang, Sterling International, Inc.

### Presenter: Qing-He Zhang, Sterling International, Inc.

The brown marmorated stink bug (BMSB), Halyomorpha halys, native to northeastern Asia, is a serious invasive pest insect recorded so far in 41 states in America, as well as Canada, Switzerland, Germany, Italy, France and Hungary. There is an urgent need for a reliable and efficient trap to detect, monitor or mass-trap H. halys. Earlier reports from Japanese and USDA researchers indicated that BMSB adults and nymphs are strongly attracted to the brown-winged green stink bug pheromone, methyl (E,E,Z)-2,4,6-decatrienoate (M246-DT). Our field screening bioassays of several other known stink bug pheromones showed that the harlequin bug pheromone, murgantiol, was also significantly attractive to BMSBs and, surprisingly, we discovered that murgantiol acts synergistically with M246-DT to powerfully attract BMSBs. Rescue stink bug traps baited with this newly patented synergistic pheromone combination (Zhang et al., 2014) effectively catch both adults and nymphs of BMSB throughout the entire growing season. Additionally, we found that several common essential oils, such as clove, lemongrass, spearmint, ylang ylang, wintergreen, geranium, pennyroyal and rosemary oils were strongly repellent to BMSBs in the field (Zhang, et al., 2013). Both pheromone-baited traps and essential oil repellents are potentially useful for BMSB integrated pest management.

Zhang et al. 2014. U.S. Patent # 8,663,620.

Zhang et al. 2013. J. Appl. Entomol. DOI: 10.1111/jen.12101

## 11:25 AM 109 Non-host plant volatiles disrupt sex pheromone communication in a specialist herbivore • Illini Room A

Fu-min Wang, Zhejiang Agriculture and Forestry University Jian-yu Deng, Zhejiang Agriculture and Forestry University Coby Schal, North Carolina State University Yong-gen Lou, Zhejiang Agriculture and Forestry University Guo-xin Zhou, Zhejiang Agriculture and Forestry University Bing-bing Ye, Zhejiang Agriculture and Forestry University Xiao-hui Yin, Zhejiang Agriculture and Forestry University

## Presenter: Jian-yu Deng, Zhejiang Agriculture and Forestry University

Volatile chemicals emitted by plants have multiple ecological functions, and their effects on herbivores can be both positive and negative. However, little is known about potential influences of non-host plant volatiles (NHPVs) on intersexual communication in specialist herbivores. We hypothesized that both the release and reception of sex pheromone in specialist herbivores might be affected by NHPVs. We focused on the effects of several predominant constitutive terpenoids released by conifers and eucalyptus trees on electrophysiological and behavioral responses of an oligophagous species, *Plutella xylostella*, that feeds on cruciferous plants. NHPVs affected the calling behavior of adult females,

including a lower maximum and cumulative calling rate, delayed calling initiation and lowered calling duration compare with females exposed to host atmosphere volatiles. Orientation responses of male *P. xylostella* to sex pheromone were also significantly inhibited by NHPV terpenoids in a wind tunnel and in the field. Disruption by NHPVs of both sex pheromone emission and orientation to pheromone may help to explain, at least in part, an observed reduction in herbivore attack in polyculture compared with monoculture plantings. Moreover, the combination of NHPVs with sex pheromones may be a promising new strategy of mating disruption of moths.

11**:**40 AM

## 110 Dispensers with reduced sex pheromone load for mating disruption of oriental fruit moth (Lepidoptera: Tortricidae) in peaches. • Illini Room A

Alex Il'ichev, David Williams, Joanne Dawson, and Neil Penfold, Department of Environment and Primary Industries, Victoria, Australia

## Presenter: Alex Il'ichev, Department of Environment and Primary Industries, Victoria, Australia

Oriental Fruit Moth (OFM) is very important worldwide pest of stone and pome fruit. Pheromone-mediated mating disruption (MD) is successful in controlling OFM when insecticide resistance is present. If MD for OFM is based on a competitive mechanism, the number of sex pheromone point sources would be more important for successful disruption than the sex pheromone load in the point sources (dispensers). Furthermore, the use of dispensers with a reduced load of sex pheromone active ingredient, but applied at the registered dispenser density (point sources per hectare), should be as effective for MD as the application of the standard registered dispensers. Therefore, investigation of efficacy of low dosage pheromone dispensers applied at the registered rate for MD of OFM in peaches is important for better understanding of MD mechanisms in commercial orchards. Analysis of peach fruit damage and OFM catches revealed that MD treatment with 25% and 50% load of sex pheromone did not provide effective control of initial low levels of OFM infestation. Only dispensers with 75% load of sex pheromone were as effective as registered Isomate OFM Rosso-S® dispensers with 100% load when all dispensers were applied at the same standard registered rate of 500 dispensers per hectare.

Keywords: Oriental fruit moth, mating disruption, sex pheromone, stone fruit.

## Forest insect chemical ecology

2:30 PM

## 111 Survey and detection of Cerambycidae: It's a trap • Illini Room A

Jeremy D. Allison, Natural Resources Canada Kevin J. Dodds, US Forest Service

Presenter: Jeremy D. Allison, Natural Resources Canada

Among invasive insect species, large woodborers (e.g., Cerambycidae) represent one of the most serious threats to forest health globally and it appears that their rate of introduction into new geographic areas is increasing. Survey and detection programs for exotic and native forest insects (including Cerambycidae) frequently rely on traps baited with odorants, which mediate orientation toward a resource. In North America, recent introductions of several species of Cerambycidae have resulted in hundreds of millions of dollars in direct losses and costs of control and containment measures. In recent years remarkable progress has been made in the identification of attractants for cerambycid beetles. Comparatively few studies have explored the relationship between trap design and performance for cerambycid beetles, and measures directly relevant to detection efforts

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and the logistics of trap deployment have received little attention. The majority of studies that have examined trap performance have used the metric of abundance of target taxa that are usually common or abundant in the environment. Ideally, operational survey and detection programs would detect non-native species before they become abundant (i.e., when they are rare). This presentation will present the results of recent work: 1) using the metrics of species richness, diversity and abundance to compare several intercept trap designs for effectiveness of sampling Cerambycidae in eastern North America; 2) examining how the logistics of trap deployment (intertrap distance) influences the capture of target Cerambycidae; and 3) design factors that influence the performance of different intercept traps for the capture of Cerambycidae.

2:45 PM

#### 4 112 The role of complexity in host blends for attracting longhorn beetles to pheromones • Illini Room A

R. Maxwell Collignon, University of California, Riverside Ian Swift, California State Collection of Arthropods Longwa Zhang, Anhui Agricultural University Yunfan Zou, University of California, Riverside Jocelyn Millar, University of California, Riveside

Presenter: R. Maxwell Collignon, University of California, Riverside

Host-plant volatiles have been shown to be important synergists for pheromone attraction of some longhorn beetle species (Coleoptera: Cerambycidae). This phenomenon is typically most pronounced for species infesting conifers. To determine the extent of this synergistic effect and explore some of the blend factors that influence it, a complex blend of synthetic host volatiles was developed based on the volatile emissions of four conifer species. The blend was field tested at three release rates alongside a generic cerambycid pheromone blend in the San Bernardino Mountains of southern California. Only a high release rate of the synthetic conifer blend with pheromone was effective in attracting conifer-infesting cerambycids (subfamilies Spondylidinae and Lamiinae). To gain a better understanding of the specificity of this host volatile-pheromone synergism and to determine the importance of host volatile complexity in cerambycid pheromone attraction, the synthetic blend blend was tested against alpha-pinene—a common host volatile utilized for attracting cerambycids-both alongside pheromone. Here we report the effects of host volatile blend complexity on the pheromone attraction of certain cerambycid species. Increased understanding of which factors of host volatile blends are most important in cerambycid mate and host location will aid in the development of better attractants to monitor these ecologically important beetles.

3:00 PM

# 113 The effect of chirality on the attraction of lamiines to fuscumol and fuscumol acetate • *Illini Room A*

Gabriel P. Hughes, Purdue University Yunfan Zou, University of California, Riverside Jocelyn G. Millar, University of California, Riverside Matthew D. Ginzel, Purdue University

#### Presenter: Gabriel P. Hughes, Purdue University

Within the family Cerambycidae (Coleoptera), (E)-6,10-dimethyl-5,9-undecadien-2-ol (fuscumol) and (E)-6,10-dimethyl-5,9-undecadien-2-yl acetate (fuscumol acetate) attract several species in the subfamily Lamiinae. However, it is unclear whether beetles within the subfamily actually produce these compounds as pheromones or rather respond to them as kairomones. Here we report that male *Astyleiopus variegatus* (Haldeman) produce

both (S)-fuscumol and (S)-fuscumol acetate, suggesting that the compounds are indeed pheromones for this species. Only one other lamiine is known to produce fuscumol and fuscumol acetate, and both compounds are likewise in the (S)-configuration. We tested the hypothesis that chirality influences the attraction of lamiines to these compounds by baiting traps with enantiomerically pure fuscumol and fuscumol acetate. We found that three lamiines were attracted to specific enantiomers: *Astylopsis macula, Aegomorphus modestus*, and *Graphisurus fasciatus*. Interestingly, one cerambycine, *Obrium maculatum*, was also significantly attracted to one or more of the chiral compounds.

3:15 PM

## 114 (R)-Desmolactone is a sex pheromone or sex attractant for the endangered valley elderberry longhorn borer *Desmocerus californicus dimorphus* and several congeners (Cerambycidae: Lepturinae) • *Illini Room A*

Ann M. Ray, Xavier University Richard A. Arnold, Entomological Consulting Services, Ltd. Ian Swift, California State Collection of Arthropods Philip Schapker, Oregon State University Sean McCann, Simon Fraser University Christopher Marshall, Oregon State University J. Steven McElfresh, University of California, Riverside Jocelyn G. Millar, University of California, Riverside

Presenter: Ann M. Ray, Xavier University

We report here that (4R,9Z)-hexadec-9-en-4-olide [(R)-desmolactone] is a volatile attractant for multiple species and subspecies in the cerambycid genus *Desmocerus*. This compound was previously identified as a female-produced sex attractant pheromone of *Desmocerus c. californicus*. Headspace volatiles from female D. *a. aureipennis* contained (R)-desmolactone, and the antennae of adult males of two species responded strongly to synthetic (R)-desmolactone in coupled gas chromatography-electroantennogram analyses. In field bioassays in California, Oregon, and British Columbia, traps baited with synthetic attractant captured significantly more individuals of *Desmocerus* species and subspecies than did control traps. Only male beetles were captured, suggesting that this compound acts as a sex-specific attractant, rather than as a cue for aggregation. In a separate field bioassay, male beetles of *D. californicus dimorphus* responded to the synthetic attractant in a dose-dependent manner. Our finding represents the first example of a generic pheromone for species in the subfamily Lepturinae, and has promising implications for the monitoring and conservation of the U.S. Federally Threatened Valley Elderberry Longhorn Beetle, *D. californicus dimorphus*.

3:30 PM

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#### Prey pheromone as a kairomone in a beetle complex of a Hungarian oak forest (Coleoptera: Cerambycidae and Cleridae) · Illini Room A

Zoltán Imrei, Plant Protection Institute, Budapest, Hungary Jocelyn Millar, University of California, Riverside Éva Bálintné Csonka, Plant Protection Institute, Budapest, Hungary Gergely Janik, Forestry Research Institute, Mátrafüred, Hungary György Csóka, Forestry Research Institute, Mátrafüred, Hungary Edit Orgován, Plant Protection Institute, Budapest, Hungary

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Mike Domingue, The Pennsylvania State University Miklós Tóth, Plant Protection Institute, Budapest, Hungary

#### Presenter: Zoltán Imrei, Plant Protection Institute, Budapest, Hungary

Several cerambycid species of the tribe Clytini including *Plagionotus detritus* share habitat and overlap in flight period in the oak forests of the Mátra mountains of Hungary, where they gather on the sunlit parts of log piles. A predatory beetle species, *Clerus mutillarius* (Coleoptera: Cleridae), occurs on the same logs in large numbers, preying on bark beetles and longhorn beetles. We identified 3-hydroxy-2-hexanone and (S)-2-hydroxy-3-octanone from air entrainment samples of male *P. detritus* beetles. The combination of the synthetic compounds attracted significantly more *P. detritus* than either of the two compounds alone or unbaited funnel traps. The blend of the compounds was significantly attractive to both males and females, suggesting that 3-hydroxy-2-hexanone and (S)-2-hydroxy-3-octanone are aggregation pheromone components of *P. detritus*. Male and female specimens of the predatory *C. mutillarius* also were significantly attracted to the two components of the aggregation pheromone of *P. detritus*, indicating that *C. mutillarius* may use the aggregation pheromone of *P. detritus* as a kairomone to find prey items.

#### Acknowledgement

This work was partially funded by USDA-APHIS cooperative agreement 10-8100'-1422-CA and by OTKA grant K 104294 of HAS.

# 4:10 PM 116 Host selection by the pine processionary moth is related to the pine species volatile composition • *Illini Room A*

E. Mateus, CENSE, Universidade Nova de Lisboa, Portugal M. D .R. Gomes da Silva, Universidade Nova de Lisboa, Portugal M. Oliveira, CIMA, Universidade de Évora, Portugal Maria Rosa Paiva, Universidade Nova de Lisboa, Portugal

Presenter: Maria Rosa Paiva, Universidade Nova de Lisboa

The pine processionary moth, *Thaumetopoea pityocampa*, is a defoliator causing economic damage to conifers and health problems to humans in the Mediterranean region. Its distribution is expanding in latitude and altitude, due to climate change. Host trees include both native and exotic pines, although the intensity of attack varies widely among species. The chemical composition of the needles of twelve *Pinus* species, *P. pinea*, *P. pinaster*, *P. halepensis*, *P. nigra*, *P. brutia*, *P. patula*, *P. radiata*, *P. taeda*, *P. elliotti*, *P. kesiya*, *P. sylvestris* and *P. eldarica*, was studied. Headspace solid-phase microextraction (HS-SPME) and steam distillation extraction (SDE) were used to collect the volatile fractions. Samples were analyzed using gas chromatography (GC), enantioselective gas chromatography (eGC) and gas chromatography/mass spectrometry (GC/MS) [1]. By means of principal component analysis, pine species were differentiated according to their volatile components. Using a partial least squares regression, the enantiomeric composition of several terpenes identified was related to the differences in the level of attack by the

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moth, observed among pine species. Considering the results obtained, the existence of a possible relationship between the phylogeny of the pine species and respective volatile composition is not supported.

[1] Mateus E, Barata RC, Zrostlíková J, Paiva MR, Gomes da Silva MDR 2010. Characterization of the volatile fraction emitted by Pinus spp. by one and two dimensional chromatographic techniques with mass spectrometric detection. Journal of Chromatography A, 1217 (11): 1845-1855.

http://www.ncbi.nlm.nih.gov/pubmed/20144462.

#### Corymbia phloem phenolics, tannins and terpenoids: interactions with a 4:25 PM 117 cerambycid borer • Illini Room A

R. Andrew Hayes, Department of Agriculture, Fisheries and Forestry, Queensland Andrew M. Piggott, Macquarie University

Timothy E. Smith, Department of Agriculture, Fisheries and Forestry, Queensland Helen F. Nahrung, Forest Industries Research Centre, University of the Sunshine Coast

### Presenter: R. Andrew Hayes, Department of Agriculture, Fisheries and Forestry, **Oueensland**

Plant secondary chemistry mediates the ability of herbivores to locate, accept and survive on potential host plants. We examined the relationship between attack by the cerambycid beetle Phoracantha solida and the chemistry of the secondary phloem (inner bark) of two differentially attacked plantation forestry taxa, Corymbia citriodora variegata and its hybrid with C. torelliana. We hypothesised that this differential rate of attack may have to do with differences in secondary chemistry between the taxa. We used spectrophotometric and chromatographic methods to examine the secondary chemistry of the bark. We found differences in the bark chemistry of the taxa, both with respect to phenolic compounds and terpenoids. We could detect no difference between bored and non-bored C. citriodora variegata trees (the less preferred, but co-evolved host). Hybrid trees were not different in levels of total polyphenols, flavanols or terpenes according to attack status, but acetone extracts were significantly different between bored and non-bored trees. We propose that variations in the bark chemistry explain the differential attack rate between C. citriodora variegata and the hybrid hosts.

#### Differential performance and use of *Populus tremula* host chemistry by 4:40 PM 118 Chrysomela tremula leaf beetles • Illini Room A

Ken Keefover-Ring, Ninon Martin, Solange Roberts, and Benedicte Albrectsen, Umea University

#### Presenter: Ken Keefover-Ring, Umea University

Leaf beetles in the genus Chrysomela specialize on Populus and Salix species from the Salicaceae family. Their larvae secrete salicylaldehyde, which they use as a potent deterrent against predators. Larvae use the phenolic glycoside (PG) salicin, obtained from host plant leaves, to synthesize salicylaldehyde. Chrysomela tremula beetles feed upon European aspen (Populus tremula) in much of its extensive range. While salicin does occur in *P. tremula*, it is only one of several more complex PGs, many present in much higher concentrations. In addition, the types and amounts of PGs vary between individual trees, creating four chemical phenotypes (chemotypes). It remains unclear whether leaf beetles can use PGs besides salicin as precursors to produce salicylaldehyde and whether this differs with chemotype. In this project we analyzed the defensive secretions and frass of C. tremula larvae, the frass of adults, and the foliage from the different P. tremula PG

chemotypes that they were feed on to determine use of host chemistry. We also measured the performance and survival of larvae and adults on these chemotypes.

4:55 PM

### 119 Gypsy moth (Lymantria dispar) herbivory affects the spatial distribution of induced phytochemical defenses in trembling aspen (Populus tremuloides) • Illini Room A

Kennedy F. Rubert-Nason, John J. Couture, and Richard L. Lindroth, University of Wisconsin–Madison

Presenter: Kennedy F. Rubert-Nason, University of Wisconsin-Madison

Herbivore induction of phytochemical defenses is widely reported; however, less is known about the spatial distribution of induced defenses within a plant. We examined the impacts of gypsy moth (Lymantria dispar) defoliation on local and systemic phytochemical responses of five Populus tremuloides genotypes cultivated under varied soil nutrient conditions. We deployed second-instar gypsy moth caterpillars onto one branch of each experimental tree for 15 days and then measured putative defense phytochemicals in foliage from branches receiving direct damage (local response), as well as adjacent undamaged branches (systemic response), and branches of insect-free control trees. Analysis of variance revealed that insect damage generally increased foliar tannins and salicinoids (phenolic glycosides), with the response magnitudes and directions varying by plant genotype and soil nutrient levels. The largest increases in tannin concentrations occurred on branches with leaves directly receiving damage, while the largest increases in salicinoid concentrations occurred in leaves that did not receive damage. Partial least squares regression revealed a negative relationship between the salicinoid tremulacin and insect performance. Collectively, our findings show that herbivory can alter the spatial distribution of phytochemical defenses in *P. tremuloides* and support the notion that plant responses to herbivory will depend on a combination of factors including environment, genotype and location of the damage.

5:10 PM

120

# Genotypic variation in tree response to elevated temperature and defoliation $\cdot$ $\mathit{Illini Room A}$

Mary A. Jamieson, University of Wisconsin–Madison Kenneth F. Raffa, University of Wisconsin–Madison Peter B. Reich, University of Minnesota Richard L. Lindroth, University of Wisconsin–Madison

Presenter: Mary A. Jamieson, University of Wisconsin-Madison

Little is known about the influence of temperature on plant defense, in particular with respect to genotype by environment effects. We examined growth and phytochemical responses of three aspen (*Populus tremuloides*) genotypes to elevated temperature and defoliation. Trees were grown in replicate climate-controlled environments under simulated ambient or elevated (+ 5°C) temperatures. After two months, we implemented a defoliation treatment (two weeks of gypsy moth herbivory + clipping to reduce foliage by ~75%). Then, we evaluated growth and phytochemical composition two months post-defoliation treatment. Aspen genotypes showed differing responses to elevated temperature and defoliation. There was a significant three-way interaction effect on foliar biomass. However, even after defoliating plants by 75%, there was little variation in foliar biomass at the end of the experiment, indicating trees exhibited compensatory growth. Total tree biomass was influenced by genotype and reduced by ~10% with defoliation, but there was no effect of temperature. Elevated temperature, however, did affect biomass allocation, resulting in shorter trees with higher root:shoot ratios. Across genotypes and temperature treatments,

growth was negatively associated with concentrations of defense compounds, specifically foliar condensed tannins and phenolic glycosides. Defoliation led to a ~20% reduction in condensed tannins. Elevated temperature had a stronger effect, yielding a ~55-80% decrease, depending on genotype. Similarly, temperature affected phenolic glycosides more strongly than did defoliation, and warming responses depended on genotype and defoliation treatment. Our study indicates climate warming may interact with herbivory to differentially influence tree growth and defense, with variable responses among genotypes.

### **CSiV** General Session I

#### 2:30 PM

# 121 Odor perception during mate choice affects reproductive investment in zebra finches • *Room 407*

Barbara Caspers, Bielefeld University Anna Gagliardo, Pisa University Tobias Krause, Bielefeld University

#### Presenter: Barbara Caspers, Bielefeld University

Inbreeding depression with its loss of genetic variability is a key selective pressure resulting in assortative mating. Zebra finches have been shown to suffer from costs of inbreeding, e.g. decreased maternal investment and decreased chick survival, creating the necessity to avoid mating with close kin. It has been suggested recently that olfactory kin recognition seems also feasible for birds, zebra finches included. The impact of olfactory cues during mate choice in birds, is, however, less known so far. We therefore performed a mating experiment and asked whether females also use olfactory cues during mate choice, in order to recognize close and unfamiliar kin and to avoid inbreeding with it. We allowed female zebra finches to mate with either of two unfamiliar males, one unrelated and one full brother. Females were randomly assigned to one of two treatment groups in which they were either made anosmic or remained able to perceive odors. If olfactory cues are involved in kin recognition, we expected to observe smelling females to be able to mate exclusively with the unrelated mate, whereas anosmic females should mate randomly with respect to relatedness. Females of the two treatment groups differed significantly in their reproductive behavior, indicating that olfactory cues play an important role during mate choice and reproduction.

### 2:45 PM 122 Skin lipids of the striped plateau lizard (Sceloporus virgatus) may signal female receptivity and reproductive quality alongside visual ornaments • Room 407

Jay Goldberg, Max Planck Institute for Chemical Ecology Alisa Wallace, University of Puget Sound Stacey Weiss, University of Puget Sound

#### Presenter: Jay Goldberg, Max Planck Institute for Chemical Ecology

Reptiles are especially attuned to chemical signals and accordingly many species utilize pheromones in the mate selection process. Pheromones can carry a variety of information and be responsible for location of receptive mates, but they can also function as chemical ornaments honestly signaling mate quality. In this study, we determined whether the skin lipids of female Striped Plateau Lizards (*Scelorporus virgatus*) can function as pheromones. By preparing lipids for gas chromatography/ mass spectrometry with methanolysis and silylation reactions, we identified 17 compounds in skin lipid samples. Using principal component analysis, we compared the skin lipid profile of receptive and

non-receptive females and determined that an uncharacterized compound may allow for chemical identification of receptive mates. We then used multivariate regression models to compare extracted principal components to measures of female fitness and reproductive qualities and found that the level of two 18- carbon fatty acids present in a female's skin lipids may indicate her clutch size. Finally, we compared the information content of the skin lipids to that of her orange patches to assess whether chemical and visual cues signal different information or not. We found that the chroma of a female's orange throat patch related to her clutch size, thereby suggesting that chemical signals reinforce the information communicated by visual ornamentation in this species and supporting the 'backup signals' hypothesis for multiple signals.

#### 3:00 PM

#### Does deconvolution help to disentangle the complexities of a mammal odor? • 123

#### Room 407

#### Peter Apps, Botswana Predator Conservation Trust

#### Presenter: Peter Apps, Botswana Predator Conservation Trust

Mammal odors (in the broad sense) are notorious for chemical complexity and small quantities of individual components. No single separation resolves all the components, and so some components are partially or completely obscured by others. In principle, as long as components differ in retention time and have at least one different mass spectrum fragment ion, their GC-MS signals can be deconvoluted. I have tested deconvolution (AnalyzerPro, SpectralWorks http://www.spectralworks.com/analyzerpro.html) on GC-MS data from methanol extracts of African wild dog (Lycaon pictus) urine. It is better and much faster than an experienced human at finding hidden peaks, but several features of real data confound consistent component assignments across samples. In addition to random variation and drift in retention times and increased retention due to overloading, some compounds showed marked changes in relative retention. As chromatographic peaks get smaller, minor fragments in their mass spectra are lost in the noise, and this affects the matches and reverse matches between spectra. Fluctuating background that coincides with the elution of real peaks generates false components. To get the best from deconvolution, separations need repeatable retention times, sharp peaks (although they do not have to be resolved), similar peak sizes in different samples, and low and consistent background noise. Deconvolution is an extremely powerful tool, but it does not offer a black box to dump data into, it does not fix analytical shortcomings, and the results from real data need to be checked and refined manually.

#### 3:15 PM

#### Putative urinary pheromone of the Indian porcupine, Hystrix brachyura · 124 Room 407

Biswatosh Ghosh, Zoological Survey of India, Kolkata Mousumi Poddar-Sarkar, University of Calcutta, Kolkata Sibdas Ray, University of Calcutta, Kolkata Ratan Lal Brahmachary

#### Presenter: Biswatosh Ghosh, Zoological Survey of India, Kolkata

The study on behavioral pattern and chemical analysis of urinary pheromone of the Indian porcupine, Hystrix brachyura Linn.1758, were performed during 2011-2014 in different phases from a zoo near Kolkata (22°20.886 N & 88°04.379 E). Ethological data based on five porcupines, in two different enclosures [Encl.1 (Area: 23.38 m2) with []:[]=2:1 & Encl. 2 (Area: 1.6 m2) with ∏:∏=1:1] were recorded. Urine and faeces are deposited at two specific sites, about equidistant from the main entry hole. Males, particularly, sniffed and licked

female urine and they frequently sniffed at the area adjacent to the urination site. The male collected urine in the hollow quills of the tail and sprayed it in the neighborhood. We identified urine as the primary source of osmic signals (tested by vomeronasal organ via the tongue). Gas Chromatography Mass Spectrometry (GCMS) analysis of hexane extract of the urine of a female showed 5 peaks of which only two could be identified as cresol and phenylacetylene. Dichloromethane extract of a male and a female revealed a single cresol peak. Bioassay of o-, p-, and m-cresol on *H. brachyura* is being initiated. Thin Layer Chromatography showed the presence of triglycerides and diglycerides in the urine, which might act as fixatives. The urine smell is strong even to the human nose and lasts for about 30 min on soil in natural habitats; however, standard o- and p-cresol dropped on soil for experimentation lose their smell to the human nose after ~5 min and ~2 min, respectively. The persistence of smell in urine might be due to the adherence of fixative lipid.

PM 125 Sending mixed signals: testing the 'fixative hypothesis' of composite olfactory cues in male ring-tailed lemurs • Room 407

Lydia K. Greene, Kathleen E. Grogan, Kendra N. Smyth, Christine A. Adams, Skylar A. Klager, and Christine M. Drea, Duke University

#### Presenter: Lydia K. Greene, Duke University

When communicating with conspecifics via scent, animals often deposit composite signals, the functionality of which remains a mystery because the challenge of deciphering meaning from chemical cocktails is further compounded. Male ring-tailed lemurs (Lemur catta) have a complex olfactory repertoire, involving highly volatile antebrachial (A) secretions deposited alone or after being mixed with squalene-rich paste exuded from brachial (B) glands. Here, we tested the 'fixative hypothesis' that B secretions help adhere volatile A signals in the environment. We used behavioral bioassays conducted during the breeding season at the Duke Lemur Center (Durham, NC) to examine the response of 12 adult, male recipients to odorants collected from unknown conspecifics. In experiment 1, we examined responses to A, B, and A+B signals, presented concurrently in fresh and 12-hr time-delayed conditions. Male lemurs preferentially investigated mixed secretions over single odorants at both time points; mixed odorants were preferentially sniffed when fresh, but preferentially licked when decayed. Countermarking was directed towards fresh, but not decayed, mixtures. Perhaps a longer time delay may have enhanced response differences to A. Substituting synthetic squalene (S) for B secretions in experiment 2 did not replicate the results, as S alone or in combination with A may have been somewhat aversive at both time points. Although S is a well-known fixative, B secretions contain additional volatile and nonvolatile chemicals that likely encode salient information. While underscoring the uniqueness of an animal's various glandular secretions, these results highlight the synergistic effect of depositing odorants as composite signals.

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3:30 PM

4:10 PM

#### Chemical communication, reproduction and conflict in a monotreme, the 126 short-beaked echidna • Room 407

Rachel L. Harris, Barbara R. Holland, Elissa Z. Cameron, Noel W. Davies, and Stewart C. Nicol, University of Tasmania

#### Presenter: Rachel L. Harris, University of Tasmania

Mammals use chemical signals to coordinate reproductive and social behaviors; these signals can be sexually-selected and influence sexual conflict. However, mammal-based chemical ecology research is dominated by studies on rodents or captive populations and more input from non-model systems is needed. Short-beaked echidnas (Tachyglossus aculeatus) are evolutionarily distinct, highly seasonal, have well-developed olfactory senses, and show sexual conflict: males mate with hibernating females; females mate post-fertilization and males seem to practice infanticide. We investigated how chemical signals influence reproductive behavior by combining gas chromatography-mass spectrometry, behavioral observations, reproductive physiology and multivariate statistics in a multi-year field-based study on wild echidnas in Tasmania. We collected 778 odorant samples from 69 adults throughout their annual cycle and coupled temporal changes in composition with reproductive behavior. Odor profiles were chemically complex and differed between sexes, seasons and individuals<sup>1</sup>. Odor profiles of females did not change during hibernation, suggesting males use sex-specific odor to locate hibernating females rather than a specific mating attractant. Similarly, there were no detectable changes in odor profiles during pregnancy, and pregnant females continue to attract and mate with males. Unusually, female reproductive status may not be advertised by olfactory cues. Instead, we hypothesize that chemical signals allow multi-male mating by females, thereby confusing paternity as a counter-strategy to sexually-selected male infanticide. Therefore, male and female echidnas use chemical signals antagonistically to increase their reproductive success, resulting in an evolutionary arms race between signallers and receivers.

<sup>1</sup>Harris, R.L., Holland, B.R., Cameron, E.Z., Davies, N.W., and Nicol, S.C. (In press) Chemical signals in the echidna: differences between sexes, seasons, individuals and gland types. Journal of Zoology

#### Evolutionary aspects of the use of predator odors in anti-predator strategies of 127 Lumholtz's tree-kangaroos (Dendrolagus lumholtzi) · Room 407

Jay Goldberg, Centre for Rainforest Studies at the School for Field Studies, Australia, Max Planck Institute for Chemical Ecology, Germany Sigrid Heise-Pavlov, Centre for Rainforest Studies at the School for Field Studies, Australia Megan Nicholson, Centre for Rainforest Studies at the School for Field Studies, Australia

Presenter: Jay Goldberg, Centre for Rainforest Studies at the School for Field Studies, Australia and Max Planck Institute for Chemical Ecology, Germany

Habitat loss and non-native introduced predators are considered main threats to the survival of many unique species of Australian marsupials. The Lumholtz's tree-kangaroo (Dendrolagus lumholtzi) (LTK), an arboreal marsupial inhabiting tropical rainforests of North Queensland, is classified as "near threatened" under the Queensland Nature Conservation (Wildlife) Regulation Act (2006). Up to 10% of annual tree-kangaroo fatalities are caused by wild and domestic dogs, which suggest that tree-kangaroos may be unable to recognize terrestrial predators and to apply appropriate anti-predator strategies. However, tree-kangaroos co-evolved with various terrestrial predators; amongst them are the extinct Marsupial Lion (*Thylacoleo carnifex*), the Tasmanian Tiger (*Thylacinus cynocephalus*)

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and the extant Tasmanian Devil (*Sarcophilus harrisii*). Our study investigates behavioral responses of LTK to odor cues from terrestrial predators to understand whether LTKs possess a "ghost of predation past" recognition of odors from terrestrial predators that can help minimizing threats from introduced non-native predators. Captive LTKs were exposed to scat material from the Tasmanian Devil (*Sarcophilus harrisii*) and two novel predators, the Dingo (*Canis lupus dingo*) and the Domestic Dog (*Canis familiaris*). Duration and frequency of comfort, locomotor, chemosensory and stress behaviors were analyzed. Tree-kangaroos reduced the duration of comfort behaviors and increased the duration of chemosensory behaviors when exposed to Tasmanian Devil odors by increasing their mobility, which suggests that tree-kangaroos show a "ghost of predation past" recognition. Results may assist in anti-predator training of rehabilitated tree-kangaroos before their release.

4:40 PM

#### 128 Bacteria and social odors in meerkats • Room 407

Sarah LeClaire, CEFE (CNRS) Christine M. Drea, Duke University

#### Presenter: Sarah LeClaire, CEFE (CNRS)

The fermentation hypothesis for chemical signatures suggests that variation in chemical signals is due to underlying variation in the composition of odor-producing bacterial communities [1]. Although the contribution of bacteria to the production of vertebrate scent signals has long been suspected, there is still little information about the covariation between odorant profiles and bacterial communities in vertebrate scent secretions (but see [2]). Here, we studied the anal gland secretions of the meerkat, Suricata suricatta, a social mongoose that lives in territorial family groups and relies heavily on scent for social communication. We asked if bacterial communities and odorant profiles covaried, and if bacteria and odorants were related to host traits, such as sex, age, group membership, and kinship. The scent profiles of 50 meerkats were studied using solvent extraction and gas-chromatography/mass-spectrometry, while the corresponding bacterial compositions were studied using a culture-independent technique. We found that the odorant profiles covaried with the composition of bacterial communities, and that both the odorants and bacteria were related to a meerkat's sex, age, and group membership, but not kinship. Our results suggest that variation in meerkat chemical signals could result, at least in part, from the underlying variation in odor-producing bacteria. Next-generation sequencing of bacterial composition is ongoing to determine if the bacteria in meerkat anal secretions contain phylotypes known to produce odorants.

[1] Albone ES, Eglinton G, Walker JM & Ware GC 1974. Life Sci. 14:387–400

[2] Theis KR et al. 2013. Proc Natl Acad Sci USA. 110: 19832-19837

# 129 How to read chemical signals in vertebrates? Ideas from chemical signals in the Eurasian otter *Lutra lutra* • *Room* 407

Eleanor F. Kean, Elizabeth A. Chadwick, and Carsten T. Muller, Cardiff University

Presenter: Carsten T. Muller, Cardiff University

Eurasian otters, *Lutra lutra*, like other mustelid and mammalian species, use chemical signals for communication and for that purpose deposit spraints—a mixture of anal gland secretion and fecal material—at prominent places. The capture and analysis of volatile organic compounds (VOCs) from spraints reveals a highly complex mixture of VOCs (431 VOCs in total across 157 samples), and therefore results in highly multivariate datasets.

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Techniques such as Principal Component Analysis are frequently used to simplify such datasets, but focusing attention on the most variable compounds ignores less variable but potentially more important discriminators. Permutational multivariate analysis of variance (PerMANOVA) and canonical analysis of principal co-ordinates (CAP) provide alternatives that compare profiles of VOCs as a whole rather than treating each individual compound as an independent variable. Using an existing dataset of otter VOC profiles, PerMANOVA was able to discriminate VOC profiles by sex, age and reproductive status (PerMANOVA P<0.01) and CAP assigned profiles correctly in 71% (sex), 65% (age) and 50% (reproductive status) of cases. A third method, weighted correlation network analysis (WGCNA), was used to test whether subsets of VOCs would retain the discriminative power of the full profile, and identified 12 VOCs each for sex, age and reproductive status, which allowed discrimination between groups. By reducing the number of VOCs necessary to discriminate biotic variables, the potential for application of scent analysis in areas such as behavioral analysis and monitoring is considerably enhanced.

5:10 PM 130 **Open slot** 

## Thursday, July 10

### **ISCE Student Travel Award Presentations**

9:00 AM 131 **You are what you eat: diet-induced chemical crypsis in a coral-feeding reef fish** • Illini Room A

> Rohan M. Brooker, James Cook University Philip L. Munday, James Cook University Douglas P. Chivers, University of Saskatchewan Geoffrey P. Jones, James Cook University

Presenter: Rohan M. Brooker, James Cook University

Many organisms match features of their environment to conceal themselves from predators or prey. While the vast majority of camouflage research has examined forms which confound visual perception, many organisms primarily interact with their surroundings using non-visual sensory systems. In particular, organisms may have evolved mechanisms to 'blend in' with chemical components of their habitat. One potential mechanism is through the sequestering of elements from the diet, causing a consumers odor to chemically match the odor of its prey. Coral-feeding fishes provide an excellent model for examining this diet-induced crypsis because corals are both food and shelter for these species. To test for chemical crypsis in the coral-feeding filefish, Oxymonacanthus longirostris, we used the olfactory preferences of specialized coral-obligate crabs as a bioassay to determine the effect of coral diet on fish odor. Crab species exhibited a strong preference for the odor of filefish that had been fed their preferred coral, suggesting that habitat-specific dietary elements are sequestered and influence odor. Crabs exhibited the same strong preference for the odor of filefish that had fed on their preferred coral habitat and odor directly from that preferred coral, suggesting an accurate chemical match. In a predator behavior experiment, predatory cod were less attracted to filefish odor when presented together with the coral it had been feeding on, suggesting diet can reduce detectability. To our knowledge, these results provide the first evidence of diet-induced chemical crypsis in a vertebrate.

# 9:15 AM 132 Are male responses to chemicals associated with females' silk related to different hybridization histories in black widow spiders? • Illini Room A

Luciana Baruffaldi and Maydianne C.B. Andrade, University of Toronto, Scarborough

Presenter: Luciana Baruffaldi, University of Toronto, Scarborough

The species-specificity of male responses to sex pheromones of females may predict the likelihood of hybridization. In spiders, pheromones associated with the female's silk are typically complex blends of chemicals that trigger male courtship. We study functional divergence in contact pheromones in black widow spiders (Latrodectus), a genus of 30 species with worldwide distribution. Here we focus on two invasive species with different hybridization histories. L. hasselti, the only Latrodectus species endemic to Australia, invaded New Zealand in the 1980s and has hybridized with a local species. L. geometricus, sympatric with 4 congeners in its native Africa, is now distributed worldwide with no reports of hybridization. If a high historic risk of hybridization predicts current male responses, then L. geometricus but not L. hasselti should respond preferentially to conspecific females. We quantified male responses to L. hasselti, L. geometricus, and L. hesperus females and their silk using (1) a bioassay of male searching activity on methanol extracts of females' silk and (2) male courtship and mating attempts when on the webs of females. We show that (1) male L. geometricus respond only to extracts from conspecifics, whereas male L. hasselti also respond to extracts from heterospecifics; however (2), males of both species initiate courtship and attempt to mate when heterospecific females are present. Thus, some males discriminate conspecifics based on extracted silk chemicals alone, but intact silk and/or females eliminate this effect. It is unclear whether male responses to pheromones alone are linked to variation in hybridization for these species.

#### AM 133 Apis mellifera cuticular chemistry in a foraging context • Illini Room A

María Sol Balbuena, Universidad de Buenos Aires Andrés González, Universidad de la República, Uruguay Walter M. Farina, Universidad de Buenos Aires

Presenter: Maria Sol Balbuena, Universidad de Buenos Aires

When a honey bee (*Apis mellifera*) collects nectar with high sucrose concentration, it returns to the hive and dances vigorously to communicate the discovered food source. During this conspicuous display its body temperature rises, promoting the release of certain cuticular hydrocarbons (CHs) that have been postulated as a relevant stimulus for the inactive foragers . A rise in body temperature also occurs upon nectar collection, and is affected by the food source profitability . Our aim is to study CHs during the entire foraging cycle of honey bees under different food quality contexts, both outside (feeding site, hive entrance) and inside the hive (during dance and food exchange). We present quantitative variations of 48 CHs in foragers fed at an artificial feeder that offered low (0.5M) or high (2M) sucrose concentration. Hive bees or foragers captured in empty feeders served as controls. The bees were sacrificed using CO2 and the CHs were extracted in dichloromethane and analyzed by GC-MS. Using multivariate analysis, we found significant differences in CHs of hive and forager bees, but not among forager

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bees. Analyses of CHs during the remaining phases of the foraging cycle are underway and will be presented as preliminary results. Differences in CHs of honey bees exploiting food sources that differ in profitability would suggest a modulation in the release of chemical cues associated to differential recruitment.

9:45 AM

Four mutations in the target site for cardenolides explain the insensitivity of Oncopeltus fasciatus to highly toxic plant chemical defenses • Illini Room A

Safaa Dalla and Susanne Dobler, Universität Hamburg

Presenter: Safaa Dalla, Universität Hamburg

Intricate adaptations of herbivorous insects to plant chemical defenses are widespread. Understanding the genetic basis of these adaptations is a central question in chemical ecology. Target site insensitivity to cardenolides is a prime candidate for studying such adaptations on the genetic level. Oncopeltus fasciatus, the large milkweed bug, is specialized on plants producing toxic secondary compounds called cardenolides which are specific inhibitors of the Na,K-ATPase. We investigated the target site for cardenolides, like ouabain, in the gene sequence of the Na,K-ATPase  $\alpha$ -subunit of O. fasciatus. Genetic analyses indicate that O. fasciatus possesses three gene copies bearing different amino acid substitutions in the cardenolide target site. We expressed the ouabain sensitive Na,K-ATPase  $\alpha$ -subunit of Drosophila melanogaster in combination with either of the  $\beta$ -subunits Nrv3 or Nrv2.2 in baculovirus-infected Sf9 cells and introduced the amino acid substitutions N122H, N122H-T797S, Q111T-N122H-F786N, Q111T-N122H-F786N-T797S observed in the different ATPase  $\alpha$ -copies of *O. fasciatus*. To determine the functional importance of the introduced substitutions, the sensitivity of the ATPase activity to increasing ouabain concentrations was measured and compared to that of nervous tissue of O. fasciatus. The latter was highly insensitive to ouabain compared to the Na,K-ATPase of D. melanogaster. The substitutions significantly decreased Na,K-ATPase sensitivity to ouabain in a stepwise manner. The combined substitutions at positions 111, 122, 786, 797 closely mimicked the behavior of the Na,K-ATPase of nervous tissue. They thus appear to be responsible for the pronounced insensitivity of O.fasciatus to cardenolides and are central for the adaptation to its host plant's chemical defenses.

# 10:00 AM 135 **Do pollinators rather than herbivores mediate selection for a toxic chemical** defense? • *Illini Room A*

Paul A. Egan, Trinity College Dublin Erin Jo Tiedeken, College Dublin Philip C. Stevenson, Jodrell Laboratory, Royal Botanic Gardens Geraldine A. Wright, Newcastle University Jane C. Stout, Trinity College Dublin

#### Presenter: Paul A. Egan, Trinity College Dublin

While there is evidence that toxic nectar provides an adaptive benefit to plants, expression of toxic or deterrent compounds in floral nectar is often confounded with the defense needs of other plant parts due to phenotypic correlation. Thus there exists a conflict: antagonists may drive selection for higher toxin levels in one plant tissue, but corresponding increased concentrations in nectar may negatively impact pollinators and hence reproductive fitness. We investigated this in a toxic-nectar producing invasive species, *Rhododendron ponticum*, using field and bioassay techniques. In populations in the native (Iberian) and introduced (Irish) range, we quantified grayanotoxin levels in leaves and nectar, herbivore damage and pollinator visitation, and plant fitness. In addition, we performed bioassays with both a known generalist weevil herbivore (*Otiorhynchus sulcatus*) and bumble bee

pollinator (*Bombus terrestris*) to determine feeding and survival responses to grayanotoxin in diets. Our results revealed a 2.5-fold decrease in nectar toxin levels in the introduced range, alongside a novel chemotype lacking grayanotoxin in its nectar. Enemy-release could not explain this finding, as there were significantly higher levels of herbivory in the introduced range; yet high doses of grayanotoxin did deter weevil feeding in bioassays. Instead, our results suggest pollinator-mediated selection as the main driving force, as plants with low levels of nectar toxin were both fitter and less pollinator-limited, while nectar toxins had no impact on the survival of bumble bee pollinators. Taken together, these findings highlight the importance plant mutualists can play in shaping chemical trait evolution typically associated with plant-herbivore interactions.

#### 10:40 AM

#### 136 The aggregation pheromone of *Homalinotus depressus* (Coleoptera: Curculionidae) • Illini Room A

Diogo Montes Vidal, Universidade Federal do Paraná Marcos Antônio Barbosa Moreira, Embrapa Paulo Henrique Gorgatti Zarbin, Universidade Federal do Paraná

Presenter: Diogo Montes Vidal, Universidade Federal do Paraná

Since 2005, the populations of Homalinotus depressus (Coleoptera: Curculionidae) is constantly increasing in the Brazilian northern region, leading to large financial losses to coconut production. Due to biological aspects of the species, the use of pheromones in pest management programs is promising. The behavioral responses of H. depressus to conspecifics aeration extracts suggested the presence of a male-produced aggregation pheromone. GC analyses of these extracts revealed the presence of three male-specific compounds. Analytical datasets suggested the identity of the minor component as isophorone, which was confirmed by co-injection with an analytical standard. Several reactions were proposed starting from isophorone in order to identify the remaining components. The epoxidation reaction resulted in epoxyisophorone, which co-eluted with one of the male-specific compounds. The FTIR spectrum for the major compound showed an O-H stretch band at 3300cm-1, suggesting the presence of a hydroxyl group. Mass spectrum showed a molecular ion at m/z156, indicating two additional mass units, when compared to epoxyisophorone. By NaBH4 reduction of epoxyisophorone, we identified the major compound as epoxyisophorol. We prepared a mixture of syn and anti stereoisomers and their respective retention times, and NMR data revealed the natural major compound as one of the syn enantiomers. Syn-enantiopure compounds were obtained in high enantiomeric excesses by biocalalysis reactions and the absolute configuration was determined as (1S,2S,6R)-epoxyisophorol. Y-tube olfactometer bioassays demonstrated that the synthetic major compound is as attractive as male volatiles. Field bioassays employing the major compound in different stereoisomeric compositions are underway.

10:55 AM

#### 137 **Chemical ecology of azoxyglycosides** • *Illini Room A*

Alberto Prado, McGill University Donald Windsor, Smithsonian Tropical Research Institute Jacqueline C. Bede, McGill University

#### Presenter: Alberto Prado, McGill University

Azoxy compounds are extremely rare in nature, found only in a few microorganisms and cycad plants. In cycads (Order Cycadales), all tissues are protected by highly toxic azoxyglycosides (AZGs). Only highly specialized insect herbivores are able to cope with these dipolar N-oxides. We are studying the chemical ecology mediating the interaction between Neotropical cycads (Zamiaceae) and specialist Aulacoscelidinae leaf beetles

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(Chrysomeloidea: Orsodacnidae). Cycads annually produce a limited number (<20) of longlived leaves, which implies that the plant must have multiple strategies to defend them. We have observed a trade-off between chemical and physical protection of the leaves. AZG levels are highest in young, unexpanded leaves and decrease as the leaf matures. Once the leaf has fully expanded, AZG levels are at a minimum and leaf toughness becomes the primary means of protection. Because AZGs concentrations decrease before the leaf has become sufficiently tough, there is a vulnerable period at which leaves are susceptible to herbivory by specialist insects. Therefore, high AZGs levels in the youngest cycad leaves appears to prevent insect herbivory even from specialist Aulacoscelidinae beetles, but as these concentrations fall during expansion the beetles are able to tolerate the cycad AZGs. Aulacoscelidinae larvae feed on and develop within the megagametophyte of the plant. Adults beetles sequester AZGs from the leaves of their hosts for use in their own defensive secretions. Phylogenetic and fossil data suggest an ancient relationship; therefore, understanding this cycad-Aulacoscelidinae beetle association may provide insights into the evolution of plant-leaf beetle interactions.

### 11:10 AM 138 Ontogenetic differences in chemical alarm cue production determine antipredator responses and learned predator recognition • Illini Room A

Matthew Mitchell and Mark McCormick, James Cook Uniersity

Presenter: Matthew Mitchell, James Cook University

How individuals assess, respond and subsequently learn from alarm cues is crucial to their survival and future fitness. Yet this information is not constant through time; many individuals are exposed to different predators throughout their life as they outgrow some predators or move to habitats containing different predators. To maximize overall fitness, individuals should discriminate between different cues and respond and learn from only those that are relevant to their current ontogenetic stage. We tested whether juvenile spiny chromis, Acanthochromis polyacanthus, could distinguish between chemical alarm cues from conspecific donors of different ontogenetic stages and whether the cue ontogenetic stage of the cue donor affected the efficacy of learning about predators. Juveniles displayed a significant antipredator response when conditioned with juvenile chemical alarm cues paired with predator odor but failed to respond when conditioned with predator odor paired with either adult alarm cues or with saltwater. Subsequently, individuals only recognized the predator odor alone as a threat when conditioned with juvenile alarm cues. This demonstrates that prey may be highly specific in how they use information from conspecific alarm cues, selectively responding to and learning from only those cues that are relevant to their developmental stage.

11:25 AM

## 139 Following in their footprints: Cuticular hydrocarbons as overwintering aggregation site markers in *Hippodamia* convergens • *Illini* Room A

Christopher A. Wheeler and Ring T. Carde, University of California, Riverside

## Presenter: Christopher A. Wheeler, University of California, Riverside

The convergent lady beetle (*Hippodamia convergens*) forms large overwintering aggregations at revisited montane microsites far removed from their summer foraging grounds. Although orientation responses to visual and altitudinal features of habitat can explain the arrival of migrants at the general overwintering macrosite, the role that pheromones play in the accumulation of individuals in inconspicuous hibernacula microsites is not fully understood. Through two-choice bioassays and gas chromatography and mass spectrometry, we found that *H. convergens* orient toward hydrocarbons previously deposited on their walking surfaces by conspecifics. n-Tricosane (C23) is primarily responsible for

this chemically-mediated orientation. Footprint extracts, as well as C23 alone, induce the eventual accumulation in the field of migrant *H. convergens* at artificial hibernacula, confirming their probable role as aggregation signals. Aggregations persisted over many days when footprint extracts were applied in conjunction with the previously identified 2-isobutyl-3-methoxypyrazine aggregation pheromone. The C23 hydrocarbon functions as a pheromone that interacts with responses to methoxypyrazines to effectively mediate formation of persistent aggregations of diapausing conspecifics at specific microsites. Also discussed is the potential effect that C23 has as a persistent scent marker in establishing the traditional use of hibernacula.

# 11:40 AM 140 Reexamination of the female sex pheromone of the sweetpotato vine borer moth: Identification of tricosatriene and its field evaluation • Illini Room A

Qi Yan, Tokyo University of Agriculture and Technology Le Van Vang, Can Tho University Chau Nguyen Quoc Khanh, Can Tho University Hideshi Naka, Tottori University Tetsu Ando, Tokyo University of Agriculture and Technology

Presenter: Qi Yan, Graduate, Tokyo University of Agriculture and Technology

The sweetpotato vine borer moth, Omphisa anastomosalis (Pyraloidea: Crambidae), is a serious pest in tropical and subtropical Asia-Pacific regions. From an Okinawa population in Japan, (10E,14E)-10,14-hexadecadienal (E10,E14-16:Ald) has been identified as a main pheromone component, in addition to hexadecanal, (E)-10-hexadecenal and (E)-14-hexadecenal as minor components; however, traps baited with the synthetic compounds were less effective at attracting males in the field than virgin females. While Pyraloidea females usually produce only Type I compounds, which are unsaturated fatty alcohols and their derivatives, mating communication of some Pyraloidea species is interestingly mediated by a combination of the Type I compounds and Type II compounds, which are unsaturated hydrocarbons and their epoxides. Based on this knowledge, we examined a pheromone extract of females inhabiting Vietnam by GC-MS and successfully found (3Z,6Z,9Z)-3,6,9-tricosatriene (Z3,Z6,Z9-23:H). A mixture of synthetic E10,E14-16:Ald and Z3,Z6,Z9-23:H could effectively attract the males in a Vietnamese field. In field test with the minor pheromone components mixed to the binary blend did not increase the number of male moths captured. A combination of Z3,Z6,Z9-23:H and another geometrical isomer of E10,E14-16:Ald scarcely attracted the males. Furthermore, E10,E14-16:Ald mixed with other polyunsaturated hydrocarbons showed that the mixtures which included a C21 triene, a C22 triene, or a C23 pentaene interestingly attracted as many males as did the mixture with Z3,Z6,Z9-23:H. The males seemed to have a strict recognition of E10,E14-16:Ald, but a low specificity in perception of Z3,Z6,Z9-23:H. The identification of a highly attractive sex pheromone will help in integrate pest management of O. anastomosalis.

#### **CSiV** General Session II

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9:00 AM

# Chemical characterization of a species-identifying pheromone in the common carp (*Cyprinus carpio*) • *Room* 407

Ratna Ghosal, Haude M. Levesque, and Peter W. Sorensen, University of Minnesota Presenter: Ratna Ghosal, University of Minnesota

Pheromones are commonly used by fishes to mediate social interactions. When tested, almost all fish have been found capable of discerning species identity by odor alone: their pheromones are species-specific. However, all fish pheromonal compounds identified to

date are common metabolites of hormones or biliary sterols. To address this puzzle, we hypothesized that species-specificity may be conveyed by combinations of metabolites or 'complexes' rather than novel products. There may be a core suite of compounds that convey species-identity into which other products including hormones mix and convey physiological condition. To test this, we examined the complexity of the odor released by juvenile common carp which we previously found to exclusively attract conspecifics. Holding waters of juveniles were fractionated into nonpolar and polar fractions and their behavioral activity tested in mazes. We found activity in both fractions. Further evaluation of the nonpolar fraction found it to contain two active components. Finally, we tested the long-standing hypothesis that mixtures of polar L-amino acids might have pheromonal activity. While analysis of holding water and food odors found them to contain differing ratios of primary L-amino acids, when tested we found that, while the food amino acid mixture was attractive, the carp mixture was not. We now speculate that species-specific peptides, perhaps related to MHC, may play a role in pheromone complexes. These complexes could be used to attract and remove invasive fish such as the carp (Funded by the Minnesota Environmental and Natural Resources Trust Fund).

#### 9:15 AM 142 Chemical communication in tilapia: state of the art • Room 407

Tina Keller-Costa, Peter C. Hubbard, João Saraiva, Ana Rato, and Adelino V.M. Canário, Algarve Centre of Marine Sciences (CCMAR)

#### Presenter: Tina Keller-Costa, Algarve Centre of Marine Sciences (CCMAR)

Fishes use pheromones to coordinate reproduction, migration, and social interactions. However, few pheromones have been chemically identified. Tilapias are extremely important aquaculture and invasive species worldwide. In the Mozambique tilapia (Oreochromis mossambicus), a social, lek-breeding cichlid, reproduction and male aggression are mediated through urinary cues released by dominant males. The objective of this study was the chemical identification of such pheromones and establishment of their biological functions. Compounds in the most active urine fraction were identified by mass spectrometry and NMR spectroscopy and synthesized. Electro-olfactogram recordings were used to assess olfactory potency of urine and synthetic analogues. Female release of the oocyte maturation-inducing steroid 17,20 $\beta$ -P in response to chemical stimulation was measured by radioimmunoassay. The aggression-reducing effect of dominant male urine (DMU) or synthetic analogues was investigated using a mirror assay wherein males were allowed to fight their own mirror image. Two epimeric  $5\beta$ , $3\alpha$ -reduced steroid 3-glucuronides were identified as the most potent odorants in male urine. Both steroids are detected by a highly specific, common olfactory receptor mechanism. Females exposed to DMU or to a mix of the two steroids showed a rapid 10-fold increase in 17,20 $\beta$ -P release. Thus, the male urinary steroids prime the female endocrine system to accelerate oocyte maturation. However, the steroids alone could not reduce male aggression in receivers as DMU. Only reconstitution of all urine fractions restored this effect, suggesting that a multicomponent pheromone mediates aggression. In conclusion, dominant tilapia males release a sex pheromone to synchronize spawning and enhance reproductive success.

### 9:30 AM 143 Hormonally-mediated behavioral sensitivity to an androgenic male sex pheromone complex in the goldfish • *Room 407*

Peter Sorensen and Haude Levesque, University of Minnesota

Presenter: Peter Sorensen, University of Minnesota

Although it is well established that fishes employ hormonally-derived sex pheromones, how these common products function in species-specific manners and how the behavioral

sensitivity they induce is regulated is not understood. This study addressed these questions in a model cyprinid fish, the female goldfish, by examining their behavioral sensitivity to male goldfish and common carp odors. While immature female goldfish were attracted to odors of all life stages of conspecifics, after being injected with prostaglandin  $F2\alpha$  $(PGF2\alpha)$ , a hormone that drives sexual receptivity, they became specifically responsive to conspecific male odor. When male goldfish holding waters were extracted with C18 columns, both the nonpolar and polar fractions of conspecific holding waters were attractive to receptive female goldfish, but only the polar fraction of common carp water was attractive. This finding demonstrates that the male sex pheromone discerned by females is a mixture of nonpolar and polar and compounds, but that species-identity may lie in the polar component. Analysis of male goldfish and common carp holding waters demonstrated the presence of androstendione (AD). When AD was tested on its own it was not attractive but when added to conspecific water it was, confirming the role of mixtures in the whole pheromone. In conclusion, when sexually receptive, female goldfish recognize a male sex pheromone that is composed of the relatively common steroid AD along with a combination of other polar body odors. This combination of compounds we have termed a pheromone complex. (Funded by the Minnesota Environmental and Natural Resources Trust Fund).

# 9:45 AM 144 Chemical communication between frogs and tadpoles: The search of chemical compounds that influence parental care behavior in poison frogs • Room 407

Lisa M. Schulte, East Carolina University

Presenter: Lisa M. Schulte, East Carolina University

Anurans have evolved a remarkable diversity of reproductive strategies including advanced levels of parental care. Males of the neotropical poison frog Ranitomeya variabilis (Dendrobatidae), for example, deposit their tadpoles singly into phytotelmata (small water bodies in plants). The exploitation of these small pools lowers the risk of predation, but it is costly because of limited resource availability. Additionally, the tadpoles of this species are cannibalistic, so the identification and avoidance of conspecifics during tadpole deposition represent adaptive behaviors for these frogs. By conducting in-situ pool-choice experiments we showed that parental *R. variabilis* are able to recognize the presence of conspecific tadpoles in phytotelmata using chemical compounds. Furthermore, these frogs can distinguish chemically between tadpoles of different species, avoiding only some heterospecific tadpoles (Schulte and Lötters, 2014). Compounds of the avoided con- and heterospecific tadpoles were extracted and fractionated in the lab and the effects on tadpole deposition behavior of different fractions were tested by presenting them to the frogs in the forest. In this way, the chemical compound(s) responsible for the frog's reactions could be described and compared between the different avoided species. We found that the frogs avoided con- and heterospecific tadpoles using different chemical compounds, suggesting that the communication between adult R. variabilis with con- and hetero-specific tadpoles might have evolved independently from each other.

Schulte, L.M., Lötters, S. (2014) A danger foreseen is a danger avoided: how chemical cues of different tadpoles influence parental decisions of a Neotropical poison frog. Animal Cognition, 17:267-275.

#### 10:00 AM 145 Chemosignals in the archaic New Zealand frog Leiopelma hamiltoni · Room 407

Bruce Waldman, Seoul National University

Presenter: Bruce Waldman, Seoul National University

The most archaic lineages of frogs, represented by the New Zealand family Leiopelmatidae, communicate by chemical rather than bioacoustic signals. We analyzed how

chemical signatures mediate social recognition in Hamilton's frog, Leiopelma hamiltoni. In the wild, Hamilton's frogs live 30 years or more, rarely moving outside small (<25 m<sup>2</sup>), well-defined home ranges. At night, individuals travel slowly above-ground throughout their home ranges and return to diurnal refuges as morning approaches. We collected frogs, held them in captivity for 72 hours, and then tested their preferences for substrates that they had marked themselves to those marked by conspecifics. Individuals showed evidence of self-recognition as they spent more time on their own substrates than on those marked by frogs collected from other home ranges. This social discrimination was strongest when subjects were exposed to chemosignals of conspecifics from home ranges that did not overlap with their own. Next, we collected samples of skin secretions, urine, and feces from subjects, and we examined their saliency as signals of individual identity. Skin secretions were most effective in eliciting self-recognition, as subjects readily discriminated between their own odors and those of conspecifics when exposed only to swabs taken from the skin. Subjects also discriminated between substrates marked by self and non-self urine. However, subjects did not consistently discriminate between their own feces and that of conspecifics. Our findings raise the possibility that chemical communication may complement bioacoustical signaling abilities to serve a variety of social functions in anuran amphibians.

#### Age-related effects on individual discrimination among meadow voles 146 10:40 AM Microtus pennsylvanicus · Room 407

Michael Ferkin, Christian Vlautin, and Lyndsey Pierson, University of Memphis

Presenter: Michael Ferkin, University of Memphis

Individuals need to discriminate among the scent marks of different conspecifics in order to navigate their habitat. To do so, individuals could habituate to the scent marks of familiar conspecifics and then discriminate between them and unfamiliar scent marks of novel conspecifics. In a series of experiments using a habituation-test paradigm, we tested the hypothesis that aging affects an animal's ability to identify individual differences in the scent marks of conspecific meadow voles (Microtus pennsylvanicus). Voles that were 2-3 month-old, 5-7 month-old, and 10-13 month-old but not 15-18 month-old were able to habituate to the scent mark of donor A and then during the test phase discriminate between the scent mark of donor and that of donor B. Thus, voles that were 15-18 month-old were no longer able to discriminate differences between the scent marks of two same-sex conspecifics. They were, however, able to distinguish between scent marks of an opposite sex and same-sex conspecific. Fifteen-18 month-old voles may be less likely than younger voles to attend to changes in their social environment. Consequently, such age-related impairments may have pronounced effects on the nature of interactions older voles have with conspecifics.

#### Production of Major Urinary Proteins (MUPs) in male mice depends on social 10:55 AM 147 conditions and social status · Room 407

Ken Luzynski, Michaela Thoss, and Dustin Penn, Konrad Lorenz Institute of Ethology

#### Presenter: Ken Luzynski, Konrad Lorenz Institute of Ethology

Major Urinary Proteins (MUPs) transport and modulate the release of volatile pheromones in the urinary scent marks of house mice, but their functions have mainly been studied in the laboratory. Our aim was to compare the production of MUPs in semi-natural populations of wild-derived house mice and determine whether MUP production is associated with increased social status or reproductive success. Behavioral observations, reproductive success, and urinary concentration of MUPs were recorded from 8 populations of

mice (consisting of 4 males and 4 females per enclosure population). Preliminary results indicate that MUP concentration in the enclosure setting is significantly higher for both sexes of mice compared to singly housed mice kept in colony conditions. Dominant males have significantly higher MUP concentrations than subordinate and isolated mice. Longitudinal sampling results show that MUP concentrations of isolated mice were highly variable between sample periods compared to mice in the enclosure. Results imply that sampling MUP concentrations of isolated mice at one time point may not be an accurate measure of overall MUP production. Paternity analysis is ongoing, but data revealing a relationship between reproductive success, social status, and MUP expression will be included in the presentation as well.

11:10 AM

#### The effects of phytoestrogens on the sexual behavior of meadow voles 148 (Microtus pennsylvanicus) · Room 407

#### Lyndsey Pierson and Michael Ferkin, University of Memphis

#### Presenter: Lyndsey Pierson, University of Memphis

Phytoestrogens are nonsteroidal compounds, such as the soy-derived isoflavones and the coumestans, commonly found in plants such as clover and alfalfa, that, when consumed, either mimic or antagonize the effects of various steroid hormones. Therefore, consumption of these compounds can have very interesting effects on mammalian physiology and behavior. Meadow voles (Microtus pennsylvanicus) inhabit ephemeral grasslands and live in patchy habitats that vary in the quality and quantity of available forage. Thus, voles may encounter plants that vary in phytoestrogen content within their home ranges. We hypothesized that the amount of phytoestrogens that voles consume affects features of the three components of their sexual behavior: the attractiveness of their scent marks to the opposite sex, their interest in the opposite-sex (proceptivity), and their mating behavior (receptivity). We tested this hypothesis by placing 5-9 month-old meadow voles into one of three groups for 30 days immediately prior to testing, during which time they were fed a (1) control diet with about 600 mg/kg phytoestrogen, (2) low phytoestrogen diet with about 20 mg/kg phytoestrogen, or (3) high phytoestrogen diet with roughly 1500 mg/kg phytoestrogen. Preliminary results indicate that phytoestrogens has an effect on all three components of the sexual behavior of meadow voles and that these effects may have vary by sex.

11:25 AM

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### The barcode hypothesis revised: Variability of major urinary proteins in wild male house mice · Room 407

Michaela Thoß, Ken Luzynski, and Dustin Penn, Konrad Lorenz Institute of Ethology

Presenter: Michaela Thoß, Konrad Lorenz Institute of Ethology

Male house mice produce large quantities of major urinary proteins (MUPs) in their urine, which bind and transport volatile pheromones to the urine and scent marks. Several studies suggest that an individual's particular MUP isoform number and pattern provides a unique signature that mediates individual recognition (barcode hypothesis). To provide an individual signature, MUPs must show both high intra-individual consistency and high inter-individual variability, but these assumptions have never been tested. We collected urine from wild male house mice (Mus musculus musculus) and compared MUP isoforms from the individuals over time. MUP isoform patterns changed significantly during sexual maturation and even during adulthood for 93% of males; however, 90% of isoform bands were consistent over 5 months. We examined the inter-individual variability of MUP

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isoforms and found variability only in the minor bands. Thus, our results indicate that the major MUP isoforms show high intra-individual consistency, but low inter-individual variability (and minor isoforms show the opposite pattern). It is therefore unclear whether and how variability in MUP isoforms could mediate individual recognition.

### 11:40 AM 150 Responses to predator chemical signals in the house mouse are modulated by early olfactory experience • *Room 407*

Vera Voznessenskaya, Artyom Klinov, Ilya Kvasha, and Tatuana Laktionova, A. N. Severtzov Institute of Ecology & Evolution RAS

Presenter: Vera Voznessenskaya, A. N. Severtzov Institute of Ecology & Evolution RAS

Finding universal carnivore signals does not explain why only odors from natural predators produce profound effects on the behavior and the neuroendocrine systems of prev. It suggests the ability of prey to distinguish predator species by odors. The domestic cat is the most specialized predator to the house mouse. We examined the influence of the species-specific compound from cat urine, L-felinine, on the reproduction and investigatory behavior of mice in comparison with the cat urine and how the response to cat odors could be modulated by early olfactory experience. Olfactory thresholds to cat urine and L-felinine were measured with an automated olfactometer (Knosys, USA). The number of newborn pups and sex ratio were recorded. Fecal corticosterone metabolites were monitored non-invasively. Patterns of investigatory and avoidance behavior were analyzed using an open field paradigm. The percentage of animals with a block of pregnancy was significantly higher (n=26, P<0.001) in adult mice exposed to L-felinine (0.05%). Exposure of adult mice to L-felinine also affected sex ratio (n=26, p<0.001) in favor of males. The observed effects could be explained in part by long-lasting elevation of corticosterone under L-felinine exposures (n=13, p<0.001). Exposures of mice to cat chemical cues during the critical period for odor sensitization (14-28 days after birth) significantly lowered the olfactory thresholds (n=10, p<0.001). Early ontogenetic exposures also decreased (n=10, p< 0.05) patterns of avoidance behavior to cat odors in the open field. At the same time, corticosterone response stayed unchanged, indicating the innate nature of the response.

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## Chemical ecology of social behavior

2:30 PM

151 Pheromonal plasticity in *Apis mellifera adansonii* (Latr.) • Illini Room A

Abdullahi Ahmed Yusuf, University of Pretoria Robin Crewe, University of Pretoria Christian Pirk, University of Pretoria

Presenter: Abdullahi Ahmed Yusuf, University of Pretoria

The composition of the secretions from the mandibular glands of honey bees have been studied extensively, with those of the queens dominated by  $\dot{\omega}$ -9 fatty acids and  $\dot{\omega}$ -10 fatty acids dominating those of non-laying workers. *Apis mellifera adansonii* is one of the widely distributed sub-species of African honey bees found along the West African coast to the Congo basin. However, its mandibular gland pheromones have not been analyzed previously. Using gas chromatography, we analyzed the composition of mandibular gland pheromones in workers and queens of *A. m. adansonii* sampled from three ecological regions in Nigeria. Qualitatively, workers and queens have similar pheromone profiles to those previously reported in other sub-species of honey bees. Interestingly, we found

9-ODA and high amounts of its precursor 9-hydroxy-2(E)-decenoic acid (9-HDA) in workers, thus showing that they produce queenlike signals under queenright conditions, which is similar to workers of *A. m. capensis*, which show high levels of worker reproduction even under queenright conditions. While queens from the regions sampled did not differ in terms of either the composition or amounts of the pheromones produced, we found geographic variation in the profiles of workers, suggesting different pheromone-clusters or ecological and climatological adaptations in *A. m. adansonii*. Our results provide more empirical evidence for the nature and extent of variability present in the the mandibular gland products of honey bees and how maintaining this diversity is important to the conservation of honey bee subspecies.

2:45 PM

#### 48 Perception and diversification of fertility signals in Odontomachus trap-jaw ants • Illini Room A

Adrian A. Smith, University of Illinois at Urbana-Champaign Jocelyn G. Millar, University of California, Riverside Lawrence M. Hanks, University of Illinois at Urbana-Champaign Andrew V. Suarez, University of Illinois at Urbana-Champaign

Presenter: Adrian A. Smith, University of Illinois at Urbana-Champaign

The cuticular hydrocarbon profile encodes information essential to ant colony organization: nestmate signals, sex cues, and individual fertility status. Understanding the chemical nature of these signals and how multiple signals are encoded within a single phenotype are crucial steps in understanding the evolution of eusociality. My studies of North American Odontomachus trap-jaw ants provide data on how queen and worker fertility is signaled and how these signals evolve both within and between species. For O. brunneus I provide experimental evidence that (Z)-9-nonacosene serves as the fertility signal for queens and workers. Across Florida populations, O. brunneus has divergent and population-specific cuticular hydrocarbon nestmate profiles, but the fertility signal is conserved. Treating non-reproductive workers with the fertility signal elicits queen-like reactions only when the reacting workers are from the same population. Similarly, live queens are treated as a queen only by workers from the same population. Finally, the fertility signal alone, without any other accompanying hydrocarbons, fails to elicit queen-like responses from any workers. These results suggest that perception of the fertility signal is dependent on the presence of a nestmate or nestmate-like cuticular hydrocarbon profile. Finally, I examine the potential fertility signals used by closely related species, O. relictus, O. ruginodis, and O. haematodus. I find that fertility signals are not conserved across these closely related species. Surprisingly, I also report that the cuticular compounds that best distinguish a reproductive from a non-reproductive individual in O. ruginodis are compounds that were, before now, unknown to occur on any insect.

3:00 PM

# 153 Spinganine-like biogenesis of (E)-1-nitropentadec-1-ene in termite soldiers of the genus *Prorhinotermes* • *Illini Room A*

Anna Jirosova, Andrej Jancarik, Jana Brabcova, Klara Dolejsova, Pavel Majer, and Robert Hanus, Institute of Organic Chemistry and Biochemistry AS CR, Prague, Czech Republic

### Presenter: Anna Jirosova, Institute of Organic Chemistry and Biochemistry AS CR, Prague, Czech Republic

Though structurally diverse and frequently identified in bacteria, fungi, and plants, naturally occurring nitro compounds have only rarely been documented in arthropods. In 1974, (E)-1-nitropentadec-1-ene (NPD) was the first-described insect-produced nitro compound, identified in the defensive secretion of soldiers of the genus *Prorhinotermes*1. However, its

biosynthesis has long remained unknown. In the present study, we demonstrate that NPD originates from an initial condensation of glycine or L-serine with tetradecanoic acid as an analogy to the sphingolipid biosynthetic pathway, and we propose three alternative biosynthetic scenarios of NPD formation. In addition, we are studying the enzymatic apparatus underlying the central transformation of the proposed biosynthesis, i.e., the conversion of an amino group into a nitro moiety by means of a putative N-oxygenase. Using a series of in vitro bioassays with isolated termite tissues, we detected an N-oxygenase activity, converting substrates containing an amino group into nitroso or nitro products. Last but not least, the temporal dynamics in NPD production have been inferred from enzyme activity bioassays and NPD quantifications in *Prorhinotermes* soldiers of different ages.

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#### Soldier-dependent tolerance for competitor threats in a termite • Illini Room A

Li Tian, Department of Entomology, University of Kentucky Kenneth F. Haynes, Department of Entomology, University of Kentucky Xuguo Zhou, Department of Entomology, University of Kentucky

Presenter: Li Tian, Department of Entomology, University of Kentucky

Fleeing from natural enemies can be costly because it means loss of foraging and social opportunities. Thus, modulated tolerance of predator/competitor threats is essential for organisms to maximize their fitness in a stressful environment. Previous studies suggest that organisms maintaining morphological and behavioral traits for efficient defense show high tolerance for natural enemy threats. Termite colonies display tolerance for competitor threats in areas where competing species cohabit in the same nesting and foraging locations. The soldier caste, a specialized defense in termite, may play an essential role in the threat tolerance. The fitness consequences and behavior responses of the eastern subterranean termite, Reticulitermes flavipes, to competitor cues were measured in laboratory experiments, under soldier and soldier-less situations. The presence of competitors resulted in significant mortality and body weight reduction in workers. Workers exposed to competitor cues markedly increased time spent in alarm vibration. Workers accompanied by a soldier exhibited higher survival rate and lost less weight. Our results suggest that constant exposure to competitor cues, probably chemical cues, can be stressful to workers. However, the soldier caste enhances tolerance of workers for competitor threats. This advantage of the soldier caste is mediated possibly through chemical communication between workers and soldiers.

## 3:30 PM 155 **Postmortem chemical changes and differential undertaking response in** termites • Illini Room A

Qian Sun, Kenneth Haynes, and Xuguo Zhou, University of Kentucky

Presenter: Qian Sun, University of Kentucky

Undertaking behavior is a colony hygiene activity in social colonies to collectively prevent the spread of infectious disease and is considered an essential adaptation to social living. In termites, undertaking response varies depending on the nature of corpses, but little is known about the underlying mechanism. In *Reticulitermes flavipes*, the most common subterranean termite in North America, workers exhibit differential undertaking response toward corpses with different postmortem times. Specifically, newly deceased corpses were retrieved and cannibalized by their nestmates, whereas aged corpses were buried. To link chemical profile with behavioral repertoire, death-related chemicals were documented postmortem in *R. flavipes* using gas chromatography-mass spectrometry. The results showed that two volatiles, 3-octanone and 3-octanol, occurred immediately after death, while fatty acids, phenol, and indole gradually built up with longer postmortem time. Using

an integrative approach combining chemical ecology and behavioral bioassay, specific effects of these chemicals on undertaking response were investigated.

### Chemical signals—Analysis and synthesis

4:10 PM 156 Diastereoselective synthesis of (-)-iridomyrmecin and (+)-isoiridomyrmecin: Recently identified semiochemicals of the parasitic wasp *Leptopilina heterotoma* · *Illini Room A* 

> John Hofferberth, Kenyon College Ingmar Weiss, University of Regensburg Joachim Ruther, University of Regensburg Johannes Stökl, University of Regensburg

Presenter: John Hofferberth, Kenyon College

To aid in the elucidation of the semiochemistry of the parasitic wasp *Leptopilina heterotoma*, a concise diastereoselective synthesis of both antipods of iridomyrmecin and isoiridomyrmecin was developed. Using this synthetic approach, isomerically pure samples of two structurally related phytochemicals from *Teucrium marum* (cat thyme), teucriumlactone and dolicholactone, were also prepared. The highlights of this synthetic project and the subsequent biological evaluation of the title compounds will be described.

#### References:

1. Journal of Organic Chemistry 2013, 78, 7318-7323, DOI: dx.doi.org/10.1021/jo400884g

2. Nature Communications 2013, 4:2767, DOI: 10.1038/ncomms3767

# PM157HS-SPME-GC/MS analysis of volatiles from *Eucalyptus* spp. with different<br/>susceptibility to the eucalyptus snout beetle • Illini Room A

Eduardo P. Mateus, Universidade Nova de Lisboa, Portugal Sofia Branco, Universidade Nova de Lisboa, Portugal Stefan Schütz, Buesgen-Institute, Georg August University, Göttingen, Germany Maria Rosa Paiva, Universidade Nova de Lisboa, Portugal

Presenter: Eduardo P. Mateus, Universidade Nova de Lisboa, Portugal

In Portugal, eucalyptus plantations are mostly exploited for pulp and paper production and represent an important contribute to the GDP. Different closely related species of the genus Gonipterus, the eucalyptus snout beetle, which originates from Australia have spread around the word causing serious economic losses. This work studied the volatiles emitted by the leaves of different eucalyptus species and hybrids, which could play a role in mediating host tree selection by the defoliator. Specifically, Eucalyptus nitens and three hybrids of Eucalyptus globulus showing different susceptibility to Gonipterus platensis were analyzed. The volatiles were extracted by solid phase microextraction (SPME) using 100 mm Polydimethylsiloxane (PDMS) fiber and analyzed by Gas Chromatography (GC-FID), multidimensional Gas Chromatography and Mass Spectrometry (GC/MS). Seventy-one compounds were detected in the volatile fraction emitted by the eucalyptus leaves, 49 of which were identified. Results show that samples belonging to more susceptible trees emitted a larger number of compounds in higher amounts. Using a principal component analysis (PCA) it was possible to visualize a separation among the sample provenances driven by a-pinene, limonene, 1,8 cineol and viridiflorene. Coupled gas chromatography with electroantennographic detection (GC-EAD), using antennae of adult snout beetles, was employed to screen for active compounds present in headspace extracts of Eucalyptus

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*globulus*. Volatile extracts for GC-EAD were performed using Mono Trap Disks (DCC18), following disks desorption with 300 ml of dichloromethane. GC-EAD revealed 21 active compounds. Results suggest that the snout beetle may use a blend of volatiles to locate and identify appropriate host eucalyptus.

4:40 PM

158 Antennal and behavioral response of Asian citrus psyllid Diaphorina citri Kuwayama (Hemiptera: Liviidae), to degradation products of citrus volatiles • Illini Room A

Justin George, Paul Robbins, USDA, ARS, and Stephen Lapointe, USDA, ARS

Presenter: Stephen Lapointe, USDA, ARS

The Asian citrus psyllid is the primary vector of the bacterial pathogen believed to cause citrus greening disease. We studied antennal and behavioral responses to principal components of headspace volatiles collected from citrus flush to identify behaviorally active compounds for use in traps or as deterrents. Compounds identified by electroantennographic detection (GC-EAD) were confirmed by antennal response to neat compounds from glass stimulus tubes. Tubes loaded with  $\beta$ -ocimene or citral produced no response immediately after preparation at a range of concentrations. The sealed tubes became stimulatory after 3 to 9 days at room temperature, apparently through oxidative degradation. GC-MS demonstrated complete degradation of both compounds over 3 to 9 days on glass (with or without filter paper) to acetaldehyde, acetone, acetic acid, formic acid and other compounds. GC-EAD of extracts of filter paper loaded with neat compounds aged in glass pipettes identified peaks that elicited consistent and large antennal responses determined by GC-MS to be acetic and formic acids. Both were highly stimulatory to D. *citri* antennae and positively correlated with log dose. Probing behavior was studied by incorporating blends of antenally active compounds in varying proportions and amounts into an emulsified wax substrate (SPLAT™, ISCA Technologies, Inc.). More probes were observed on SPLAT containing blends of acetic and formic acid compared with either acid separately or other compounds. Our study suggests that phytophagous insects may use degradation products for host finding and that Asian citrus psyllid may orient to formic and acetic acid present in the citrus canopy.

# 4:55 PM 159 More than cuticular hydrocarbons: the high chemical diversity in the courtship pheromones of parasitoid wasps • Illini Room A

Johannes Stoekl, University of Regensburg Ingmar Weiss, University of Regensburg Anna-Teresa Dandekar, University of Regensburg John Hofferberth, Kenyon College Joachim Ruther, University of Regensburg

Presenter: Johannes Stoekl, University of Regensburg

Males of the parasitoid wasp genera *Asobara* and Leptopilina, both larval parasitoids of *Drosophila*, display high frequency wing fanning to court females. This courtship behavior is characteristic for many parasitoid wasps and is usually elicited by a close range female sex pheromone. In many species cuticular hydrocarbons (CHCs) have been identified as components of the courtship pheromone, but other compounds may also be important. Here we present data on the courtship pheromone of *A. tabida* and three species of *Leptopilina*. Our results demonstrate that the courtship pheromone of *A. tabida* is characterized by remarkable chemical diversity. A multi-component blend of female-specific compounds including methyl 6-methylsalicylate (M6M), fatty alcohol acetates (FAAs) and cuticular hydrocarbons (CHCs) elicited male courtship behavior. Individually,

none of the three chemical classes alone was sufficient to elicit a full behavioral response in males. However, a blend of M6M and FAAs or combinations of one or both of these with female-derived CHCs resulted in male wing fanning responses comparable to that elicited by a crude extract of females. All three investigated *Leptopilina* species produce iridoids and CHCs, but the compounds are used to different degrees in the courtship pheromone. In *L. heterotoma*, only iridoids elicited wing fanning in males, while in *L. victoriae* CHCs were sufficient for the full courtship display. In *L. boulardi* only iridoids and CHCs together elicited the full courtship behavior compound classes. Although the closely related parasitoid wasps we considered in this study all possess a similar potential reservoir of compound classes from which to compose their pheromone blends, our results indicate that they use different combinations of compound classes during courtship. Our findings also indicate that CHCs are not always a required component of the courtship pheromone for these wasps.

5:10 PM

# 160 Fatty acids as precursors of the aliphatic components of marking pheromone of the bumble bee males *Bombus terrestris* • *Illini Room A*

Petr Zacek, Academy of Sciences of the Czech Republic Jiri Kindl, Academy of Sciences of the Czech Republic Oldrich Hovorka, Academy of Sciences of the Czech Republic Alena Votavova, Masaryk University Irena Valterova, Academy of Sciences of the Czech Republic

#### Presenter: Petr Zacek, Academy of Sciences of the Czech Republic

Bumble bee males use marking pheromones in their premating behavior. The pheromone is stored in the cephalic part of the labial gland and its composition is species-specific (1). The labial gland (LG) of *B. terrestris* males contains a mixture of terpenic and aliphatic compounds, where 2,3-dihydrofarnesol and ethyl dodecanoate are the most abundant. A recent study confirmed that both aliphatic and terpenic components of the pheromone can by synthesized de novo in the LG (2). However, our experiments showed that fatty acids, stored in the fat body (FB), can be used as precursors for biosynthesis of the aliphatic compounds. We fed freshly emerged males with deuterated fatty acids (FA) of various carbon chain lengths and analyzed labelled metabolites in FB, hemolymph (HEM), and LG with two-dimensional comprehensive gas chromatography coupled with mass detection. The results confirmed that the labelled precursors were modified into the main aliphatic pheromone components. Furthermore, comparison of the distribution of the deuterated fatty acids among all examined tissues showed that palmitic acid is transported predominantly to the labial gland. This observation is in agreement with previous studies on de novo biosynthesis (2). Therefore, we propose that palmitic acid is the key precursor, where both ways of the biosynthesis-de novo pathway and the FA-precursor pathway-interconnect.

Financial support from the Czech Science Foundation (#14-04291S) is gratefully acknowledged.

1. Bringer B., Zoon Suppl. 1 (1973), 25.

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## Evolution, genomics, and transcriptomics of chemical ecology and new directions

#### 9:00 AM

Genome sequence of *Dendroctonus ponderosae* Hopkins and insights into mountain pine beetle biology • *Illini Room A* 

Christopher I. Keeling, University of British Columbia Maria Li, University of British Columbia Hannah Henderson, University of British Columbia Christine C. Chiu, University of British Columbia Harpreet K. Dullat, University of British Columbia Macaire M.S. Yuen, University of British Columbia Inanc Birol, Canada's Michael Smith Genome Sciences Centre Steven J.M. Jones, Canada's Michael Smith Genome Sciences Centre Jörg Bohlmann, University of British Columbia

Presenter: Christopher I. Keeling, University of British Columbia

Mountain pine beetle (MPB) is a serious pest of western North American pine forests whose complex semiochemistry orchestrates a mass attack to overcome host defenses. We assembled draft male and female genome sequences from field-collected insects. Insights gained from the genome sequences include details of sex chromosome evolution, discovery of horizontal gene transfer, and phylogenies of select gene families important to MPB survival under adverse environmental conditions. Through a combination of metabolite, proteome, and transcriptome analyses of midgut and fat body tissues from beetles induced to produce pheromone by juvenile hormone III treatment, we have identified candidate genes involved in pheromone biosynthesis. We present results from functional characterization of select genes and an application of RNAi as a tool for MPB research.

# 9:15 AM 162 Cytochromes P450 of terpenoid detoxification and olfaction in the mountain pine beetle (*Dendroctonus ponderosae*) · *Illini Room A*

Christine C. Chiu, Christopher I. Keeling, and Jörg Bohlmann, University of British Columbia

Presenter: Christine C. Chiu, University of British Columbia

The mountain pine beetle (MPB, *Dendroctonus ponderosae*) is a major insect pest of pine forests in western North America. While MPB serves a vital role in the natural cycle of forest rejuvenation, population outbreaks can have ecologically and economically devastating impacts. The current MPB outbreak has affected over 18 million hectares of mostly lodgepole pine (*Pinus contorta* var. *latifolia*) in British Columbia and is expanding east of the Rocky Mountains into a novel habitat of jack pine (*Pinus banksiana*) forests. Using a functional genomics approach, which is building on the recently sequenced MPB pine beetle genome and transcriptome, RNAi of MPB genes, and biochemical enzyme characterization, we identified several MPB P450s in antennae, midgut and fat body associated with olfaction and detoxification of pine host terpenoids. Biochemical assays revealed novel functions of MPB P450s with monoterpenoids and diterpenoids as substrates. The substrates of these P450s represent some of those that are present and abundant in oleoresin defense and volatile emissions of the pine hosts of MPB.

## 9:30 AM 163 **Evolution of cardenolide resistance and sequestration in milkweed butterflies** (Lepidoptera: Danaini) • Illini Room A

Georg Petschenka and Anurag A. Agrawal, Cornell University

Presenter: Georg Petschenka, Cornell University

Co-evolutionary theory predicts that herbivorous insects developed counterstrategies to cope with plant defensive compounds. This is clearly exemplified by the monarch butterfly (Danaus plexippus) which tolerates and sequesters toxic cardenolides from Asclepias species (Apocynaceae). Monarch butterflies adapted to cardenolides by modifying their Na+/K+-ATPase, a ubiquitous cation transporter which is strongly inhibited by cardenolides in other animals but is relatively resistant in monarchs. Like D. plexippus, many other milkweed butterfly species (Danaini) also are associated with cardenolide- producing Apocynaceae, and recently we showed that cardenolide-resistant Na+/K+-ATPase in this lepidopteran tribe evolved in a stepwise manner. We tested three milkweed butterfly species whose Na+/K+-ATPases have different levels of cardenolide resistance (Euploea core: sensitive, D. gilippus: intermediately resistant, D. plexippus: highly resistant) for growth, sequestration, and cardenolide metabolism on several milkweed species. Caterpillars of all three species were reared on eight species of Asclepias strongly differing in their cardenolide production. Our results clearly show that Na+/K+-ATPase resistance is not obligatory for tolerating dietary cardenolides. The level of Na+/K+-ATPase resistance rather parallels the extent of cardenolide sequestration. The latter was tested by comparative HPLC analyses and by pharmacological assays where we applied cardenolides from caterpillar hemolmyph on butterfly Na+/K+-ATPases, in vitro. We found that the three butterfly species strongly differ in the amount of sequestered cardenolides. Remarkably, the pharmacological potential of hemolymph cardenolides also did not correlate with caterpillar performance, indicating that the effect of plant toxins is modified by other plant traits.

#### 9:45 AM

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Insects and Iridoids: Ecology and evolution of sequestration in the Lepidoptera • Illini Room A

Deane Bowers, University of Colorado

Presenter: Deane Bowers, University of Colorado

The iridoid glycosides are a group of terpenoid compounds found in over 50 families of plants. While these plants may be toxic or deterrent to most insects, some species have evolved the ability to specialize on plants containing these compounds and a subset of those have the ability to sequester these compounds for defense against their own enemies. In the Lepidoptera, the ability to sequester iridoid glycosides has arisen several times. However, species vary in the amounts of iridoid glycosides that they sequester and whether or not these compounds are retained into the adult stage. Using data from several species of Lepidoptera, I address four questions about the ecology and evolution of iridoid glycoside sequestration:

1) In what clades of Lepidoptera has iridoid glycoside sequestration evolved?

2) Is there variation among species in the amounts of iridoid glycosides that they can sequesters?

3) How does host plant variation in iridoid glycosides affect sequestration of iridoid glycosides?

4) How does variation in iridoid glycoside sequestration affect other trophic levels?

# 10:00 AM 165 Sexual deception or rendezvous attraction? Deconstructing the floral mimicry of Cephalanthera rubra (Orchidaceae) • Illini Room A

Manfred Ayasse, University of Ulm, Germany Paulo Milet Pinheiro, University of Ulm, Germany Stefan Dötterl, University of Salzburg, Austria

#### Presenter: Manfred Ayasse, Institute of University of Ulm, Germany

Flowers of the deceptive orchid Cephalanthera rubra are believed to mimic Campanula flowers and thereby attract Campanula-pollen specialist bees of Chelostoma rapunculi as pollinator. Since, however, only males of this bee are described to visit flowers of Ce. rubra, sexual deception or rendezvous attraction are possible alternatives. Using bioassays, colorimetric measurement of flowers, chemical and electrophysiological analyses, we aimed to clarify the pollination mechanism. The bioassays evidenced that pentane extracts of *Ch. rapunculi* virgin females but not of Ce. rubra flowers elicit copulation attempts by male bees, and olfactory and visual floral cues of Ca. trachelium and Ce. rubra are equally attractive to flower-naive male and female bees of Ch. rapunculi. Color measurements indicate a high similarity among flowers of Campanula spp. and Ce. rubra. We found no chemical similarity between extracts of Ch. rapunculi virgin females and those of Ce. rubra flowers. Furthermore, we found compounds in female extracts but not in flower extracts triggering antennal responses in Ch. rapunculi males. Finally, no volatiles were present in headspace samples of virgin females, whereas we identified in Ce. rubra flowers eight compounds, including the spiroacetal (E)-conophthorin. This compound, rarely found as a floral scent constituent, is quite common among Campanula species and is involved in recognition of Campanula host flowers by Ch. rapunculi. Our results comprehensively show that Ce. rubra is not pollinated by sexual deception, and that flowers mimic visual and olfactory floral cues of *Campanula* to attract pollinators by means of rendezvous.

# 10:40 AM 166 **Plasticity in the molecular basis of olfaction in a Chagas disease vector** • *Illini Room A*

Marcelo Gustavo Lorenzo, Centro de Pesquisas René Rachou—Fiocruz, Brazil Aman Bonaventure Omondi, Swedish University of Agricultural Sciences, Alnarp, Sweden. Rickard Ignell, Swedish University of Agricultural Sciences, Alnarp, Sweden. Jose Manuel Latorre Estivalis, Centro de Pesquisas René Rachou—Fiocruz Foundation, Brazil

#### Presenter: Marcelo Gustavo Lorenzo, Centro de Pesquisas René Rachou–Fiocruz

The kissing bug *Rhodnius prolixus* is the most important vector of Chagas disease in northern South America. The olfactory system is essential in the attraction of vector insects to humans, thus underlying disease transmission. Two protein families are involved in insect olfaction: the odorant (ORs) and ionotropic receptors (IRs). Insect olfactory sensory neurons, both those expressing ORs and IRs, require the presence of co-receptor proteins in order to function: the canonical OR coreceptor Orco and Ir8a, Ir25a and Ir76b for IRs. The sequences of these co-receptors have previously been identified in the genome of *R. prolixus*. The main objective of this work was to investigate whether the transcription of these coreceptors is regulated by changes in triatomine physiology. cDNA from antennae of insects exposed to different physiological (nutrition and age) and developmental conditions was obtained. The expression levels of the four coreceptors were determined by means of qRTPCR, revealing a decrease after blood ingestion, both for fifth instar nymphs and adult insects. In addition, an up-regulation of the genes encoding these coreceptors was observed during the first phase of adult life (1-day-old vs 20-day-old insects). Thus, the expression profile of these coreceptor genes is significantly modulated by physiological

state, tentatively linked to previously observed behavioral changes in *R. prolixus*. An increased understanding of the olfactory system of these insect vectors may be crucial for developing sustainable control methods.

Acknowledgements: INCTEM, FAPEMIG, PROEP-FIOCRUZ, CNPq, FIOCRUZ Visiting researcher fellowship program and the Linnaeus initiative 'Insect Chemical Ecology, Ethology and Evolution' IC-E3

10:55 AM

# 167 **Chemosensory receptors of** *Rhodnius prolixus,* a Chagas disease vector · *Illini Room A*

Jose Manuel Latorre Estivalis, Centro de Pesquisas René Rachou—FIOCRUZ Foundation— Brazil

Hugh M. Robertson, University of Illinois at Urbana-Champaign

Marcelo Gustavo Lorenzo, Centro de Pesquisas René Rachou-FIOCRUZ Foundation

Presenter: Jose Manuel Latorre Estivalis, Centro de Pesquisas René Rachou– FIOCRUZ Foundation–Brazil

Three protein families mediate insect chemoreception: odorant (OR), gustatory (GR) and ionotropic (IR) receptors. The first objective of this work was to characterize the members of these three families bioinformatically in the assembled genome sequence of Rhodnius prolixus, a vector of Chagas disease. The second objective was to evaluate the antennal expression of a subset of these ORs (10) and IRs (23), comparing through all developmental stages. Finally, we evaluated whether other sensory organs (e.g., rostri) also express these receptors. Receptor sequences of other insects were used as queries of the genome of *R. prolixus*. We identified 115 OR, 28 GR and 27 IR genes. RNA was extracted from entire eggs, and from larval (including samples from all larval instars) and adult insect antennae (separately for males and females). In addition, RNA was isolated from proboscises, tarsi, tibial pads and ovipositors from adults of each sex separately. Gene expression was studied by RT-PCR. All 10 OR and 21 of 23 IR genes showed antennal expression in adults. The antennal expression profiles through developmental stages were variable, as several genes had consistent expression over all stages while many others seemed to increase their expression with development. Finally, the expression of most ORs and IRs was confirmed in the sensory tissues evaluated. Data confirm OR and IR expression in antennae and indicate that olfactory gene transcripts start being produced in embryos, suggesting that olfaction is functional in early phases of triatomine development. Understanding the molecular basis of triatomine olfaction may reveal new targets for the development of vector control tools.

Acknowledgments: INCTEM, FAPEMIG, PROEP-FIOCRUZ, CNPq and FIOCRUZ Visiting researcher fellowship program.

### 168 **Organization of the antennal lobe and the odorant receptors underlying olfaction in the Asian longhorned beetle** *Anoplophora glabripennis* • *Illini Room A*

Robert F. Mitchell, University of Arizona Loyal P. Hall, The Pennsylvania State University Thomas C. Baker, Pennsylvania State University Duane D. McKenna, University of Memphis John G. Hildebrand, University of Arizona

Presenter: Robert F. Mitchell, University of Arizona

The Asian longhorned beetle (*Anoplophora glabripennis*; "ALB") is a devastating forest pest in China and populations have repeatedly established in North America and Europe.

joint meeting of the **International Society for Chemical Ecology** and the **Chemical Signals in Vertebrates** group

11:10 AM

Several chemicals have recently been proposed as pheromones and attractants of adult ALB, suggesting a complex chemical ecology that could translate into new methods of monitoring or control. We are therefore describing the odorant receptors and antennal lobe architecture of ALB to better understand chemosensation in this species. We report here 141 genes in the insect odorant receptor family annotated from the recently-completed ALB genome project, representing 126 receptor sequences and 15 apparent pseudogenes. Initial reconstructions of the ALB antennal lobe indicate that adult insects process odors through 60-70 glomeruli, suggesting an equivalent number of odorant receptors expressed in the antennae. ALB also retains some receptors with close homologs in the related subfamily Cerambycinae, suggesting odorants that may play a key role across the ecology of many longhorned beetle species.

### 11:25 AM 169 **Biological production of moth pheromones in yeast and plant factories: a** synthetic biology approach • *Illini Room A*

Christer Löfstedt, Lund University

Presenter: Christer Löfstedt, Lund University

The use of pheromones for control of pest insects has many advantages over the use of traditional pesticides. The global market for pheromone-based control products is currently estimated to approximately \$200 millions and tons of synthetic pheromones are produced commercially for this purpose. We currently explore two "green chemistry" alternatives to conventional synthetic production of pheromones. One option is the pheromone brewery, a yeast cell factory for pheromone production [1]. Another option is a plant factory, using genetically modified plants for production of pheromones or pheromone precursors [2]. As a test of the pheromone brewery concept, we co-expressed a  $\Delta$ 11 desaturase and a FAR in the brewer's yeast Saccharomyces cerevisiae and produced (Z)-11-hexadecenol [1]. Using Nicotiana benthamiana as a plant factory, we produced several typical 14C and 16C moth sex pheromone components by transient expression of up to four genes coding for consecutive biosynthetic steps [2]. The fatty alcohol fractions from the genetically modified plants were acetylated and mixed to mimic the respective sex pheromones of the small ermine moths Yponomeuta evonymella and Y. padella. These mixtures were very efficient and specific for trapping of male moths and matched the activity of conventionally produced synthetic pheromones. Semi-synthetic preparation of sex pheromones may be a novel and cost-effective way of producing moderate to large quantities of pheromones with high purity and a minimum of hazardous waste.

[1] Hagström et al. (2013) Microb. Cell Fact. 12(1): 125

[2] Ding et al. (2014) Nat. Commun. 5: 3353

11:40 AM 170 Substrate specificity of acetyltransferases expressed in yeast: implications for biological production of moth pheromones • *Illini Room A* 

Bao-Jian Ding, Lund University Ida Lager, Swedish University of Agricultural Sciences Sunil Bansal, Kansas State University Timothy P. Durrett, Kansas State University Sten Stymne, Swedish University of Agricultural Sciences Christer Loefstedt Lund University

Presenter: Bao-Jian Ding, Lund University

Moth pheromone precursors and pheromone components can be produced in yeast and plants by heterologous expression of insect pheromone-production genes. Acetyltransferases

that catalyze the formation of acetate esters by transfer of the acetate group from the acetyl-CoA to the fatty alcohol have been postulated in pheromone biosynthesis. However, so far no fatty alcohol acetyltransferases responsible for production of acetate pheromone components in insects have been characterized. We expressed a plant-derived diacylglycerol acetyltransferase (EaDAcT) and a yeast-derived fatty alcohol acetyltransferase (ATF1) to investigate their efficiency for conversion of moth pheromone alcohols into acetates commonly occurring as pheromone components in Lepidoptera. These acetyltransferases did transform fatty alcohols with chain lengths from 10 to 18 carbons and with double bonds at varying positions into their corresponding acetate esters. They prefer shorter chain lengths to longer and unsaturated substrates over the saturated ones. When the microsomes of the yeast overexpressing these genes were incubated with the same fatty alcohols, the preparations showed an activity pattern similar to the activity observed in the in vivo experiments.

#### Factors mediating consumption

9:00 AM

# 171 Chemical cues associated with ant-mediated seed dispersal in neotropical pioneer tree species • Illini Room C

Selina A. Ruzi, P. Camilo Zalamea, James W. Dalling, and Andrew V. Suarez, University of Illinois at Urbana-Champaign

Presenter: Selina A. Ruzi, University of Illinois at Urbana-Champaign

Ant-mediated seed dispersal has evolved multiple times in many regions around the world, affecting plant distributions through both primary and secondary dispersal. Most research investigating the chemical cues associated with ant-mediated seed dispersal has focused on dispersal of myrmecochorous plants. Myrmecochorous plants have food bodies called elaiosomes attached to their seeds. Elaiosomes contain chemicals that ants cue in on and these signals may be more important than any nutritional benefits for eliciting seed removal. Seeds without elaiosomes can also be attractive to ants and this may be a form of chemically mediated manipulation by the plant to get ants to disperse seeds without providing a reward. To examine the chemical cues that play a role in seed dispersal, we field-tested hexane and methanol extracts from seeds of four neotropical pioneer tree species on Barro Colorado Island, Panama. All four species lack elaiosomes, but three elicit a seed-carrying response from ants and one does not. We measured both removal and attempted removal rates of each seed and beads treated with their chemical extract by a common generalist ant species, Ectatomma ruidum. By comparing the chemical profiles of the extracts deemed attractive or unattractive based on ant behavior, we have begun to determine candidate chemicals cues involved in eliciting seed removal.

9:15 AM

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**Fecal contamination of food increases herbivore performance on plants** · *Illini Room C* 

Swayamjit Ray, Pennsylvania State University Iffa Gaffor, Pennsylvania State University Anjel Helms, Pennsylvania State University Wen-Po Chuang, Kansas State University John Tooker, Pennsylvania State University

Gary W. Felton, Pennsylvania State University Dawn S. Luthe, Pennsylvania State University

#### Presenter: Swayamjit Ray, Pennsylvania State University

Caterpillar behaviors such as feeding, crawling, and oviposition are known to induce defenses in maize and other plant species. We examined plant defense responses to another important caterpillar behavior on plants, their defecation. Fall armyworms (FAW, *Spodoptera frugiperda*), a major threat to maize (*Zea mays*), are voracious eaters and deposit copious amounts of frass in the enclosed whorl tissue surrounding their feeding site, where it remains over long periods of time. FAW frass is composed of molecules derived from the host plant, the insect itself and associated microbes and hence provides abundant cues that may alter plant defense responses over time. We observed that proteins from FAW frass initially induced wound-responsive defense genes in maize; however, pathogenesis-related (pr) defense genes were induced as the time after application increase of salicylic acid and suppression of jasmonic acid accumulation in leaves, as well as increased performance of herbivores on the plant and reduced fungal pathogen performance over time.

#### 9:30 AM

#### 173 Playing with plant appetability to protect crops against insect pests? • Illini Room C

Maxime R. Hervé, INRA—UMR IGEPP Régine Delourme, INRA—UMR IGEPP Antoine Gravot, Université Rennes—UMR IGEPP Nathalie Marnet, INRA—UR BIA Solenne Berardocco, Université Rennes—UMR IGEPP Anne Marie Cortesero, Université Rennes—UMR IGEPP

### Presenter: Maxime R. Hervé, INRA-UMR IGEPP

Plant resistance to phytophagous insects is widely seen as driven by secondary metabolites. We propose that both primary and secondary metabolites play an important role in plant resistance, by determining plant appetability through their phagostimulant and phagodeterrent effects. This important phase of plant attack could be manipulated to develop new varieties more resistant to pest attacks. We tested this hypothesis using oilseed rape (Brassica napus; OSR) and the pollen beetle (Meligethes aeneus), a pollenfeeder that destroys flower buds and leads to potentially important yield losses. For that purpose, we compared insect appetence for six OSR genotypes in a feeding experiment and assessed the balance between potential phagostimulant and phagodeterrent compounds in the eaten tissues (perianth and anthers). Several dozen primary and secondary metabolites were individually quantified by GC, UPLC and UPLC-MS: sugars, free amino acids, glucosinolates, flavonols and hydroxycinnamic acids. Intergenotypic variability was found both in the feeding experiment and in the metabolic profile of the eaten tissues. Biochemical composition of the perianth was in particular highly correlated to insect attacks. Only a few compounds explained this correlation, among which was sucrose, a metabolite known to be highly phagostimulant. Our study suggests that plant appetability could be a component of resistance to insects and that key determinants of this appetability can be assessed. It also highlights the important role that primary metabolites could play in plant-insect interactions. Our results open the way for a new crop protection strategy based on artificial selection of key determinants of plant appetability.

## 9:45 AM 174 Inducibility of volatile and non-volatile compounds in the brown macroalga *Fucus vesiculosus · Illini Room C*

Ursula S.R. Röse and Janithri Wickramanayake, University of New England

#### Presenter: Ursula S.R. Röse, University of New England

*Fucus vesiculosus* are very abundant in the intertidal zones of the coast of Maine despite considerable herbivore pressure. This implies that they may contain defense mechanisms that protect them against herbivore and microbial attack. We investigated the inducibility of compounds in the alga *F. vesiculosus* in response to directly applied stressors such as snail damage, isopod damage, mechanical injury, and plant signaling compounds to determine how quickly these defense compounds are synthesized in the algae. We found that extracts of brown algae contained quantitatively and qualitatively different compounds in response to methyl jasmonate and mechanical injury. *F. vesiculosus* synthesized significant amounts of the compounds tocopherol and fucosterol, which may have antimicrobial properties or play a role in tissue repair. In headspace collections, we found that *F. vesiculosus* released a significant amount of tribromomethane in response to all treatments, but in larger amounts in response to methyl jasmonate. Further, hexadecane is emitted from *F. vesiculosus* and detectable after 1 day post- mechanical injury or methyl jasmonate exposure but is not seen in control samples. These compounds may have antibacterial or antioxidant properties.

# 10:00 AM 175 Interactive effects of host plant resistance and predation risk on Colorado potato beetles • Illini Room C

#### Jennifer Thaler, Cornell University

#### Presenter: Jennifer Thaler, Cornell University

We seek a predictive framework for when non-consumptive effects of predators on prey will be strong. We tested how environmental conditions and traits of prey influenced prey responses to predation risk by measuring Colorado potato beetle responses to predation risk and how these responses are altered by host plant resistance. Specifically, eggs from Colorado potato beetle sibships were reared in predation risk or control environments on high- or low- resistance plants. We used potato plants treated with a foliar application of jasmonic acid to enhance plant resistance. On each plant-type we exposed Colorado potato beetle larvae to predaceous stink bugs (Podisus maculiventris), including either 'lethal' predators or sham predators with their mouthparts surgically altered to prevent killing. We measured CPB responses to the predator over their lifetime and fitness. We found 3-fold phenotypic variation between sibships in consumption and growth rate. Sibships varied in their type of response to predation risk, with 9 of 14 sibships reducing feeding and 5 of 14 sibships increasing assimilation efficiency in the predation risk treatment compared to controls. Mean sibship growth rate in the absence of predation was strongly correlated with their behavioral response to predation risk. Beetles on highresistance plants had a weaker response to predation risk. Beetles whose siblings grew the most quickly in the absence of predators were the ones that reduced feeding the most in the presence of predators. Overall, environmental conditions and prey traits that promote rapid growth are associated with stronger responses to the predator.

### 10:40 AM 176 Prey perception of predation risk: volatile chemical cues mediate nonconsumptive effects of a predator on a larval insect pest • Illini Room C

Sara L. Hermann and Jennifer S. Thaler, Cornell University

#### Presenter: Sara L. Hermann, Cornell University

Predators can affect prey in two ways-by reducing their density (consumptive effects) or by changing their behavior, physiology, or other phenotypic traits (non-consumptive effects). Understanding cues and sensory modalities prey use to detect predators is critical for predicting the strength of non-consumptive effects and outcomes of predator-prey encounters. While predator-associated cues have been well studied in aquatic systems, less is known about how terrestrial prey, particularly insect larvae, detect their predators. We evaluated how the Colorado potato beetle (Leptinotarsa decemlineata) larvae perceive predation risk by isolating cues from its predator, the spined soldier bug (Podisus maculiventris). When exposed to male "risk" predators that were surgically manipulated so they could hunt but not kill, beetles reduced feeding 29% compared to controls. Exposure to risk females caused an intermediate response. Beetles ate 24% less on leaves pre-exposed to predators compared to leaves never exposed to predators, indicating that tactile and visual cues are not required for prey response. Volatile cues from male predators reduced feeding by 16% compared to controls, whereas larvae exposed to odors from female predators did not impact beetle feeding. Finally, visual cues from the predator did not affect beetle feeding. These results demonstrate that volatile cues explain the net effect of predation risk from predators on prey feeding and that these cues are sex-specific. Variation in the information about predators within the environment may explain the importance of multiple cues in prey perception of risk especially due to differential effects from male and female predators.

# 10:55 AM 177 Semiochemically mediated adult-juvenile interactions enhance first instar feeding success in the bed bug, *Cimex lectularius* L. • *Illini Room C*

Sydney E. Crawley and Kenneth F. Haynes, University of Kentucky

Presenter: Sydney E. Crawley, University of Kentucky

One of the most common interactions between adults and immatures is the facilitation of offspring feeding by their parents. For insects, this interaction is often mediated by pheromones. Bed bugs, Cimex lectuarius L., release (E)-2-hexenal and (E)-2-octenal with their signaling functions suggested to be varied, including alarm, aggregation and sex recognition. These pheromones, along with compounds associated with late instar nymphs, also discourage potentially damaging male copulation attempts. In a very different context, we tested the hypothesis that female bed bugs facilitate immature feeding through the use of semiochemicals. A behavioral assay indicated that first instar bed bugs held with groups containing adult females fed significantly more frequently than those without adults or with adult males. Additionally, older bed bug nymphs did not have the same effect on first instar feeding. Female bed bugs reached a blood source significantly faster than first instars, suggesting that there is opportunity to signal the presence of a host. GC-MS analyses showed that quantity and blend ratio of (E)-2-hexenal and (E)-2-octenal emitted during feeding distinguish adult females from males and late-instar nymphs. Behavioral tests are needed to determine if these differences could have a signaling function. If they do, then mother-offspring communication may play an important role. Furthermore, such a finding would prompt questions about the specificity of the communication channel between a mother and her offspring, and how this interaction might be maintained.

# 11:10 AM 178 A specialized herbivore with a generalized problem: vertebrate-toxin interactions • *Illini Room C*

Natasha Wiggins, University of Utah Lisa Shipley, Washington State University Jennifer Sorensen Forbey, Boise State University

#### Presenter: Natasha Wiggins, University of Utah

Sagebrush (Artemisia spp.) is considered to be a toxic plant genus that most vertebrate herbivores avoid eating due to the high concentrations of chemical defenses it contains and its relatively low nutritional value. The types and concentrations of chemical defenses can be considerably variable: from within an individual plant and among individuals, to between populations, subspecies and species. The pygmy rabbit (Brachylagus idahoensis) is a sagebrush specialist that relies on sagebrush-steppe habitat in the northwestern US. Pygmy rabbits subsist on sagebrush exclusively during winter, and it remains a significant portion of their diet throughout the year. This research aimed to investigate the chemically-mediated interactions between pygmy rabbits and their diet, and the influence that chemical defenses have on herbivore foraging decisions. We conducted a series of captive- and field-based feeding trials and quantified the chemical composition of a range of sagebrush subspecies and species that rabbits consume. Pygmy rabbit preferences were largely driven by volatile oil (monoterpene) concentrations, although other classes of defensive compounds (total phenolics) and plant nutrients (crude protein) may also play an important role in diet selection. The chemically-mediated interactions we explored directly contribute to our understanding of herbivore foraging decisions across multiple spatial scales. This information is important for the management of a native vertebrate herbivore which is currently a species of concern.

# 11:25 AM 179 The role of predation cue sources in inducible defenses in the eastern oyster (Crassostrea virginica) • Illini Room C

Avery Scherer, Jessica Lunt, Alex Draper, and Delbert L. Smee, TAMU-CC

Presenter: Jessica Lunt, TAMU-CC

The amount of information and its reliability conveyed through predation cues can be impacted greatly by the source of the cues. Cues from predators can indicate predator species, proximity, and diet while cues from prey indicate organisms recently consumed and potentially indicate threats from cryptic predators. Costs may differ between these cues as well, as predator cues can vary greatly, be highly specific, or require previous exposure for recognition. In contrast, conspecific alarm cues are often innately recognized but can be misleading in the source of damage to the cue-producing organism. This study investigates the role that direct cues from predators and indirect cues from prey organisms play in the induction of eastern oyster (Crassostrea virginica) morphological defenses. A mesocosm experiment exposed newly settled oysters to cues from crushed conspecifics, crushed clams (Mercenaria mercenaria), blue crabs (Callinectes sapidus) fed oysters or clams, or blue crabs that were starved. To determine if morphological changes occurred in response to these cue treatments shell diameter, weight, and compression force were measured after eight weeks. Initial analysis suggests oysters respond to both direct and indirect predator cues (p < 0.0001), though they change morphological characters differentially. Oysters can discern between cue types and may change the characters which are the most cost-effective for the perceived threat. Threat of predation causes oysters to grow

heavier and thicker shells to reach a size less susceptible to predation. However, the source of predation determines oyster shape as diameter changes depending on predator diet.

### 11:40 AM 180 **Toxin or medication? Nicotine enhances caterpillar immunity** • *Illini Room C*

Michael Garvey, Purdue University Curtis Creighton, Purdue University, Calumet Ian Kaplan, Purdue University

### Presenter: Michael Garvey, Purdue University

The insect immune response is strongly affected by diet nutritional quality, such as the quantity and ratio of carbohydrates to proteins. Rarely, though, are antagonist compounds, such as plant toxins, incorporated into nutritional studies. To approximate realistic food sources, and to understand the nutritional ecology of insect immunity, we used *Manduca sexta* as a model system to examine host performance and immune function across nutritional space augmented with the alkaloid nicotine. We assayed caterpillar performance by measuring larval body mass and determined immunocompetence by measuring total circulating hemocytes and phenoloxidase activity at the fourth instar. Nicotine had negative effects on larval development which were mitigated by dietary protein concentration. In addition, nicotine increased phenoloxidase activity, suggesting enhanced immunity against parasites, but did not affect total hemocyte concentrations. Together with previous findings that ingested nicotine can function as parasitic medication in hornworms, our findings indicate that toxic nicotine can further enhance the immune function of *M. sexta* feeding on tobacco (*Nicotiana* sp.), even at the cost of delayed development through interactions with total plant nutritional quality.

### Invasives and biological control

2:30 PM

### 181 Chemical ecology of stored product insects: Host odor and habitat volatiles mediate host location behavior of an ectoparasitoid of *Tribolium confusum*. • *Illini Room A*

Benjamin Fürstenau, Freie Universität Berlin

Cornel Adler, Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection (ÖPV), JKI Berlin-Dahlem Hartwig Schulz, Institute for Ecological Chemistry, Plant Analysis and Stored Product

Protection (ÖPV), JKI Berlin-Dahlem

Monika Hilker, Freie Universität Berlin

Presenter: Benjamin Fürstenau, Freie Universität Berlin

Most storable plant products suffer post-harvest attacks by a wide range of pests, especially insects, resulting in considerable global losses of food every year. To date, effective protection of stored food commodities from pests often relies on the use of potentially hazardous pesticides which can cause tremendous costs, food contamination, and pest resistance. Therefore, new and environmentally friendly control strategies for insect pest management are needed. The use of parasitoids as an alternative method for biological control of insects infesting stored food has been shown to be a promising approach. In order to improve such biological control methods it is essential to deepen and broaden our knowledge of factors that drive host location by parasitoids. In the present study we will investigate how naturally occurring (info-)chemicals mediate interactions between the larval parasitoid *Holepyris sylvanidis* (Brèthes, 1913) (Hymenoptera: Bethylidae) and its host, *Tribolium confusum* (Jacquelin du Val, 1868) (Coleoptera: Tenebrionidae), one of the most important pests in flour mills, bakeries, and pasta factories worldwide.

Preliminary studies indicated that *H. sylvanidis* females are attracted to odor from larval feces of *T. confusum* and infested wheat grist. We aim to identify the naturally occurring chemicals which mediate host location behavior of *H. sylvanidis* and which could be useful for biological control of *T. confusum*. Host odor will be chemically analyzed by coupled gaschromatography-mass spectrometry (GC-MS). The parasitoid ´s physiological and behavioral response to host odors and identified authentic volatile compounds will be analyzed by electroantennography (EAG) and olfactometer bioassays.

2:45 PM

### 182 Responses of two parasitoids of the emerald ash borer, Agrilus planipennis Fairmaire, the introduced Spathius agrili Yang, and native Spathius floridanus Ashmead, to volatile host-associated cues • Illini Room A

Todd D. Johnson, University of Wisconsin–Madison and University of Illinois Urbana-Champaign Jonathan P. Lelito, USDA APHIS PPQ EAB Unit Kenneth F. Raffa, University of Wisconsin–Madison

Presenter: Todd D. Johnson, University of Wisconsin–Madison, University of Illinois Urbana-Champaign

Two parasitoids, the introduced specialist Spathius agrili Yang (Braconidae), and the native generalist Spathius floridanus Ashmead (Braconidae), have been proposed as biological control agents of the emerald ash borer, Agrilus planipennis Fairmaire. However, little is known about their host-location behaviors. We evaluated responses to components of this host complex, Fraxinus pennslyvanica (green ash) stem tissue, F. pennslyvanica foliage, and an A. planipennis larva within a stem. Experiments were conducted in Y-tube olfactometer bioassays, using wasps reared on A. planipennis larvae feeding in F. pennslyvanica twigs at USDA APHIS in Brighton, MI. Naïve S. agrili were attracted to the entire complex, and leaf tissue alone, relative to blank controls. S. agrili were also significantly attracted to a stem with a larva and leaf tissue together, relative to leaf tissue alone. In contrast, naïve S. floridanus were attracted to a larva within a stem, but not to any other component. Thus, naïve S. agrili and S. floridanus appear to employ different host-location strategies. Because S. floridanus attack multiple species of Agrilus, host-associated cues perceived during emergence from their natal hosts may improve attraction to emerald ash borer larvae. Subsequent experience may elicit attraction to plant foliage and thereby facilitate location of host habitat. In contrast, naïve S. agrili may learn additional host-associated cues during oviposition. Further understanding of host-location behavior may improve the utility of these parasitoids for biological control, both by suggesting strategies for pre-release conditioning and by providing tools for assessing post-release establishment.

3:00 PM

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### **Olfactory correlates of Drosophila suzukii's preference for ripe fruit** · Illini Room A

Teun Dekker, SLU, Alnarp Santosh Revadi, FEM, San Michele all'Adige (TN), Italy Suzan Mansourian, SLU, Alnarp Sukanya Ramasamy, FEM, San Michele all'Adige (TN), Italy Sebastien Lebreton, SLu, Alnarp, Sweden Paul G. Becher, SLU, Alnarp, Sweden Sergio Angeli, Free University of Bozen-Bolzano

Omar Rota-Stabelli, FEM, San Michele all'Adige (TN), Italy Gianfranco Anfora, San Michele all'Adige (TN), Italy

### Presenter: Teun Dekker, Swedish University of Agricultural Sciences

The spotted wing Drosophila (SWD, *Drosophila suzukii*) is a recent invader of the EU and US. It has rapidly spread and established itself throughout the continents. Instead of its closely related sibling species, this species does not oviposit on rotten fruits, but prefers ripe fruits. A morphological adaptation of the ovipositor enables *D. suzukii* to pierce and oviposit in intact fruit, resulting in over a billion dollars of damage worldwide. The species' shift from 'rotten to ripe' is likely to be paralleled by olfactory adaptations. We used a combination of techniques, including neurophysiology, neuroanatomy, bioinformatics, and chemical analysis, to detect and assess the significance of some of the most dramatic adaptations of this species' olfactory circuit.

3:15 PM

### 184 Comparative sensitivity and pheromone emission of four invasive tephritid fruit flies, Bactrocera dorsalis, B. papayae, B. philippinensis and B. invadens, following their exposure to and consumption of methyl eugenol · Illini Room A

Alvin Kah-Wei Hee, Universiti Putra Malaysia Suk-Ling Wee, Universiti Kebangsaan Malaysia Ritsuo Nishida, Kyoto University Keng-Hong Tan, Tan Hak Heng Co.

Presenter: Alvin Kah-Wei Hee, Universiti Putra Malaysia

Among the notorious pest species in the Bactrocera dorsalis complex include some of the world's most severe fruit pests such as the Oriental fruit fly, B. dorsalis, along with its very close sibling taxa, B. papayae, B. philippinensis and B. invadens, and their males are strongly attracted to, and readily consume, methyl eugenol (ME) that is found in over 480 plant species worldwide. While the first three taxa continue to threaten the fruit industry in the Asia-Pacific countries, the establishment of B. invadens in Africa within the last decade had raised alarm that it can potentially devastate the entire fruit industry there. The identification of these four cryptic putative species has been the subject of great controversy for many years due to their very similar morphology, high mating compatibility and identical sex pheromone system following pharmacophagy of ME. Here we report on the laboratory trials that have been undertaken to evaluate response of the flies to ME and their pheromone emission profiles following ME feeding. To date, our trials have demonstrated that the ED50 (effective median dose required to elicit attraction of 50% of males tested for their response to ME) of the four species is not significantly different. In addition, the pheromone emission of the flies showed similar profiles in which two major phenylpropanoid compounds--(E)-coniferyl alcohol and 2-allyl-4,5-dimethoxyphenol-- that are biosynthesized from ME and sequestered in the male rectal glands were emitted as sex pheromone during courtship at dusk. Thus, together with this and the flies' similar sensitivity to ME, we provide further evidence that these four putative taxa belong to the same biological species (currently being synonymized as one species, B. dorsalis).

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### PM 185 Husbandry of Pest Enemies: Is there hope for "HOPE"? • Illini Room A

Jeffrey R. Aldrich, University of California, Davis and Jeffrey R. Aldrich Consulting, LLC Presenter: Jeffrey R. Aldrich, University of California, Davis and Jeffrey R. Aldrich Consulting, LLC

The spined soldier bug, *Podisus maculiventris* Say (Heteroptera: Pentatomidae: Asopinae), is a common generalist predator ranging from coast-to-coast in North America. Adults

and nymphs actively search for prey, especially for caterpillars and exposed beetle larvae. The male-produced aggregation pheromone of *P. maculiventris* was the first heteropteran pheromone identified (Aldrich et al. 1978, Aldrich et al. 1984); the pheromone attracts both sexes of adults as well as second-to fifth-instar nymphs, and it is one of the cheapest of all known pheromones to make. There is a multitude of published research on the natural impact of spined soldier bugs on various pest species and on the potential of P. maculiventris for augmentative biocontrol. Nevertheless, although spined soldier bugs are commercially available, utilization of this beneficial insect for biocontrol falls far short of its predicted potential, principally due the cost of mass-rearing the bugs. In the absence of a practical artificial diet, this predator must be reared on live prey. Here I will discuss my recent and ongoing efforts to devise semiochemical and improved rearing and handling techniques to increase the husbandry of the spined soldier bug for, at least, garden-scale biocontrol.

Aldrich, J. R., M. S. Blum, H. A. Lloyd, and H. M. Fales. 1978. J. Chem. Ecol. 4: 161-172.

Aldrich, J. R., J. P. Kochansky, and C. B. Abrams. 1984. Environ. Entomol. 13: 1031-1036.

#### 4:10 PM 186 Contemporary evolution of plant chemical defenses after invasion in response to herbivory and climate • Illini Room A

Eva Castells, Universitat Autònoma de Barcelona

Presenter: Eva Castells, Universitat Autònoma de Barcelona

Biological invasions are excellent systems to study rapid evolution of plant chemical defenses. Current hypotheses predict a rapid divergence of plant chemical defenses in response to changes in herbivore consumption pressure caused by a decrease in the enemies from the area of origin (e.g. evolution of increased competitive ability -EICAhypothesis) or in response to differences in the climatic conditions. Senecio pterophorus (Asteraceae) is a perennial shrub native to eastern South Africa that has recently invaded western South Africa, Australia and Europe. The four distributional regions of S. pterophorus differ in their levels of herbivory<sup>1</sup> and summer drought stress. Here we evaluated 1) whether native and introduced populations diverged in their genetically-based levels of chemical defenses, and 2) whether these biogeographical differences were driven by postinvasive changes in herbivory or climate. We performed a common garden experiment with 54 populations of S. pterophorus sampled throughout the entire known worldwide distributional area, including the native and three non-native ranges. Genetically-based chemical defense concentrations varied among regions. Contrary to the EICA hypothesis we found higher levels of chemical defenses in the introduced populations. The role of enemy release and summer drought stress are simultaneously evaluated as potential drivers for the evolution of chemical defenses. This is the first study to evaluate rapid evolution of an exotic plant covering the entire known distributional area of a species.

<sup>1</sup>Castells et al. (2013) Reduced seed predation after invasion supports enemy release in a broad biogeographical survey. Oecologia 173: 1397-1409

#### **Chemical ecology of the Argentine ant** • *Illini Room A* 4:25 PM 187

Kevin Welzel, University of California, Riverside

Presenter: Kevin Welzel, University of California, Riverside

The Argentine ant (Linepithema humile) is a widespread invasive species in various urban/agricultural settings worldwide. In the invaded locations, Argentine ants have had a negative impact on native ant fauna by competitively displacing some native ant species. By exploring the chemical ecology of the Argentine ant, we plan to investigate

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mechanisms that have allowed them to invade these new environments successfully. Behavioral observations combined with chemical investigations will help us to elucidate some important aspects of interspecific (or intraspecific) competition involving Argentine ant. We plan to use headspace analyses to investigate if Argentine ants emit any chemical compound(s) when they are involved in interspecific (or intraspecific) aggression. The study will help us to gain a better understanding on the aggressive interactions between Argentine ants and other native arthropods in the invaded locations. As one of our efforts in exploiting the chemical ecology of Argentine ant to develop functional control strategies, we are currently investigating a "pheromone-assisted technique" as an economically viable approach to maximize the efficacy of conventional baits targeting the Argentine ant. Laboratory and field experiments with insecticidal bait indicated that the overall kill on Argentine ant colonies was significantly improved by incorporating a synthetic Argentine ant pheromone, (Z)-9-hexadecenal, in the baits. This technique has a potential to provide maximum control efficacy with lower amounts of insecticides applied in the environment, leaving little or no effect on nontarget ants or other organisms.

### 4:40 PM 188 Volatile and cuticular chemical constituents are caste-specific in the Argentine ant Linepithema humile • Illini Room A

R. Ryan Neff, Jocelyn G. Millar, Dong-Hwan Choe, and Mike Rust, University of California, Riverside

#### Presenter: Ryan Neff, University of California, Riverside

Social insect colony cohesion is governed by intricate feedback loops in which semiochemicals play an important role. In the Argentine ant *Linepithema humile* (Mayr), these semiochemicals have been investigated to some extent in workers and queens, but the primary focus has been on cuticular hydrocarbons (CHCs). Here we report the volatile and CHC constituents of Argentine ant queens, virgin queens, workers, males, and worker larvae, as well as the CHCs of gyne larvae. We show marked qualitative and quantitative differences between the different groups, particularly in queen-specific compounds. Queens can be differentiated from other castes by the high relative abundance of 5-methyl and 5,11-dimethylalkanes, of (Z)-14 and (Z)-9-alkenes, and the iridoid dolichodial. Worker cuticular profiles are dominated by long-chain di and trimethylalkanes, completely lack the long-chain alkenes seen in queens, and have a high abundance of another iridoid, iridomyrmecin, compared with dolichodial. Male cuticular profiles were similar to workers with the exception of having a lower proportion of n-alkanes. Iridoids were absent in males, with only an alkyl pyrazine and two hydrocarbons detectable in aerations. Our results demonstrate the impressive chemical diversity present within this highly polygyne invasive ant.

### 4:55 PM 189 Associative learning of *Podisus maculiventris* (Hemiptera: Pentatomidae) to herbivore-induced plant volatiles • *Illini Room A*

Ulianova Vidal Gomez and Ian Kaplan, Purdue University

Presenter: Ulianova Vidal Gomez, Purdue University

The spined soldier bug, *Podisus maculiventris*, is a polyphagous biocontrol agent that likely uses herbivore-induced plant volatiles (HIPVs) for foraging. We hypothesized that the behavioral responses of this predator to HIPVs are acquired via associative learning, as suggested by theoretical predictions linking natural enemy host range with odor preferences. We also proposed that it is possible to attract stink bugs to methyl salicylate (MeSA), if it is paired with a positive reward. To evaluate *P. maculiventris* odor preferences, we combined laboratory and field experiments. First, naïve (i.e., lab-reared in colony)

and experienced (i.e., field=collected) individuals were allowed to choose between scents released by caterpillar (*Manduca sexta*)-damaged vs. undamaged tomato plants. In the second part, naïve adults and nymphs were conditioned to MeSA by linking this odor with *M. sexta* as a food reward. After experiencing this association, preferences toward MeSA were assessed. We found that field-collected males showed orientation to odors released by caterpillar-damaged plants, but naïve lab-reared individuals showed no preference. When conditioned females were tested in the field, they chose *M. sexta*-damaged plants. Similarly, nymphs conditioned to MeSA showed an orientation response to this HIPV. Unexpectedly, adults did not display a distinct preference upon conditioning. These results suggest that stink bug attraction to volatile cues is acquired by learning rather than innate. We have learned not only that associative learning is the mechanism underliyng this interaction, but also that this predator can be conditioned to specific HIPVs, components of a more complex odor blend.

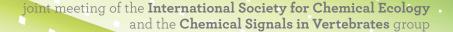
5:10 PM

### 190 Effects of the phytochemical xanthotoxin on the growth and survival of the navel orangeworm (*Amyelois transitella*) • Illini Room A

Vikram A. Bagchi, University of Illinois at Urbana-Champaign Mark Demkovich, University of Illinois at Urbana-Champaign May R. Berenbaum, University of Illinois at Urbana-Champaign Joel Siegel, USDA-ARS, Parlier, CA

#### Presenter: Vikram A. Bagchi, University of Illinois at Urbana-Champaign

The navel orangeworm (Amyelois transitella) is the most destructive lepidopteran pest of nut crops, such as almonds, pistachios, and walnuts, in California orchards. An environmentally safe and sustainable approach to control this insect in orchards is to use natural compounds to interfere with its growth and survival. Xanthotoxin is a natural furanocoumarin found in several plant hosts of this insect, including figs (Ficus carica). In this study, we tested its effects on growth and survival of navel orangeworms from three populations: a laboratory strain derived from individuals collected in almonds, a strain derived from individuals collected in fig orchards, and a strain derived from a bifenthrinresistant population in almonds. Xanthotoxin was added to a semi-defined artificial diet at six concentrations (2.5, 5, 7.5, 10, 12.5 and 15 mg/g). After 48 h, the lethal median concentration (LC50) for each strain was determined. The laboratory strain displayed an LC50 of 4.5 mg/g xanthotoxin. In contrast, the LC50 of the fig strain was 9.4 mg/g. The highest LC50, 11.6 mg/g, was recorded for the bifenthrin-resistant strain. Collectively, these results showed that, although xanthotoxin reduces the growth and survival of A. transitella from populations associated with almonds, it is less toxic to populations associated with hosts containing xanthotoxin or with a history of pesticide adaptation. Both the fig and the pesticide-resistant strains may owe their greater tolerance to xanthotoxin to enhanced metabolic capabilities. Future studies will involve characterization of the biochemical detoxification of xanthotoxin, knowledge of which can contribute to developing effective strategies to manage this pest across its multiple host crops.



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### Plasticity of plant defenses

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9:00 AM

Synergistic defensive function of raphides and protease: Raphides function as intensifiers of other defense substances through the needle effect · Illini Room A

Kotaro Konno, Takashi A. Inoue, and Masatoshi Nakamura, National Institute of Agrobiological Sciences

### Presenter: Kotaro Konno, National Institute of Agrobiological Sciences

Raphides, needle-shaped calcium oxalate crystals in tissues of many plants, have been thought to play defensive roles against herbivores without detailed bioassays for their defensive roles and modes of function using purified raphides. Therefore, we performed clear bioassays giving the larvae of the eri silkmoth, Samia ricini, leaves of their host plant, the castor oil plant, *Ricinus communis*, painted with the raphides purified from kiwifruits, Actinidia deliciosa, in the presence or absence of cysteine protease (or other defense proteins), which often coincide with raphides in plant tissues. Raphides alone or cysteine protease alone showed only weak defensive activities around experimental concentrations. However, when raphides and cysteine protease coexisted, they synergistically showed very strong growth-reducing and insecticidal activities. In contrast, amorphous calcium oxalate did not show synergism with cysteine protease, indicating that the needle-shape of raphides is essential for the synergism. The present study provides the first clear experimental evidence for the synergism between raphides (physical defense) and other defensive factors (chemical defense). Furthermore, the study suggests the existence of "the needle effect", which intensifies the bioactivities of other bioactive factors by making holes to the barriers (cell membrane, cuticle, epithelium) and facilitate the factors to reach the targets. Since raphides are widely found in the plant kingdom, raphide-borne defenses can be regarded as a defense syndrome that may be important in defense plasticity by intensifying other defense agents.

1. Konno K, Inoue TA, Nakamura M (2014) Synergistic defensive function for aphides and protease therough the needle effect. PLoS ONE 9(3): e91341

### 9:15 AM 192 **Oviposition-induced direct and indirect defense trait in a commercial hybrid** maize variety · Illini Room A

Daniel M. Mutyambai, International Centre of Insect Physiology and Ecology, Nairobi, Kenya Toby J.A. Bruce, Rothamsted Research

Charles A.O. Midega, International Centre of Insect Physiology and Ecology, Nairobi, Kenya Johnnie Van den Berg, North-West University, Potchefstroom, South Africa John A. Pickett, Rothamsted Research

Zeyaur R. Khan, International Centre of Insect Physiology and Ecology, Nairobi, Kenya

### Presenter: Daniel M. Mutyambai, International Centre of Insect Physiology and Ecology, Nairobi, Kenya

Maize, like most other Gramineae, naturally defends itself against herbivore attack through a number of mechanisms, including the production of herbivore-induced plant volatiles (HIPVs) following larval feeding. Previously, we discovered a valuable defense trait in wild and landrace maize varieties that was not present in hybrid varieties. These landraces, after stemborer egg deposition on the plants, release HIPVs which attract both egg

(*Trichogramma bournieri*) and larval (*Cotesia sesamiae*) parasitoids. This is considered preventive defense before any damage is inflicted on the plant. Here, we report on the occurrence of this trait in some elite maize varieties. HIPVs were collected through headspace sampling from plants with and without *Chilo partellus* eggs. GC-MS and GC-EAG were used for volatile analysis. Four-arm olfactometer bioassays were carried out using parasitic wasps *T. bournieri* and C. *sesamiae*. Two-choice oviposition bioassays were carried out in oviposition cages between oviposited and unexposed plants. Coupled GC-MS revealed that elite variety 'SC DUMA 43' emitted more EAG-active compounds when exposed to *C. partellus* oviposition, compared to unexposed plants. Both egg and larval parasitoids preferred samples containing HIPVs from plants with eggs to those from plants without eggs. Plants without eggs were significantly preferred for subsequent oviposition by moths compared to plants oviposited on earlier. These results indicate that, while breeding for improved agronomic performance could have resulted in the loss of innate defense traits in high-yielding commercial maize hybrids, some hybrids do exhibit these traits. Avenues of exploiting these traits in pest management need further investigation.

### 9:30 AM 193 Impacts of methyl salicylate volatile cues on tomato (Solanum lycopersicum) defense against specialist Manduca sexta • Illini Room A

Elizabeth Rowen, Michael Gutensohn, Natalia Doudareva, and Ian Kaplan, Purdue University Presenter: Elizabeth Rowen, Purdue University

Recent efforts in biological control have explored using the attractive qualities of herbivoreinduced plant volatiles (HIPVs) to draw natural enemies to protect crops throughout the field. These HIPVs also function in interplant communication whereby plants 'eavesdrop' on volatiles released by their herbivore-infested neighbors and exploit this cue as an early warning system to induce defenses in anticipation of attack. It is thought this may occur by inducing defenses directly, or by priming plant defense such that the plant responds much more strongly and quickly to subsequent damage. To further understand the mechanisms underlying HIPV-mediated plant communication, we studied the defenses of tomatoes (Solanum lycopersicum) exposed to the HIPV methyl salicylate (MeSA). We assessed the interactive effects of HIPV exposure and simulated Manduca sexta herbivory in the following treatments: (1) naïve control; (2) MeSA exposure; (3) M. sexta damage; and (4) MeSA exposure and subsequent M. sexta damage. We measured the plants' responses to these treatments over time using peroxidase, polyphenol oxidase, proteinase inhibitor activity, headspace volatiles and mRNA expression of defense genes. Tomatoes upregulated chemical defenses in response to herbivory, but preliminary data suggest that previous exposure to MeSA does not prime plants against attack

#### 9:45 AM 194 The chemical defense of extrafloral nectaries • Illini Room A

Moshe Gish, Mark C. Mescher, and Consuelo M. De Moraes, The Pennsylvania State University

Presenter: Moshe Gish, Pennsylvania State University

Many plant species, in more than 100 plant families, bear extrafloral nectaries (EFN) that attract predatory and parasitoid insects. The tissue of the EFN is rich in sugar and therefore prone to predation by chewing insects or invasion by bacteria and fungi. It would be advantageous for a plant to raise its chemical defense levels in the vicinity of the EFN. We tested whether this tactic is used by the EFN-bearing plant *Vicia faba* (fava bean). Fava bean plants are rich in the toxic non-protein amino acid L-DOPA (L-3,4-dihydroxyphenyl-alanine), which is found in all parts of the plant, except for the seeds (Goyoaga et al. 2008). We analyzed L-DOPA levels in different parts of the EFN-bearing stipule using GC-MS and compared them to L-DOPA levels in other parts of the plant. While the extrafloral

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nectar contains no L-DOPA, the EFN itself has L-DOPA levels that are 4.4 times higher than those found in leaves. The tissue that surrounds the EFN has 6.6 times more L-DOPA than leaves. Field and lab experiments showed that these high levels of L-DOPA do not effectively defend the plant's EFNs against chewing insects, which tend to specifically target the EFNs and remove them with impressive precision. Some evidence suggests that L-DOPA has antifungal properties (Nidiry, Ganeshan & Lokesha 2011). We are currently testing the hypothesis that the L-DOPA rich tissue of the EFN and the surrounding area function as a barrier against microbial and fungal infestations.

Goyoaga, C., Burbano, C., Cuadrado, C., Varela, A., Guillamón, E., Pedrosa, M.M. & Muzquiz, M. (2008) Content and distribution of vicine, convicine and L-DOPA during germination and seedling growth of two Vicia faba L. varieties. European Food Research and Technology, 227, 1537–1542.

Nidiry, E.S.J., Ganeshan, G. & Lokesha, A.N. (2011) Antifungal activity of *Mucuna pruriens* seed extractives and L-dopa. Journal of Herbs, Spices & Medicinal Plants, 17, 139–143.

10:00 AM 195 **Open slot** • Illini Room A

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10:40 AM

### Jasmonates: new insights into structure-activity relationships and metabolism • Illini Room A

Guillermo H. Jiménez-Alemán, Max Planck Institute for Chemical Ecology Jyothilakshmi Vadassery, Max Planck Institute for Chemical Ecology Axel Mithöfer, Max Planck Institute for Chemical Ecology Helmar Goerls, Institute for Inorganic and Analytical Chemistry Wilhelm Boland, Max Planck Institute for Chemical Ecology Prateek Sharma, Max Planck Institute for Chemical Ecology Wolfgang Brandt, Leibniz Institute of Plant Biochemistry

Presenter: Guillermo H. Jiménez-Alemán, Max Planck Institute for Chemical Ecology

Jasmonates are a family of naturally occurring lipid-derived compounds with jasmonic acid (JA) as a basic constituent. These metabolites regulate several developmental processes and responses to biotic and abiotic stress in plants. Jasmonoyl-L-isoleucine (JA-Ile), was identified as the bioactive endogenous jasmonate. Arabidopsis mutants have been employed to establish the pathway of JA-Ile biosynthesis, perception and jasmonate signaling cascade. After binding to JA-Ile, the SCFCOI1 receptor complex targets JAZ repressors for degradation by the 26S-proteasome pathway, allowing the transcription factor MYC2 to activate the expression of jasmonate-responsive genes (JRGs).<sup>1</sup> Recently, it was demonstrated that Arabidopsis CYP94B3 encodes jasmonoyl-L-isoleucine-12-hydroxylase which hydroxylates JA-Ile at carbon C12 and converts it into 12-OH-JA-Ile. This hydroxylation leads to partial inactivation of the molecule and switch-off of the jasmonate signaling cascade.<sup>2</sup> We present a tailored synthetic approach to investigate the switch-off mechanism of JA-Ile in Arabidopsis upon hydroxylation at carbon C12. Our studies reveal that partial inactivation of JA-Ile upon hydroxylation does not occur due to electrostatic interactions involving the hydroxyl group. Inactivation seems to be associated with steric hindrance. A free carboxylic acid group is not a crucial factor for JA-Ile to interact with its receptor complex SCFCOI1-JAZ; an ester bond of a 12-OH-JA-Ile-derived macrolactone is sufficient. The lactone up-regulates different subsets of JRGs depending on the configuration at the cyclopentanone ring.

[1] Yan et al. (2009) The Plant cell 21, 2220.

[2] Kitaoka et al. (2011) Plant & cell physiology 52, 1757.

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### 10:55 AM 197 Single template, multiple currencies: Multiproduct sesquiterpene synthase from Medicago truncatula • Illini Room A

Abith Vattekkatte and Wilhelm Boland, Max Planck Institute for Chemical Ecology Presenter: Abith Vattekkatte, Max Planck Institute for Chemical Ecology

There are more than 20,000 different terpene metabolites known as natural products so far. Their immense diversity is both fascinating and puzzling, especially since all known terpenoids are based on only 300 different hydrocarbon skeletons. The various terpene skeletons are generated by terpene synthases (TPS), which utilize linear prenyl diphosphates such as farnesyl diphosphate (FDP) as substrates. Surprisingly, unlike high-fidelity TPS which produce only a single product, promiscuous enzymes can generate up to 52 different compounds (1). We aim to study this exclusive activity of TPS by relating the three-dimensional topology of the active-site pocket with the catalytic mechanism.

We studied the multiproduct sesquiterpene synthase MtTPS5 from *Medicago truncatula* which on incubation with its natural substrate (E,E)-FDP provides 18 sesquiterpene hydrocarbons and 10 alcohols sharing the same cadalene skeleton (2). The structural characteristics of the enzyme are being analyzed by computational modelling studies and by X-ray after successful crystallization. This along with the investigation of the effects of substrate analogues on product formation provided critical insights into the enzyme's active site which allows for conformational changes and rearrangements of the initial (2E,6E)-germacren-11-yl cation intermediate. To better understand the interaction between substrate and enzyme, we synthesized halogenated mimics of (E,E)-FDP. Interestingly, a halogenated FDP analog acts as a competitive inhibitor of the enzyme and remains covalently bound, proving to be an ideal candidate for co-crystallization.

1. Steele, C. L et al. J. Biol. Chem. 1998, 273,2078

2. Garms, S et al.J. Org. Chem. 2010, 75, 5590

### 11:10 AM 198 **Silicon-mediated resistance against insect herbivory in rice is jasmonic aciddependent** • *Illini Room A*

#### Rensen Zeng, Fujian Agriculture and Forestry University

#### Presenter: Rensen Zeng, Fujian Agriculture and Forestry University

While the function of silicon (Si) in plant physiology has long been debated, its beneficial effects on plant resistance against abiotic and biotic stresses, including insect herbivory, have been well-documented. In addition, the jasmonate (JA) signaling pathway plays a crucial role in mediating anti-herbivore defense responses in plants. However, potential interactions between JA and Si in response to insect attack have not been directly examined. To explore the role JA may play in Si-enhanced resistance, we silenced expression of allene oxide synthase (OsAOS, JA biosynthesis) and CORONATINE INSENSITIVE1 (OsCOI1, JA perception) genes in transgenic rice plants via RNAi and examined resulting changes in Si accumulation and defense responses against the caterpillar Cnaphalocrocis medinalis (rice leaffolder, LF). Si pre-treatment increased rice resistance against LF larvae in wild-type plants but not in OsAOS and OsCOI1 RNAi lines. Upon LF attack, wild-type plants subjected to Si pre-treatment exhibited enhanced defense responses relative to untreated controls, including higher JA accumulation levels, increased levels of transcripts encoding defense marker genes, and elevated activities of peroxidase, polyphenol oxidase and trypsin protease inhibitor. Additionally, reduced Si deposition and Si cell expansion were observed in leaves of OsAOS and OsCOI1 RNAi plants in comparison to wild-type plants, as well as reduced steady-state transcript levels of Si transporters OsLsi1, OsLsi2

and OsLsi6 following LF attack in Si pre-treated plants. These results suggest a strong interaction between Si and JA in defense against insect herbivores involving priming of JA-mediated defense responses by Si and the promotion of Si accumulation by JA.

#### 11:25 AM

### 199 **Insect eggs prime distinct plant defense responses in Nicotiana attenuata** • Illini Room A

Michèle Bandoly, Monika Hilker, and Anke Steppuhn, Freie Universität Berlin

### Presenter: Anke Steppuhn, Freie Universität Berlin

Most plants respond to herbivory with defense induction. Priming refers to enhanced defense induction upon a previous stimulus predicting upcoming herbivory. Eggs of herbivorous insects on a plant reliably indicate future herbivory and can entail increased plant resistance against larvae, but the underlying mechanisms are unknown. We investigated whether egg deposition of the generalist lepidopteran herbivore Spodoptera exigua on Nicotiana attenuata primes induction of induced direct defense against feeding larvae. We found that larvae suffered higher mortality and slower development on egg-laden compared to egg-free plants. We further examined chemical defenses in constitutive and herbivory-induced leaves of prior egg-laden and egg-free plants. Egg deposition per se did not result in a change in production of secondary plant metabolites, but the induced production of caffeoylputrescin (CP) and trypsin protease inhibitors (TPI) was higher in egg-laden compared to egg-free plants In contrast, other inducible defenses, e.g. nicotine concentrations, were not altered. To investigate whether the egg-mediated plant changes contributed to the increased larval mortality on egg-laden plants, we tested the effect of prior egg deposition on CP-deficient (silenced NaMyb8 gene) and TPI-deficient (silenced NaPI gene) plants. As in wild type plants, egg deposition on PI-deficient plants resulted in increased larval mortality; however, in Myb8-silenced plants this effect was absent. Hence, higher resistance of egg-laden plants to larvae was dependent on priming of Myb8-mediated defense traits, but probably not on priming of TPI production. Our results demonstrate that insect egg deposition warns tobacco plants of future herbivory and primes specific feeding-induced defense responses.

## 11:40 AM 200 **Plant-plant signaling in willows: revisiting the original talking trees** • *Illini Room A*

Ian Pearse, Illinois Natural History Survey Kathy Hughes, University of California, Davis Patrick Grof-Tisza, University of California, Davis Richard Karban, University of California, Davis

Presenter: Ian Pearse, Illinois Natural History Survey

Thirty years ago, the idea of herbivore-induced plant-plant signals was introduced by studies in oaks and willows. Those studies were met with initial skepticism, and those systems were largely abandoned as models of plant-plant signaling. Since that time, plant-plant signaling has been increasingly recognized as a component of plant defense, but there has been little effort to return to those initial systems. We conducted a series of experiments to ask if and how plant-plant signals augment plant defense in willows. In three separate

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experiments, willows adjacent to a wounded neighbor (signal-induced) experienced less herbivore damage in the field than those with an adjacent unwounded neighbor (control). In one trial, the effect of signal induction was stronger if the wounded neighbor was a clone of the focal plant, but this trend did not hold true in a subsequent year. Removal of root contact did not affect plant-plant signaling, but removal of air contact by bagging did, suggesting a volatile signal. In feeding trials, a generalist herbivore (tussock moth *Orgya vetusta*) gained weight less rapidly due to decreased leaf consumption and also decreased conversion efficiency when fed on signal-induced willow compared to a control. The signal-induced defense in willow appeared to be most effective against caterpillars that showed the highest growth rate in an independent feeding trial. We found clear evidence that plant-plant signals modify defense against herbivores in willows, but the efficacy of this signaling system depended both on the plant involved (self or non-self) as well as the herbivore.

### Evolution, genomics, and transcriptomics of chemical ecology and new directions

9:00 AM

201

Selection on male sex pheromone composition contributes to butterfly reproductive isolation • *Illini Room C* 

Paul Bacquet, University of Louvain-la-Neuve Oskar Brattstrom, University of Cambridge Hong-Lei Wang, University of Lund Cerisse Allen, University of Montana Christer Lofstedt, University of Lund Paul Brakefield, University of Cambridge Caroline Nieberding, University of Louvain-la-Neuve

Presenter: Caroline Nieberding, University of Louvain-la-Neuve

Natural selection can facilitate diversification by inducing character displacement in mate choice traits that decreases the costs of maladaptive mating between lineages. Although reproductive character displacement (RCD) has been demonstrated in two-taxa case studies, the frequency of this process in nature is still debated. Moreover, studies have focused primarily on visual and acoustic traits, despite the fact that chemical communication is the most common means of species recognition. Using comparative analyses with 32 field-caught *Bicyclus* species, a large, mostly sympatric, butterfly genus, we showed a strong pattern of recurrent RCD for predicted male sex pheromone composition but not for visual mate choice traits. We also compared the male-specific chemical profiles among field-caught populations of four phylogenetically unrelated species of the genus, B. safitza, B. vulgaris, B. smithi and B. anynana. We found different 'pheromonal dialects' among populations that are not genetically isolated yet, which may represent the first stages of a potential speciation event. Our results suggest that RCD is not anecdotal, and that selection for divergence in male sex pheromone composition contributed to reproductive isolation within the Bicyclus genus. We propose that natural selection may target olfactory mate choice traits as a more common sensory modality to ensure reproductive isolation among diverging lineages than previously envisaged.

Bacquet et al (in revision for Nature Communications) Selection on male sex pheromone composition contributes to butterfly reproductive isolation.

Bacquet et al (submitted) Divergence in predicted male sex pheromone composition predates genetic differentiation in four *Bicyclus* butterfly species

### 9:15 AM 202 Avian but also invertebrate predators are drivers of chemical defensive strategies in tenthredinids • Illini Room C

Jean-Luc Boevé, Royal Belgian Institute of Natural Sciences

Tommi Nyman, University of Eastern Finland

Presenter: Jean-Luc Boevé, Royal Belgian Institute of Natural Sciences

Insects are often chemically-defended against predators. The sawfly larvae in the family Tenthredinidae (Hymenoptera) are plant-feeders, and among diverse lifestyles they show at least two distinct defensive strategies: easy bleeding of deterrent hemolymph, and emission of volatiles by ventral glands. Here, we mapped 12 ecological and defensive traits on phylogenetic trees inferred from DNA sequences of over 100 species from all tenthredinid subfamilies, and we then performed 10 phylogenetic correlations as well as an independent contrasts test. The results demonstrate a repeated occurrence of easy bleeding, with easy bleeding and ventral glands being mutually exclusive. None of the significant correlations involves morphological traits enhancing specific visual signals. By contrast, easy bleeding is associated with the absence of defensive body movements and with toxins occurring in the host plant. The independent contrasts test reveals that easy bleeding functions through a combination of attributes, integument mechanical resistance and hemolymph feeding deterrence against ants being negatively correlated at species-level. Our conclusion is that invertebrate predators are as important as avian predators to explain the evolution and astonishing diversity of defensive strategies in tenthredinids.

### 9:30 AM 203 A pungent secretion produced by cuckoo chicks repels predators and may turn brood parasitism into mutualism • Illini Room C

Gregory Röder, University of Neuchâtel, Switzerland Daniela Canestrari, University of Oviedo, Spain Diana Bolopo, University of Valladolid, Spain José M. Marcos, University of Valladolid, Spain Neil Villard, University of Neuchâtel, Switzerland Vittorio Baglione, University of Valladolid, Spain Ted C.J. Turlings, University of Neuchâtel, Switzerland

### Presenter: Gregory Röder, University of Neuchâtel, Switzerland

The great spotted cuckoo (Clamator glandarius L.) is a significant brood parasite of carrion crows (Corvus corone corone L.) in southern Europe. We recently found that in northern Spain, contrary to what is ordinarily known for cuckoo-host interactions, the great spotted cuckoo has no negative impact on average crow fitness. Long-term field monitoring and translocations of nestlings showed that during years of high predator pressure the nests with cuckoo chicks are more successful in completing a reproductive attempt than nests without cuckoos. The explanation for this unexpected effect is a malodorous secretion that cuckoo chicks produce when they are harassed and that may protect the entire brood against predators. To obtain information on the volatiles emitted from the excretion and to determine if these volatiles are indeed repellent to predators, we carried out a series of chemical analyses to identify the dominant volatiles. Based on the GC-MS results we generated a synthetic blend that mimicked the secretion and tested both natural and artificial secretions on model species representative of classic crow nest predators. We will provide details on the chemical analysis and composition of the cuckoo secretion, as well as conclusive evidence that the major volatile chemicals in the secretion are strongly repellent to common predators of the crows. These findings support the notion that, in this

particular case, the production of a repulsive secretion by the cuckoo chicks has turned a normally parasitic interaction into a mutualistic one.

9:45 AM

### 204 Responses of developing seeds of soybean to stink bug (*Nezara viridula*) attack and its effects on insect preference • *Illini Room C*

Jorge A. Zavala, University of Buenos Aires, INBA/CONICET Lucia Barriga, Univeversity of Buenos Aires Francisco M. Dillon, University of Buenos Aires, INBA/CONICET Jesica Barneto, University of Buenos Aires, INBA/CONICET Pedro Sardoy, University of Buenos Aires, INBA/CONICET Carolina Di Santo, University of Buenos Aires, INBA/CONICET Eduardo Pagano, University of Buenos Aires, INBA/CONICET Hugo Chludil, University of Buenos Aires

Presenter: Jorge A. Zavala, University of Buenos Aires, INBA/CONICET

Soybean (*Glycine max*), the most important legume crop in South and North America, is attacked by the southern green stink bug (Nezara viridula L. Hemiptera: Pentatomidae). Temperate regions of Brazil and Argentina and the southern of U.S. states are invaded by stink bugs, which reduce soybean crop yield. Stink bugs can puncture most aboveground plant parts with their piercing-sucking mouthparts but preferentially feed on young developing seeds. Although studies have shown that plants respond to insect damage by up-regulation of defenses, most of the available evidence comes from studies that focused on leaf damage. Here we present the responses of developing seeds to stink bug damage and the effects of plant defenses on insect behavior. While stink bug attack induced early accumulation of the defense hormone jasmonic acid (JA) in seeds, 72 h after attack salicylic acid (SA) accumulation increased and JA decreased. The hormonal changes induced expression and activity of cysteine and trypsin proteases inhibitors, and expression of genes related to synthesis and perception of JA and genes related to isoflavonoid synthesis were up-regulated. Preference choice experiments demonstrated that stink bugs feed on developing seeds with low defense levels. Our results suggest that seeds respond to stink bug attack, inducing chemical defenses regulated by both SA and JA, and insects avoid seeds with induced defenses.

10:00 AM

### 205 Plasticity of olfactory-mediated response of *Caloptilia fraxinella* (Lepidoptera: Gracillariidae) to host plant volatiles • *Illini Room C*

Joelle Lemmen, Tyler Wist, and Maya Evenden, University of Alberta

Presenter: Joelle Lemmen, University of Alberta

Herbivorous insects rely largely on olfactory cues to locate host plants, and specialists are required to locate specific host plants against a wide background of non-hosts. Olfactory modulation in specialists may occur as the timing of host plant location is important for offspring development. *Caloptilia fraxinella* (Lepidoptera: Gracillariidae) is a larval leaf miner of ash trees (*Fraxinus* spp.). Female oviposition is constrained to early spring when ash trees flush. Adult *C. fraxinella* eclose in a state of reproductive diapause, and after a summer aestivation unmated adults overwinter away from ash trees. In the spring, adults mate before orientation to ash trees for oviposition. Treatment with a juvenile hormone analogue terminates reproductive diapause and induces females to produce vitellogenic occytes in the summer and fall. In this study, we test the hypothesis that *C. fraxinella* response to host volatiles (HV) is plastic and is highest in the spring when moths orient to ash trees. We also attempt to understand the mechanisms underlying olfactory plasticity by testing moths under different hormone treatments. Moth response to HV during

different stages of reproductive diapause and activity is assessed with electroantennogram and wind tunnel bioassays. Both peripheral and behavioral responses confirm that moth response to HV is plastic, and that response is highest in reproductively active moths. Hormone treatment also impacts moth response to HV during the period of reproductive diapause, which suggests a role in olfactory perception of HV. This olfactory plasticity increases moth attraction to HV at the most appropriate time for oviposition.

### Mating and ovipositional cues

10:40 AM

### 206

### The ante orbital gland secretions of the ram increases the LH pulsatility in anoestrus ewes. • Illini Room C

Dominique Saffray, Pietro Asproni, Laurent Bougrat, and Alessandro Cozzi, IRSEA

### Presenter: Patrick Pageat, IRSEA

Ewes are sexually active only during a part of the year. Exposure of seasonally anestrous females to sexually active males results in activation of luteinizing hormone (LH) secretion and finally leads to ovulation 48 hours after the beginning of the meeting. This "ram effect" appears to be primarily due to sexual pheromones excreted by the ram. Trying to identify a gland secreting sexual pheromones is essential to have available a reliable source of pheromone able to produce this male effect. In male goats, a comparable male effect has been shown to be induced by secretions from the sebaceous glands located between horns. In rams, the ante-orbital glands are actively secreting during the mating season. To investigate the role of these glands, we studied LH pulsatility in anoestrus ewes exposed to this secretion (test group), no secretion (negative control), and introduction of a ram in the barn (positive control). In the negative control group, one micropulse was detected; in the positive control group 6 between the 7 ewes produced pulses, and all the ewes from the "test-group" produced LH pulses. The test group showed significantly more pulses compared with the negative group (S=-10.5; p<0.05). There is also a significant difference in favor of the positive control when compared to the negative control (S=-10.5; p<0.05).

#### Ovipositional responses of Culex tarsalis to semiochemicals produced by 10:55 AM 207 aquatic taxa in different guilds. • Illini Room C

Adena M. Why and William E. Walton, University of California, Riverside

### Presenter: Adena M. Why, University of California, Riverside

To date, limited work has been done looking at the ovipositional responses of female *Culex tarsalis* to semiochemicals produced by aquatic organisms in different guilds. The relationship between semiochemicals produced by predaceous insects, algivorous fish and predatory/larvivorous fish and their role in oviposition deterrence have only recently begun to be investigated. Previous experiments have shown that female mosquitoes avoid ovipositing in water that contains fish exudates and will instead choose to oviposit in control cups in binary choice trials. Culex tarsalis can experience a high risk of fish and predatory aquatic insect predation, in the larval stage, in their natural habitats and females are believed to have evolved behaviors that reduce these risks by avoiding ovipositing in areas where their offspring would be more prone to predation.

#### Influence of olfactive and gustative experience on orientation preference to 11:10 AM 208 odor in Sesamia nonagrioides • Illini Room C

Christophe Petit, Research Institute for Development (IRD), France, c/o International Centre of Insect Physiology and Ecology (ICIPE), Kenya

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Bruno Le Ru, Research Institute for Development (IRD), France, c/o International Centre of

Insect Physiology and Ecology (ICIPE), Kenya

Myriam Harry, Orsay University c/o National Centre of Scientific Research (CNRS), France Paul-André Catalayud, Research Institute for Development (IRD), France, c/o International Centre of Insect Physiology and Ecology (ICIPE), Kenya

Presenter: Christophe Petit, Research Institute for Development (IRD), France, c/o International Centre of Insect Physiology and Ecology (ICIPE), Kenya

Host fidelity in phytophagous insects can be explained by phenotypic plasticity or genetic transmission. This present study was undertaken to determine the mechanism of host fidelity in Sesamia nonagrioides (Lef.) (Lepidoptera: Noctuidae), a polyphagous species known to be a severe pest of maize in Europe and in West Africa. During two generations, the insects were reared from neonates to adults on a artificial diet enriched with vanillin (vanilla diet), a chemical compound not present in the natural habitat of S. nonagrioides, or not (control diet). Dual choice tests in a Y-tube olfactometer offering odors of control and vanilla diets were conducted to determine the orientation preference of gravid females. The females reared on vanilla diet during one generation did not show any orientation preference between the two diets, whereas, after two generations on the same diet, they showed a significant orientation toward vanilla diet as compared to the control one. This "adaptation" for a new odor in a very short time suggested that host fidelity in *S. nonagrioides* can be explained by phenotypic plasticity.

#### Identification of the aggregation pheromone of the date palm root borer 11:25 AM 209 **Oryctes agamemnon** • Illini Room C

Imen Said, Faculté des Sciences de Gafsa, Tunisia Narjes Hasni, Faculté des Sciences de Gafsa, Tunisia Philippes Couzy, UMR PISC, INRA de Versailles, France Zeineb Abdallah, Faculté des Sciences de Tunis, Tunisia Michel Renou, UMR PISC, INRA de Versailles, France Didier Rochat, UMR PISC, INRA de Versailles, France

### Presenter: Imen Said, Faculté des Sciences de Gafsa, Tunisia

Date palm is one of the most economically important fruit trees in Tunisia (first and third in the world in terms of export value and tonnage of dates, respectively). Oryctes agamemnon has been reported in Tunisia on date palm after an exchange of date palm varieties between Tunisia and the United Arab Emirates. It was transferred massively to a new oasis in infested offshoots and causes serious damage especially on young date palm trees. Our laboratory and field investigations aimed to characterize the chemical communication system of Oryctes agamemnon, in order to develop a mass trapping program to control it. Live males or extracts of males' odors attracted conspecifics in an olfactometer, whereas females only attracted males. Odors from adults feeding on sugarcane were sampled and analyzed by gas chromatography. Chromatograms showed that males emitted a blend of 1) ethyl 4-methyloctanoate, 2) 4-methyloctanoic acid, 3) 4-methyloctanyl acetate, and 4) 4-methyloctanol. Single sensillum recordings demonstrated that compounds 1, 2 and 3

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are detected by specific neurons of the antenna. Olfactometric experiments showed that compounds 1 and 3 attract both sexes of *O. agamemnon*, but females preferred compound 1 and males preferred compound 3. Compound 2 attracted females but repulsed males. Field experiments showed that the mix of compounds 1 and 2 with plant odors was significantly the most attractive but captured more females than males. Our results provide the basis for developing mass trapping to control this pest.

11:40 AM

### **Pheromone mediated mating in the western bean cutworm** *Striacosta albicosta* • *Illini Room C*

Jasmine Farhan, Joanna Konopka, and Jeremy McNeil, University of Western Ontario

### Presenter: Jeremy McNeil, University of Western Ontario

Until recently the geographic distribution of the western bean cutworm (WBC), a serious univoltine pest of beans and corn, was limited to in the western United States. However, in the last decade its range has expanded eastward and the insect is now common in the Great Lakes region of both Canada and the United States. Pheromone traps are useful in detecting the presence/absence of the WBC in recently occupied zones but there is no correlation between trap catch and subsequent infestations. As there was little information on the reproductive biology of the WBC, we undertook studies to examine (i) the effects of age, temperature and humidity on female calling behavior, (ii) the effects of abiotic factors on the periodicity of male captures in pheromone traps, (iii) the effects of age on male mating success, and (iv) jthe effects of male previous mating history on fecundity. The pheromone biology of WBC is significantly different from that of other noctuids studied and we discuss these differences within the context of voltinism and seasonal variability in abiotic factors.

### **Poster Presentations**

### Thursday, July 10

12:00–1:30 РМ • **Р1–Р34** • Illini Room С

### **Aquatic Chemical Ecology**

### P1 The effects of different aquatic predators on mosquito oviposition site choice

Lauren L. Eveland, The University of Mississippi Jason R. Bohenek, The University of Mississippi Alon Silberbush, Texas Tech University William J. Resetarits, The University of Mississippi

### Presenter: Lauren L. Eveland and Jason R. Bohenek, The University of Mississippi

Oviposition site choice (OSC) is crucial to the fitness of organisms which offer little to no parental care. Determining the mechanisms that are used in OSC is critical to understanding the dynamics of how communities are structured. Previous research has found that ovipositing mosquitoes have the ability to detect chemical cues of predators that prey on larval offspring. In this context, we conducted three separate experiments. Each experiment consisted of a paired test with eight pairs of pools. Two experiments investigated whether mosquitoes detect fish kairomones when choosing an oviposition

site. Half of the pools were conditioned with mosquitofish (*Gambusia affinis*) or green sunfish (*Lepomis cyanellus*) cues. Another experiment was preliminary work that tested whether adult amphibian predators (central newts; *Notophthalmus viridescens louisianensis*) can influence OSC of mosquitoes, a previously untested scenario. Mosquito egg rafts were removed daily, reared to fourth instar, and identified to species. We found a species specific response. *Culex restuans* avoided mosquitofish and newt treatments, but not green sunfish treatments. *Culex pipiens* did not show preference for any treatment in any experiment. Our findings display evidence that some species of ovipositing mosquitoes are clearly detecting predator cues (fish kairomones or live newts) to avoid poor quality oviposition sites.

### **Chemical Ecology: Symbionts**

## P2 Prevalence of fungal symbiosis and ergot alkaloids of beach morning glory, *Ipomoea* pes-caprae

Wesley T. Beaulieu, Indiana University Daniel G. Panaccione, West Virginia University Keith Clay, Indiana University

#### Presenter: Keith Clay, Indiana University

Defensive symbioses represent interactions among at least three species (host, symbiont, enemy) where the host gains protection from natural enemies. Recent research has revealed that chemical toxins in plants from multiple plant families are of fungal or bacterial origin. Many species of morning glories (Convolvulaceae) are infected by seed-transmitted, fungal symbionts in the genus *Periglandula* that produce bioactive ergot alkaloids. We surveyed seed collections of the pantropical vine, *Ipomoea pes-caprae* (beach morning glory or railroad vine) and made extensive field collections along the Atlantic and Gulf Coast of Florida, USA. A single seed per collection was ground to a powder, extracted in methanol and ergot alkaloids were quantified using reverse-phase HPLC. We found that 71 of 78 seeds tested contained ergot alkaloids, including 28 of 28 populations sampled in Florida. These data indicate a high prevalence of fungal symbiosis. Further, seeds contained a cocktail or ergot alkaloids with total concentrations of up to 181 ug/g dry weight. There were significantly higher concentrations in populations from Gulf Coast compared to the Atlantic Coast, FL. A more limited collection of leaf samples also revealed ergot alkaloids at approximately 3-fold higher concentrations than in seeds. While no animal assays have yet been conducted, several other morning glory species infected by Periglandula fungal symbionts that produce ergot alkaloids are toxic to grazing animals. The stressful beach habitat of *I. pes-caprae*, vertical transmission of typical *Periglandula* spp. and the symbiont-produced chemistry all suggest that populations are strongly selected to maintain fungal symbiosis.

### P3 Is fruit aroma an adaptation to primate seed dispersal?

Omer Nevo, German Primate Center Eckhard W. Heymann, German Primate Center Manfred Ayasse, University of Ulm

#### Presenter: Omer Nevo, German Primate Center

Many tropical angiosperms rely on frugivores for seed dispersal and thus developed fleshy fruits to attract them. Fruit odor has long been speculated to have evolved to signal ripeness to dispersal vectors-a hypothesis only recently confirmed for bat-dispersed figs. Primates are important seed dispersers in the tropics and possess high olfactory capacities. It is thus likely that fruits dispersed by them developed olfactory signals for ripeness as well. This hypothesis generates 3 predictions: (1) ripe primate-dispersed fruits have an odor distinct from unripe fruits; (2) fruits dispersed by animals less olfactory-dependent do not; (3) this pattern occurs repeatedly, independent of phylogeny. We propose two signaling mechanisms: (i) the signal is presented in the fruit husk; (ii) it is presented in the pulp and requires manipulation/opening. Using mass-spectrometry, we analyzed the odor profiles of intact and opened, ripe and unripe, fruits of four Neotropical species: two monkey and two bird-dispersed. We show that primate-dispersed fruits have relatively high concentrations of complex odor blends that are different between ripe and unripe fruits, either only in the pulp or in both pulp and husk, thus making them suitable for reliably signaling ripeness. In contrast, bird-dispersed fruits produce trace amounts of simple profiles which are not different between ripe and unripe fruits. Given the phylogenetic relations between the model species, this cannot be the result of phylogenetic inertia. Thus, our results support the hypothesis that fruit aroma is an adaptation acquired to signal ripeness to seed dispersing primates.

### Evolution, Genomics, and Transcriptomics of Chemical Ecology

### P4 Divergent evolution in anti-herbivore defenses within species complexes at a single Amazonian site

Maria Jose Endara, University of Utah Alexander Weinhold, European Commission, Joint Research Center, Institute for Reference Materials and Measurements (IRMM) Phyllis Coley, University of Utah Thomas Kursar, University of Utah

Presenter: Maria Jose Endara, University of Utah

The arms race between plants and insect herbivores has been invoked as one of the main mechanism driving trait diversification and coevolution for both groups. A fundamental prediction of this theory is that herbivores are driving the rate of defense evolution in plant species faster than for other traits. We evaluated this hypothesis by investigating two clades of closely related plant species coexisting at a single site in the Peruvian Amazon: *Inga capitata* Desv., and *Inga heterophylla* Willd. species complexes. We compared how these lineages differ in the suite of chemical, biotic, phenological and developmental defenses as compared to non-defensive traits that are related to habitat use and resource acquisition. We also collected insect herbivores feeding on the plants. Our data shows

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that sister lineages within both species complexes are more divergent in anti-herbivore defenses than in other functional traits. Moreover, the assemblages of herbivore communities are dissimilar between the populations of coexisting *I. capitata* lineages. Taken together, these results are consistent with the idea that, for the two species complexes, interactions with their natural enemies may have played a significant role on their phenotypic divergence and potentially in their diversification and coexistence. It also suggests that defensive traits evolve rapidly.

### P5 Investigating the interactome of Helicoverpa zea (corn earworm) on Zea mays (maize)

Andrew J. Englund, Western Illinois University Patrick F. Dowd, USDA ARS NCAUR Richard O. Musser, Western Illinois University

### Presenter: Andrew J. Englund, Western Illinois University

This experiment was designed to examine the interactome of *Helicoverpa zea* (corn earworm) with Zea mays (maize). In order to study these interactions, maize was fed on by the corn earworm for 24 hours. Not only is one interested in the effects of the feeding on the caterpillar, but one is also interested in the effects that the feeding has on the maize. The experiment was set up with having three treatments with the maize: 1) a non-wounded control plant (not fed on by caterpillars), 2) a plant fed on by mock-ablated caterpillars (caterpillars that have undergone surgery but have intact salivary glands), and 3) a plant fed on by ablated caterpillars (caterpillars that have undergone surgery and have had their salivary glands removed). Following the feeding, the leaves of the plant were removed and the tissue was prepared for RNA purification. The caterpillars were also prepared for RNA purification. Following the RNA purification, cDNA was synthesized to be used in quantitative-Real-Time-PCR (qRT-PCR) gene analysis. Both the caterpillar and maize tissue were analyzed by qRT-PCR in order to determine which genes were altered both as a result of the feeding and as a result of the surgery. The primary genes of interest for the maize were various defensive genes, both of the salicylic acid and jasmonic acid pathways. The primary genes of interest for the caterpillars were various digestive genes.

### P6 Investigating the interactome of plant-herbivore interactions

Richard O. Musser, Western Illinois University Linus Gog, University of Illinois at Urbana-Champaign Brittany L. DesRochers, Western Illinois University Charles R. Ward, Western Illinois University Sue M. Hum-Musser, Western Illinois University

Presenter: Richard O. Musser, Western Illinois University

Nicotine is an alkaloid synthesized in the roots of tobacco plants and transferred systematically in the plant in response to insect damage. While at the same time the fate of the caterpillar is dependent on its digestive performance and the ability to overcome toxic components of its diet. Insects use detoxification genes that are known to be inducible by xenobiotics, and are involved in host plant adaptation. The results in this study show how tobacco plants respond to caterpillar herbivory and we can show for the first time how caterpillars respond on a transcriptomic scale to these challenges.

## P7 Investigating the interactome of *Helicoverpa zea* (tomato fruit worm) on *Solanum lycopersicum* (tomato)

Myrtha Pierre, Western Illinois University Heather Osborn, Southern Illinois University

> joint meeting of the **International Society for Chemical Ecology** and the **Chemical Signals in Vertebrates** group

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Sue M. Hum-Musser, Western Illinois University Richard O. Musser, Western Illinois University

### Presenter: Myrtha Pierre, Western Illinois University

We studied the transcriptomic response of tomato plants (Fruits and Leaves) to the herbivory of *Helicoverpa zea* caterpillars. Particularly in relation to the caterpillar's labial saliva. In general the results showed that a variety of plant stress, defense and pathogen related genes were up-regulated by herbivory, while growth and photosynthesis genes were down regulated. The qPCR results confirmed that genes associated with plant defenses such as arginase, dehydrin, polyphenol oxidase D, polyphenol oxidase F, and threonine deaminase were stimulated more so by caterpillars that could secrete labial saliva. We then looked at the transcriptomic response of the caterpillar and found that the caterpillar compensated by stimulating more digestive and detoxification genes.

## P8 Isoprenoid biosynthetic pathway in the male marking pheromone of bumble bees (Bombus s. str.)

Darina Prchalová, Jana Brabcová, Jiří Kindl, Petr Záček, Iva Pichová, and Irena Valterová, Academy of Sciences of the Czech Republic

Presenter: Irena Valterová, Academy of Sciences of the Czech Republic

Bombus terrestris and B. lucorum are closely related species belonging to the subgenus Bombus s. str. Composition of their marking pheromone blend differs significantly. While active components in B. terrestris are mainly isoprenoids (2,3-dihydrofarnesol and geranylcitronellol), B. lucorum males use fatty acid derivatives (ethyl tetradec-9-enoate) for attraction of females. Traces of isoprenoids were detected in B. lucorum, too, but their attractiveness has not been proven. By employing RNA sequencing of the male labial glands (LGs) of B. terrestris and B. lucorum, we identified a complete set of genes from mevalonate pathway in both species. The qRT PCR analysis indicated a significantly lower level of all enzymes from mevalonate pathway in B. lucorum. Detection of acetoacetyl-CoA thiolase activity in LGs of both species and incubation of labelled acetate with dissected LGs indicated biosynthesis of terpenic compounds in B. terrestris only. These in vitro results do not exclude the biosynthesis of small amount of terpenes in vivo. Thus, the lack of active terpenic pheromone components in the B. lucorum blend is a result of significant downregulation of terpene biosynthesis in LG, which may have played a role in the speciation in the subgenus Bombus s.str.

The financial support by the Technology Agency of the Czech Republic (#TA01020969) is gratefully acknowledged.

### **General Session**

## P9 Urinary volatile chemical compound profiles across one year in a male maned wolf (*Chrysocyon brachyurus*)

Marieke Kester, Smithsonian Conservation Biology Institute Zuzy Abdala, George Mason University Nucharin Songsasen, Smithsonian Conservation Biology Institute Tom Huff, George Mason University

Presenter: Zuzy Abdala, George Mason University

The maned wolf (*Chrysocyon brachyurus*) is a neotropical canid native to the grassland habitats of South America. The species is an induced ovulator, a trait thought to be adaptive due to its unique social system (wide-ranging and monogamous, yet solitary).

Evidence suggests male urine, rather than copulation, induces ovulation in this species. Urine samples were collected weekly for one male maned wolf throughout 2013 (n = 47). Headspace volatiles were sampled using solid-phase microextraction (SPME) followed by thermal desorption into a gas chromatograph-mass spectrometer (GCMS). Profiles of urinary volatile chemicals were created for each sample across the year to identify compounds that differed seasonally. Compounds present during breeding season include long-chain fatty acid esters, short and long-chain aldehydes and ketones, as well as hemiterpenoids. The long-term objective is to identify male semiochemicals responsible for priming reproduction in females. Understanding the role that male urinary chemicals play in reproduction can potentially lead to the elucidation of an olfactory mechanism of ovulation in mammals. Findings can be applied to improve captive breeding success to promote a sustainable ex situ population as a safeguard against extinction in the wild.

## P10 Caryophyllene insect-defense signaling induced in maize by the plant growth-promoting rhizobacteria *Azospirillum brasiliense*

Franciele dos Santos, University of São Paulo Maria Fernanda G. V. Peñaflor, University of São Paulo Paul W. Paré, Texas Tech University Patrícia A. Sanches, University of São Paulo Mateus Tonelli, University of São Paulo Cristiane Nardi, Mid-West State University José Mauricio S. Bento, University of São Paulo

Presenter: José Mauricio S. Bento, University of São Paulo

Despite the well characterized role of (E)- $\beta$ -caryophyllene in mediating subterranean tritrophic interactions between plants, herbivores and natural enemies of herbivores, there is little understanding as to what role beneficial soil bacterial may play in plant defense against rhizosphere herbivory. Here we establish that colonization by the plant growthpromoting rhizobacterium (PGPR) *A. brasilense* changes maize root volatile emissions and specifically augments (E)- $\beta$ -caryophyllene. Moreover, *D. speciosa* larvae preferentially orient toward roots of non-inoculated plants and larval performance, as measured by insect weight, is lower when feeding on PGPR-inoculated plants. The role of *A. brasilense* as part of an integrative pest management (IPM) program for maize protection against south american corn rootworm, *D. speciosa* is considered.

#### P11 Strawberry and beetles-pollinators: chemoecological interaction

Laima Blažytė-Čereškienė, Violeta Apšegaitė, Gintarė Eskytė, and Vincas Būda, Nature Research Centre

### Presenter: Laima Blažytė-Čereškienė, Nature Research Centre

There are more than 20 *Fragaria* species. Pollinators of strawberries are not thoroughly studied. The best known are insects visiting flowers of strawberry *Fragaria* × *ananassa* Duchn., and *F. virginiana* Duchn (Chagnon et al. 1993; Ashman, King 2005). There are a few studies on pollinators of wild strawberry *F. vesca* L. and green strawberry *F. viridis* Weston, the species prevailing in Europe. The insect-pollinators visiting the flowers of these species are known (Blažytė-Čereškienė et al., 2012). However, the strawberry flower volatiles playing important role in mutualistic insect-plant interaction have not been studied.

The present study aims to identify *F. vesca* and *F. viridis* flower volatiles that are biologically active to Oedemeridae (*Oedemera virescens* and *O. lurida*) beetles (common insects

visiting strawberry flowers). The GC-EAD analysis of strawberry flower extracts revealed presence of three compounds in *F. viridis* flowers, and two compounds in *F. vesca* flowers, which evoked Oedemeridae beetles' antennal response. Thus, the beetles are capable to distinguish flowers of the two strawberry species. Extracts of strawberry flowers were analyzed by GC-MS. The EAD-active compounds in *F. viridis* were identified as n-nonanal, phenylacetaldehyde, and 1,4-dimethoxybenzene, in *F. vesca* as n-nonanal and phenylacetaldehyde. The role of EAD-active compounds released by strawberry flowers as volatiles playing role in mutualistic insect-plant interaction is discussed.

Ashman T.-L., King E.A. 2005. Am J Bot 92: 891–895

Blažytė-Čereškienė L., Būda V., Bagdonaitė E. 2012. Plant Syst Evol, 298: 819-826

Chagnon M., Gingras J., De Oliveira D. 1993. J Econ Ent 86: 416-420

#### P12 Open Access Centre at the Nature Research Centre in Lithuania

Laima Blažytė-Čereškienė and Vincas Būda, Nature Research Centre

Presenter: Laima Blažytė-Čereškienė, Nature Research Centre

Open Access Centre (OAC) was established in Vilnius, Lithuania in 2013 as a subdivision of the Nature Research Centre (NRC) operating on the principle of open access for both internal and external users.

Material facilities of OAC are suitable for investigations in the fields of Ecotoxicology, Chemical Ecology, Biotaxonomy and Molecular Research, as well as Geosciences. The Ecotoxicologal Researchears deals with toxic and genotoxic effects of toxic substances and pollutants on both macro- and microorganisms as well as cell cultures. Chemoecological issues cover interactions among insects and plants. Identification of plant, animal and microorganism species is the main subjects for the Biotaxonomy and Molecular Research teams. Environmental radioactivity, radioecology, nuclear geophysics, microscopic and chemical composition of natural compounds, paleomagnetic, magnetic and related environmental investigations, as well as ground and water contamination, identification of fossils, rocks and minerals can be studied in the Georesearch facility. OAC provides services for both internal and external users: enterprises and persons looking for innovations, students, interns, external teams of researchers engaged in scientific research activities, teachers, etc. Applications for OAC access is available online in accordance with the established procedure via the NRC website (www.gamtostyrimai.lt).

#### P13 Searching for pheromone of Globodera rostochiensis

Rasa Čepulytė-Rakauskienė, Rita Butkienė, and Vincas Būda, Nature Research Centre

Presenter: Rasa Čepulytė-Rakauskienė, Nature Research Centre

Potato cyst nematode *Globodera rostochiensis* (Wollenweber, 1923) Behrens, 1975 is among the major pests causing losses of potato harvest worldwide. *G. rostochiensis* reproduce sexually and we assume that pheromone could be involved in sex recognition (presence of pheromone is known to at least one plant parasitic nematode species (Jaffe et al., 1989)). Identification of pheromone could be used for nematode behavioral control. Potato root diffusate was prepared: from potato infected by *G. rostochiensis* and not infected (control). Diffusates were extracted with ethyl acetate. Comparative analysis of gas chromatography and mass spectrometry data revealed at least two compounds present in potato roots infected by *G. rostochiensis*. One of these compounds was identified as (*Z*)-9-octadecenamide. The compound till now was known as shrimp aggregation pheromone (Zhang et al., 2011). Data on *G. rostochiensis* male behavioral assay will be presented.

### P14 Phenotypic plasticity in chemical compounds produced on the wings of dry and wet season males of *Bicyclus anynana* butterflies

Emilie Dion, Temasek Lifesciences Laboratories Antonia Monteiro, National University of Singapore Joanne Yew, Temasek Lifesciences Laboratories

### Presenter: Emilie Dion, Temasek Lifesciences Laboratories

Mate recognition through sexual pheromones is a primary driver of divergence in behaviors. In numerous species of insects, the variation in pheromone blends is a major determinant of reproductive isolation and speciation. In the African tropical butterfly *Bicyclus anynana*, both sexes perform courtship. Wet season males court females more avidly than dry season males, and dry season females court males more avidly than wet season females. Currently, it is known that male *B. anynana* use volatile pheromones produced in their wings to attract females but nothing is known about lipid composition variations with seasonal form. We thus aim to identify the type and spatial distribution of cuticular lipids on butterfly wings using two recently introduced mass spectrometry methods, Direct Analysis in Real Time (DART) MS and UV-laser desorption/ ionization MS. Evidence for previously undetected lipids and their spatial localization on the wing and will be presented.

### P15 Living with the enemy: Competition between invasive corals *Tubastraea* spp and the native sponge *Desmapsamma anchorata* at Ilha Grande Bay, Southeast, Brazil

Amanda Silva, Lélis Carlos-Júniora, Cristiano Sato, Bruno Lages, Joana Mara Santos, and Beatriz Fleury, University of Rio de Janeiro State

Presenter: Beatriz Fleury, University of Rio de Janeiro State

Competition is a commonly seen interaction between native and exotic introduced species and it is understood as a fundamental process concerning the invasion establishment success. The corals Tubastraea coccinea and T. tagusensis invaded Brazil in the 80's and their establishment in the region was facilitated by the absence of local predators. The sponge Desmapsamma anchorata is the only organism known to inhibit the two invasive species development (Lages et al. 2012). Our aim was to assess which physical or chemical mechanisms were responsible for the competitive success of *D. anchorata* over the two invaders. Chemical extracts from the sponge and the competing corals were sampled and used against one another. Furthermore, we performed tests of direct interaction by putting the competing species in contact and evaluating whether the growth of *D. ancho*rata was preferentially toward the corals. Also, the first moments of the interaction were observed through microcosm analyses. There were no significant effects of the extracts of Tubastraea spp on the metabolism of D. anchorata, neither was there any observable effects the other way around. During the competition interaction, physical overlapping was the main mechanism used by *D. anchorata* whereas *Tubastraea* spp used mesenteric filaments and polip projection. Although, as shown elsewhere, in a large scale, the possibility of natural control of the invasion by the sponge might not be possible, due to the rapid settlement and growth of Tubastraea spp in the colonisation of new areas, D. anchorata might locally outcompete the two invaders.

### P16 Field trapping of the cork oak borer Coroebus undatus F. (Coleoptera: Buprestidae)

Carmen Quero, Institute of Advanced Chemistry of Catalonia (CSIC), Barcelona, Spain Benjamin Fürstenau, Institute of Biology, Free University Berlin, Germany Josep M<sup>a</sup> Riba, University of Barcelona, Barcelona, Spain

Gloria Rosell, University of Barcelona, Barcelona, Spain Angel Guerrero, Institute of Advanced Chemistry of Catalonia (CSIC), Barcelona, Spain

Presenter: Carmen Quero, Institute of Advanced Chemistry of Catalonia (CSIC), Barcelona, Spain

The flathead oak borer Coroebus undatus F. (Coleoptera: Buprestidae) is one of the main pests of cork oak Quercus suber L. in the Mediterranean region, causing important economic losses to the cork industry. However, in spite of its economic importance, no effective control treatment against this pest has been established so far, mainly because of the bark protection to the larvae. To identify possible visual and/or olfactory cues used by this insect to locate host trees or conspecific individuals, different types of purplecolored traps baited with various semiochemical lures were deployed in infested cork oak forests during the period 2010-2013. Prism traps baited with a mixture of green leaf volatiles from the host caught significantly more adults than when baited with a 3-component blend (nonanal, decanal and geranylacetone) or ethanol, indicating a kairomonal effect of the volatiles. In addition, prism traps were superior in catches to single panel and Lindgren traps. In the experiments all individuals trapped were females. This is the first demonstration of adult captures of the genus Coroebus by an attractant-based trapping method. Purple traps exhibit wavelength and reflectance peak values in the blue light (430-450 nm) and in the red light range (580-630 nm), close to those found in the elytra and dorsal abdomens of both sexes, which suggests also the involvement of visual cues for mate location.

## P17 Temperature and carbon dioxide effect on primary and secondary metabolism of soybean plants

Mariana Closs Salvador, Universidade Estadual de Londrina Jose Perez da Graça, CNPq/Embrapa Soja Mayara S. Gois Tatiana E. Ueda, Universidade Estadual de Londrina Maria Cristina N. de Oliveira, Embrapa Soja José Renato B. Farias, Embrapa Soja Mauricio Ursi Ventura, Universidade Estadual de Londrina Clara Beatriz Hoffmann-Campo, Embrapa Soja

#### Presenter: Clara Beatriz Hoffmann-Campo, Embrapa Soja

Changes in the air temperature as a consequence of increased atmospheric carbon dioxide (CO<sub>2</sub>) may change the composition of primary and secondary plant metabolism. Experiments were carried out to evaluate the effect of temperatures (25° C, 28° C, 31° C and 34° C) and elevated CO<sub>2</sub> (456 ppm) on concentration of phenolic compounds, carbon (C), nitrogen (N), and on C:N ratio and on total sugars in soybean leaves. The trials were performed by using soybean plants grown in growth chambers until the V3 stage. Phenolic compounds were identified by HPLC. The total sugars were obtained by the phenol sulphuric spectrophotometric method, and the C and N contents (%) and C:N ratio were analyzed by GC. Higher CO<sub>2</sub> concentration of phenolic compounds, mainly the isoflavones daidzein and genistein (glucoside and malonyl forms) were observed at 25° C. However, when the temperature increased, the concentration of those compounds decreased. C:N ratio and total sugars increased, and the percentage of N was reduced in plants grown under elevated CO<sub>2</sub>. Our results suggested that elevation in air temperatures and atmospheric CO<sub>2</sub> promote changes in concentration of soybean primary and secondary metabolites which can affect insect performance, mainly by increasing the

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concentration of total sugars and decreasing concentration of phenolics involved in the defense of soybean to herbivores (Piubelli et al., 2005).

Piubelli, G.C.; Hoffmann-Campo, C.B.; Moscardi, F.; Miyakubo, S.H.; Oliveira M.C.N de. Are chemical compounds important for soybean resistance to *Anticarsia gemmatalis*? Journal of Chemical Ecology, New York, v. 31, p. 1509-1525, 2005.

### P18 OsHI-MAPK1 is a key factor regulating rice induced defense and senescence

Jiancai Li, Xiaoli Liu, Qi Wang, Jiayi Huangfu, and Yonggen Lou, Zhejiang University

### Presenter: Jiancai Li, Zhejiang University

Rice (Oryza sativa), one of the most important food crops in world, suffers serious damage from rice brown planthopper (BPH) Nilaparvata lugens (Stål) in Asia. Controlling this pest with environmentally-friendly management has become the most severe challenge to agricultural scientists. OsHI-MAPK1 is a mitogen-activated protein kinase (MAPK) gene whose expression levels were up-regulated following BPH infestation. To explore its function in herbivore-induced defense response in rice, we obtained two transgenic lines (ir-mapk) with knock-down of OsHI-MAPK1. Chemical analysis revealed that after BPH infestation, ir-mapk lines accumulated more ethylene and nitric oxide, two small molecules involved in plant senescence, than WT plants did, which subsequently resulted in poor tolerance of ir-mapk lines to infestation by female BPH adults. Bioassay found that BPH preferred to feed and oviposit on WT plants over ir-mapk lines. Moreover, the hatching rate of BPH eggs laid on ir-mapk lines was only half of that on WT plants. This suggests that OsHI-MAPK1 negatively regulates antibiosis and antixenosis but positively mediates tolerance in rice to BPH. In line with these findings, 225 differently-expressed genes were observed between ir-mapk lines and WT plants when they were infested by female BPH adults, and the up-regulated genes in ir-mapk lines enriched in chitinases, antioxidant activity and defense responses. In summary, our results indicated that OsHI-MAPK1, via modulating at least two phytohormones ethylene and nitric oxide, play a pivotal role in maintaining a trade-off between defense and tolerance in plants to herbivores.

### P19 Immediate effects of nectar robbing by Palestine sunbirds (*Nectarina osea*) on nectar alkaloid concentrations in tree tobacco (*Nicotiana glauca*)

Rainee L. Kaczorowski, University of Haifa—Oranim Avi Koplovich, University of Haifa—Oranim Frank Sporer, Heidelberg University Michael Wink, Heidelberg University Shai Markman, University of Haifa—Oranim

Presenter: Rainee Kaczorowski, University of Haifa–Oranim

Plant secondary metabolites (PSMs), such as alkaloids, are often found in many parts of a plant, including flowers, providing protection to the plant from various types of herbivores or microbes. PSMs are also present in the floral nectar of many species, but typically at lower concentrations than in other parts of the plant. Nectar robbers often damage floral tissue to access the nectar. By doing so, these nectar robbers may initiate an increase of PSMs in the floral nectar. It is often assumed that it takes at least a few hours before the plant demonstrates an increase in PSMs. We addressed the question of whether PSMs in the floral tissue are immediately being released into the floral nectar following nectar robbing. To address this research question, we investigated whether there was an immediate effect of nectar robbing by the Palestine Sunbird (*Nectarinia osea*) on the concentration of nectar alkaloids, nicotine and anabasine, in Tree Tobacco (*Nicotiana glauca*). We found that the concentration of anabasine, but not nicotine, significantly increased

in floral nectar immediately following simulated nectar robbing. These findings suggest that nectar robbers could be ingesting greater amounts of PSMs than they would if they visit flowers legitimately. As a consequence, increased consumption of neurotoxic nectar alkaloids or other PSMs could have negative effects on the nectar robber. Experiments exploring the effects of the increased alkaloid concentrations on Palestine Sunbirds are currently underway and will also be presented.

# P20 Isolation of volatile constituents of cultivated sunflower, *Helianthus annus* L. to understand host selection and resistance mechanisms of sunflower to the red sunflower seed weevil, *Smicronyx fulvus* L.

Charithra Lokumana, North Dakota State University Stephen Foster, North Dakota State University Jarrad Prasifka, United States Department of Agriculture

Presenter: Charithra Lokumana, North Dakota State University

Sunflower, Helianthus annuus is an economically important oilseed crop in the northern Great Plains of the United States. Because the genus Helianthus is native to the Americas, many native insects have co-evolved and become pests of commercially cultivated sunflower (Flath et al. 1985). One insect that causes great economic loss through adult and larval feeding on sunflower heads is the red sunflower seed weevil Smicronyx fulvus L. (Charlet et al. 2009). Recent studies have found that some cultivated sunflower lines have developed resistance to the red sunflower seed weevil (Charlet et al. 2009). The present research was carried out to understand host selection and possible mechanisms of resistance of sunflower germplasm to the red sunflower seed weevil. In the first stage of this research we analyzed the volatile chemicals released by both resistant and susceptible sunflower lines in order to determine whether there were differences in host volatiles that might influence host selection by weevils. Cultivated sunflower lines were grown in a greenhouse and the volatile chemicals released by sunflower heads at R4, R5.5 and R6 flowering stages were collected at three different times of the day. Preliminary behavioral bioassays in the greenhouse were conducted to determine any host selection differences of weevils to resistant and non-resistant germplasm.

### P21 Science Is funny: Increasing scientific communication in the social media age

Jessica Lunt, Avery Scherer, and Kevin Wolfe, TAMU-CC

### Presenter: Jessica Lunt, TAMU-CC

In the age of social media, making information easily accessible and digestible is becoming the norm. As scientists do we have an obligation to keep up, especially as our society becomes less science literate? My fellow graduate students and I are working to use social media to increase scientific communication, both within our field and with the general public. To do so we have created ScienceIsFunny, a YouTube channel and Facebook page that showcases short videos about funny things that happen to us every day in the lab, field, and office. Video contributions are welcome from all areas of science in all over the world. Through these videos, we hope to increase interest in and understanding of science by communicating in a short and easily accessible format that science is not just for the brainy elite. Increased understanding of the scientific process will increase interest in and understanding of scientific results. This increase in public awareness and interest will hopefully lead to increased investment in science by the general public. To this end, we are working toward monetizing our site in order to start a mini-grant program to fund scientific outreach.

### P22 The diverse fragrances of Heliconius butterflies

Florian Mann, Technische Universität Braunschweig Sohini Vanjari, University of Cambridge Jake Morris, University of York Kanchon Dasmahapatra, University of York Chris Jiggins, University of Cambridge Stefan Schulz, Technische Universität Braunschweig

Presenter: Florian Mann, Technische Universität Braunschweig

The neotropical butterfly genus *Heliconius* with its many (sub-)species is a perfect model organism to investigate fundamental questions of evolution and ecology. The many mimicry rings show that wing colour patterns of *Heliconius* butterflies are a major part of the radiation [1], whereas the contribution of pheromones in that process is still unknown. It is known that  $\beta$ -ocimene is a male antiaphrodisiac [2] in *Heliconius* melpomene, which acts as a repellent after pupal mating. This compound is transferred from the male abdominal stink clubs onto the female. Males also possess androconia on the wings. Several observations indicate, that these organs are used to attract females. We investigated the chemistry of the androconia of several species to identify possible pheromones released from them. Our aim is to find geographical and phylogenetic characteristics to understand their radiation process. Extracts of androconia from 16 Heliconius species were investigated by GC-MS analysis. The major compounds besides alkanes with different chain lengths were acetogenins. These can be classified into seven compound classes e.g. alkenes or acetates. The mixture of compounds in these extracts is characteristic for each species. A comparative analysis showed that sympatric mimicking species produce the same compound classes but differ at least on the molecular level. In most cases, one species produces one or two additional compound classes. The same analysis of sympatric non-mimicking species showed no distinct pattern. The differences of the androconia composition within Heliconius might indicate that they are part of the female recognition during the mating process, especially in visually similar species.

[1] K. K. Dasmahapatra et al. Nature 2012, 487, 94-98.

[2] S. Schulz, C. Estrada, S. Yildizhan, M. Boppré, and L. E. Gilbert, J Chem Ecol 2008, 34, 82–93.

#### P23 Open slot

## P24 Bacterial and fungal communities are influenced by fragrance compounds produced by Texas gourd flowers

Cindy Chen, Western New England University Amanda Chung, Western New England University Steven Dixon, Western New England University Uyen Nguyen, Western New England University Erin Noval, Western New England University Kelly Robbins Western New England University Jessica Rocheleau, Western New England University

> joint meeting of the **International Society for Chemical Ecology** and the **Chemical Signals in Vertebrates** group

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Nina Theis, Elms College Dawn Holmes, Western New England University

Presenter: Nina Theis, Elms College

Floral fragrances may have evolved as antimicrobial agents to ward off plant pathogens. In this study, we examined the influence of floral volatiles emitted by Cucurbita pepo subsp. texana on microbial community structure. Molecular techniques were used to analyze communities associated with leaves and flowers collected from 50 different plants. While the leaf bacterial communities consisted almost entirely of species from the order Pseudomondales, the majority of bacterial sequences found on the flowers were from the orders Burkholderiales and Deinococcales. The fungal communities also differed; 8 different fungal genera were associated with leaf tissue, while only a single sequence most similar to Fusarium verticillioides was found on the flowers. These results are particularly interesting because Fusarium verticillioides and many species from the orders Burkholderiales and Deinococcales are known to degrade a wide range of hydrocarbons that are similar to floral volatile compounds. Agar diffusion assays were done to test antimicrobial properties of 7 important fragrance compounds on bacterial and fungal species related to those detected on Texas gourd leaves and flowers. The fungus, Eurotium chevalieri, was most sensitive to these compounds and was inhibited by every compound at concentrations as low as 1.6  $\mu$ M. Fragrance compounds with the greatest antimicrobial properties included linalool, p-anisaldehyde, 1,2,4 trimethoxybenzene, and methyl anthranilate. Floral volatiles that were least inhibitory included a-pinene and (-)- $\beta$ -caryophyllene. These results demonstrate that microbial communities colonizing Texas gourd leaves and flowers differ, and that even at low concentrations the volatile compounds associated with Texas gourd fragrance inhibit growth.

### Molecular Mechanisms of Semiochemical Perception

### P25 Involvement of jasmonic acid In airborne signal perception In tomato plants during plantplant communication

Simon Zebelo and Henry Fadamiro, Auburn University

Presenter: Simon Zebelo, Auburn University

Volatile organic compounds (VOCs) emitted from plants upon insect herbivory act as airborne signals that enhance direct and indirect defenses in remote parts of the same plant or neighboring plants. Various studies have provided compelling evidence that receiver plants are able to respond to volatile cues from conspecific or interspecific emitter plants, by activating defense-signaling pathways (1). Jasmonic acid (JA) is a key regulatory component in defense-signaling pathways (2). However, studies are limited on the role of JA on perception of airborne signals in receiver plants. Here, we tested the hypothesis that VOCs emitted from herbivore damaged tomato plants will trigger defense-signaling pathways on receiver wild-type (WT) plants, but not in jasmonic acid insensitive1-1 (jai1-1) plants. To test this hypothesis, we compared the expression levels of three defense related enzymes (phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and lipoxygenase (LOX)) and quantified transcript levels of several defense-related genes in WT and jai1-1 tomato plants after exposing them to VOCs emitted by the donor WT tomato plants damaged by Spodoptera exigua caterpillars. The selected defense enzymes and genes were expressed in higher amounts in WT tomato plants than in jai1-1 plants. These results suggest the involvement of JA in perception of airborne info-chemicals during plant-plant interactions.

### **Multimodal Communication**

## P26 Relative proportions of VOCs and behavioral responses to conspecific chemicals vary independently of a visual signaling trait in males of four *Sceloporus* lizard species

Diana K. Hews, Indiana State University Jake A. Pruett, Indiana State University Stephanie M. Campos, Indiana University Helena A. Soini, Indiana University Milos V. Novotny, Indiana University Cuauhcihuatl Vital, Universidad Autónoma de Ciudad Juárez México Jose Jaime Zuñiga-Vega, Universidad Nacional Autónoma de México México DF Emília P. Martins, Indiana University

#### Presenter: Diana K. Hews, Indiana State University

In Sceloporus lizards, male blue abdominal patches are ancestral, and white abdomen is derived. In a recent blue-loss species, conspecific chemicals (femoral gland, FG, secretions) elevated display rates over controls, but did not in a closely-related blue species. This suggests greater emphasis on chemical signaling in males of this blue-loss species. Hence, we predicted that other blue-loss *Sceloporus* species would have FG secretions with higher relative proportions of methyl ketones and carboxylic acids, two classes of potentially important volatile organic compounds (VOCs) in birds and reptiles. Using GC-MS to identify VOCs in FG secretions of four *Sceloporus* species (2 blue, 2 white), we found no simple association between color and normalized chromatogram peak areas. In these species we also measured behavioral responses of free-ranging male lizards to conspecific chemicals, predicting stronger responses in blue-loss species when presented swabs with conspecific secretions over clean (control) swabs. After exposure to conspecific swabs, male *cozumelae* (white) significantly increased social display rates, but male siniferus (white) reduced rates. Following exposure to cue swabs, merriami (blue) decreased display rates compared to control swab, but male *parvus* (blue) increase display rates compared control swab exposures. Hence, color does not predict behavioral response to FG secretions. But, in one more closely-related species pair (merriami blue, siniferus white), both species exhibited a visual/chemical tradeoff, increasing chemosensory behaviors but decreasing display rates. Data from a companion study revealed the opposite tradeoff in this species pair: males responded to conspecific male intrusions with decreased chemosensory behaviors and increase displays.

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## P27 No synergism of pheromone and host-plant volatile blends at the peripheral sensory level in the male *Grapholita molesta* (Lepidoptera: Tortricidae)

Byrappa Ammagarahalli and César Gemeno, University of Lleida

Presenter: Byrappa Ammagarahalli, University of Lleida

*Grapholita molesta* (Busck) is a major pest of stone and pome fruit trees that is managed with its sex pheromone, which is composed of a blend of Z8-12:Ac, E8-12:Ac and Z8-12:OH. Plant volatiles synergize the behavioral response of males to the sex pheromone, so single sensillum electrophysiology was used to determine if this synergism is occurring already in the pheromone receptor neurons (ORN's) housed in the antennal trichoid sensilla of males. Z8-12:Ac and E8-12:Ac ORNs did not respond to plant volatiles, and their response to sex pheromone was not affected by the presence of plant volatiles in the blend. We

conclude that the behavioral pheromone-plant synergism does not involve a change in the perception of pheromone by the pheromone ORNs.

### P28 Effects of treatment with a dopaminergic neurotoxin (MPTP) on ovarian development of a tropical damselfish

Muhammad Badruzzaman, University of the Ryukyus

Presenter: Muhammad Badruzzaman, University of the Ryukyus

In the neuroendocrine control system of fish reproduction, dopamine (DA) acts as a gonadotropin inhibitory factor and plays a role in regulating gonadal development of certain species. The present study aimed to examine effect of chemical destruction of dopamine neurons in the brain on dopaminergic activity and gonadal development of a tropical damselfish, Chrysiptera cyanea. Immunohistochemistry using an antibody against tyrosine hydroxylase (TH)-the rate-limiting enzyme of DA synthesis-revealed that population of TH-positive neurons was distributed in the antero-ventral preoptic nucleus of the diencephalon and their fibers terminated in the proximal pars distalis of the pituitary. Treatment of fish with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) at concentrations of 0.02 and 0.2  $\mu$ g/ml for 2 days caused dose dependent decreases in DA, 3,4-dihydroxyphenylacetic acid (DOPAC; DA metabolite), and DOPAC/DA (metabolic rate of DA activity) in the whole brain. These results suggest that MPTP treatment destroys TH-positive neurons in the diencephalon, and lowers DA synthesis and release in the brain. Gonadal conditions of fish with an artificially retracted ovary during the spawning season rapidly restored after MPTP treatment at 0.02  $\mu$ g/ml; compared with the control fish, the MPTP-treated fish had high gonadosomatic index (GSI) and many vitellogenic oocytes in an ovary at 5 days after MPTP treatment. These results suggest that DA in the brain drives ovarian development in this species.

### P29 Mechanisms underlying differences in peripheral olfactory perception between *Drosophila mojavensis* populations

Amber C. Crowley-Gall, University of Cincinnati Priya Date, Yale University John E. Layne, University of Cincinnati Stephanie M. Rollmann, University of Cincinnati

### Presenter: Amber C. Crowley-Gall, University of Cincinnati

Ecological speciation occurs when ecological differences lead to reproductive isolation of two populations through divergent selection forces. This can result from populations shifting to different host plants based on host availability, thereby leading to a shift in adaptive traits and eventual isolation. *Drosophila mojavensis* is a powerful model in which to examine this process because its four populations specialize on four distinct host plants across its range. Here, we examine the proximate mechanisms underlying host specialization by examining the olfactory system of *D. mojavensis* and population differences in electrophysiological responses to the volatile cues used by flies to locate their appropriate host. Significant differences between populations were found in response profiles of odorant receptor neurons to a panel of host cactus specific volatiles. These differences are consistent with gene expression differences in odorant receptor loci between populations.

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### P30 Influence of natal streams on olfactory development in Chinook salmon (*Oncorhynchus tshawytscha*)

Cory L. Ochs, Tina Suntres, Trevor Pitcher, and Barbara Zielinski, University of Windsor

#### Presenter: Cory L. Ochs, University of Windsor

Juvenile Chinook salmon are thought to imprint to stream-specific odors, as olfaction is critical during the spawning migration to natal streams after years in open waters. The specific amino acid composition of streams contributes to these olfactory cues, and causes an excitatory response of the olfactory sensory neurons that project from the nose to the lateral region of the olfactory bulb. An anatomical map of the organization of these projections has been established for mature Chinook salmon, but does not exist for juveniles. Therefore, the neuroanatomical basis for imprinting in juvenile salmon is poorly understood. Our objectives were to create an anatomical map describing the organization of chains of axonal endings of olfactory sensory neurons in the olfactory bulb of juvenile salmon and to experimentally test whether olfactory enrichment using amino acids affects the development of the lateral chains. Immunofluorescence labelling of olfactory sensory neurons from 1- and 2-month-old salmon revealed a stereotyped organization of axon chains similar to those found in adults and were consistent in size across samples. An experiment was conducted to test whether early exposure to amino acids refines the organization of lateral chains of axonal endings by decreasing their surface area with an increase in number (Braubach 2013 J. Neuroscience 33). This predicted increase in neural complexity may provide anatomical evidence that early exposure to stream-specific odors may improve imprinting success rates.

### P31 Sensitivity to cactus odorants in the desert dwelling fly Drosophila mojavensis

Nicole Rhodes, Amber C. Crowley-Gall, Stephanie M. Rollmann, and John E. Layne, University of Cincinnati

### Presenter: Nicole Rhodes, University of Cincinnati

Understanding how reproductive isolation evolves ideally consists of examining the processes that are currently causing divergence of populations of the same species. The fly *Drosophila mojavensis* represents the ideal model for such intraspecific studies. *D. mojavensis* feeds and breeds on different fermenting cactus species across its range, and distinct volatile headspaces of these cacti mediate host plant preferences. The extent to which these differences in host plant preference reflect changes in peripheral odor detection among populations remains unknown. As a first step in addressing this gap in our knowledge, we use single sensillum recordings to measure responses of individual olfactory receptor neurons (ORNs) to known cactus volatiles and characterize the number and odor tuning of individual ORN subtypes present on the antenna. Comparisons are made to known ORN response profiles for other drosophilae.

### **Student Travel Awards**

### P32 Plasticity of secreted saliva in a polyphagous lepidopteran insect and its effect on plant defense responses

Flor Acevedo and Gary W. Felton, The Pennsylvania State University Presenter: Flor Acevedo, The Pennsylvania State University

Polyphagous herbivorous insects need to adapt their physiology when feeding on different host plants. Components of the insect saliva play an important role in mediating plant defense responses. The objective of this project was to determine if the composition of

secreted saliva in herbivorous insects changes when they feed on different host plants and how it affects plant defense responses. We used the polyphagous insect *Spodoptera frugiperda* feeding on artificial diet, tomato, corn and rice as the model system. Plants were challenged with either saliva or salivary gland homogenates from caterpillars reared on diet, tomato, corn or rice plants. The expression of plant defense genes associated with the Jasmonic acid pathway was tested using quantitative real time PCR. Protein profiles of secreted saliva were obtained using SDS polyacrylamide gels. In addition, the activity of the enzyme glucose oxidase (GOX) was tested in labial glands of caterpillars reared on different host plants. Our results show differences in the saliva protein profile and activity of GOX. The highest GOX activity levels were obtained in diet-fed caterpillars followed by rice-fed, while very low activity was detected in the tomato-fed ones. In tomato, saliva from rice-fed caterpillars induced higher expression of the gene proteinase inhibitor 2 (Pin 2), while saliva from tomato-fed caterpillars suppressed the induction of the same gene. We conclude that host plants influence the saliva composition in herbivorous insects, and changes in saliva have a direct effect on the plant response to caterpillar feeding.

### P33 Convergent evolution of jasmonic acid signaling suppression in plant viruses only partially explains viral effects on host plant-insect vector interactions

Simon C. Groen, University of Cambridge and University of Arizona Jack H. Westwood, University of Cambridge Mathew G. Lewsey, University of Cambridge Alex M. Murphy, University of Cambridge Trisna Tungadi, University of Cambridge Anne Bates, University of Cambridge Noah K. Whiteman, University of Arizona Christopher A. Gilligan, University of Cambridge John P. Carr, University of Cambridge

### Presenter: Simon C. Groen, University of Cambridge and University of Arizona

Viruses encode proteins that manipulate hosts to create a favorable niche for virus replication. For insect-transmitted viruses the effects of some of these proteins are often not just restricted to their primary hosts, and may extend to the virus' insect vectors; changing their performance or behavior and often benefitting virus transmission. Previously, we found that cucumber mosaic virus (CMV) and its viral suppressor of RNA silencing (VSR) inhibit host responses to jasmonic acid (JA), a chemical signal regulating resistance to insects. This suggested that VSRs might neutralize aphid resistance by inhibiting JA signaling. We tested the effects of viruses and their VSRs on JA signaling and aphid (Myzus persicae) performance for two aphid-transmitted (CMV, potato virus Y) and two mechanically-transmitted viruses (potato virus X, tobacco mosaic virus) in Nicotiana benthamiana. All viruses and their VSRs inhibited JA signaling, leading us to theorize that VSRs may have evolved convergently to inhibit JA signaling. However, JA-signaling suppression did not always correlate with enhanced aphid performance. Thus, the effects of VSRs on JA signaling are not the sole determinants of virus-induced changes in host-aphid interactions. If convergent evolution of VSRs to suppress JA signaling has occurred, its selection has not been driven solely by aphid-mediated virus transmission. Furthermore, we identified two opposite host-specific outcomes of virus infection on aphid performance (induced susceptibility versus feeding deterrence) that must depend on variations in the interactions between different plant and viral factors. These opposite virus-transmission phenotypes could have important effects on the dynamics of viral epidemics.

### P34 Olfactory sensitivity to steroids in tilapia suggests two distinct receptor mechanisms: detecting a male sex pheromone and a putative social cue from females

Tina Keller-Costa, Adelino V.M. Canário, and Peter C. Hubbard, Algarve Centre of Marince Sciences (CCMAR)

### Presenter: Tina Keller-Costa, Algarve Centre of Marine Sciences (CCMAR)

Sex steroids and their conjugates are potent odorants for several teleosts and released into the water as pheromones, facilitating location and choice of suitable mates, or triggering endocrine changes in conspecifics that prompt gonadal maturation and improve fertility. Dominant male Mozambique tilapia (Oreochromis mossambicus) release a sex pheromone, two pregnanetriol 3-glucuronides, in their urine to stimulate the female reproductive axis. These steroids are potent olfactory stimuli for both sexes. The objective of this study was to explore the sensitivity to other steroids, and assess receptor specificity and diversity using the electro-olfactogram (EOG). In addition to the male tilapia sex pheromone, the olfactory system of O. mossambicus was highly sensitive to other 3-glucuronidated steroids, but did not respond to prostaglandins, unconjugated steroids or 17or 20-conjugated steroids. Stimulation with increasing concentrations of pregnane- and androstane 3-glucuronides or estradiol 3-glucuronide produced characteristic sigmoidal concentration-response (CR) curves. However, the characteristics of CR curves obtained for estradiol- $3\alpha$ -glucuronide were different from the other steroids, with significantly lower apparent EC50 and saturation (Imax) values. EOG cross-adaptation and binary mixture experiments suggested that pregnane- and androstane 3-glucuronides share a common olfactory receptor mechanism, whereas estradiol-3-glucuronide is detected via a distinct olfactory receptor. In conclusion, O. mossambicus has evolved high olfactory sensitivity and specificity to 3-glucuronidated steroids. Apparently two distinct receptor sites are involved, one (tentatively appointed '3G-R I') detecting the male sex pheromone, and a second (tentatively appointed '3G-R II') detecting estradiol 3-glucuronide, a putative chemical signal from females.

### Friday, July 11

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12:00–1:30 РМ • **Р35–Р64** • Illini Room С

### **Applied Chemical Ecology**

### P35 Semiochemical-mediated host preference in Callosobruchus maculatus

Olufunmilayo E. Ajayi, Rammohan R. Balusu, and Henry Y. Fadamiro, Auburn University Presenter: Olufunmilayo E. Ajayi, Auburn University

The cowpea weevil, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae) is a major field-to-store pest of cowpea and other stored legumes worldwide (Jackai and Daoust, 1989). However, little is known about the cues that guide *C. maculatus* to its hosts in the field. The goal of this study was to identify the semiochemicals cues that mediate its host location and preference. First, host preference of *C. maculatus* was studied by comparing attraction of female beetles to different seed legume varieties in four-choice

olfactometer bioassays. Biologically active headspace volatiles were then collected from the preferred hosts and identified using bioassay-guided analytical techniques.

#### References

Jackai, L. E. N. and Daoust, R. A. (1986). Insect pests of cowpea. Annual Review of Entromology 31:95-119.

Philips T. W. (1997). Semiochemicals of Stored-product Insects: Research and Applications. Journal of Stored Products Research 33:17-30.

## P36 Identification of plant-based attractants for yellowmargined leaf beetle, *Microtheca* ochroloma, a pest of crucifer crops

Rammohan Balusu and Henry Fadamiro, Auburn University

Presenter: Rammohan Balusu, Auburn University

The yellowmargined leaf beetle, *Microtheca ochroloma* Stål (Chrysomelidae), is the most damaging pest of organic crucifer production in many parts of the southern United States. Very few organically acceptable control options are currently available for this pest. The ultimate goal of this study was to develop attractant-based strategies for managing *M. ochroloma* in organic and conventional crucifer production systems. First, we studied the beetle's mechanisms of host plant selection and preference among crucifer hosts in laboratory and greenhouse tests. The results showed that certain host plants (turnip and napa cabbage) are highly preferred by the beetle over others (cabbage and collards). Next, the biologically active volatile organic compounds in the headspace of preferred host plant odors were identified by GC-EAD and GC-MS.A blend of three compounds (including anovel isothiocyanate) was later identified as an attractant for *M. ochroloma*. Finally, the results of field trials showed that this three-compound lure can be used as a monitoring tool for *M. ochrolomain* crucifer production.

### P37 Identification of host blends that attract the African invasive fruit fly, *Bactrocera invadens* (Diptera: Tephritidae)

Tibebe Dejene, Swedish University of Agricultural Science Miriam Frida Karlsson, Swedish University of Agricultural Science Ylva Hillbur, International Institute of Tropical Agriculture Emiru Seyoum, Addis Ababa University, Ethiopia Teun Dekker, Swedish University of Agricultural Science

Presenter: Tibebe Dejene, Swedish University of Agricultural Science

*Bactrocera invadens*, an invasive fruit fly species in the Afro-tropical region belonging to the *Bactrocera dorsalis* complex, causes excessive damage in fruit production and productivity. We sought to find attractants from hosts of *B. invadens* that could serve as baits in traps for monitoring and management. Attractiveness of volatiles from four different fruit species (mango, guava, banana and orange) at two stages of ripeness (ripe and unripe) was tested in an olfactometer assay. All the fruits were attractive against a clean air control. Surprisingly, females and males differed in their preference, with orange volatiles being most preferred by females and mango volatiles being most preferred by males. Extensive gas chromatography electroantennographic detection (GC-EAD) and

GC mass spectrometry (GC-MS) were used to identify compounds to which *B. invadens* antennae were sensitive. GC-EAD recordings from distal and medio-central part of antennae showed responses to a large set of odors from each fruit species. Esters dominated the antennal responses. Synthetic blends were made for each fruit species out of the shared antennally active compounds in ratios found in the extracts. In the olfactometer *B. invadens* was most attracted to the banana blend, followed by the orange, mango and guava blends in that order. The synthetic banana blend was as attractive as the extract of banana. The results demonstrate that composing attractive blends from GC-EAD active constituents shared across fruits can be effective in formulating attractive synthetic host mimics for generalist fruit fly species, such as *B. invadens*.

### P38 Oviposition choice of Anopheles gambiae s.l.

Lynda Kirie Eneh, KTH Royal Institute of Technology Gunaratna Kuttuva Rajaro, KTH Royal Institute of Technology Ulrike Fillinger, International Centre for Insect Physiology and Ecology (ICIPE), Mbita, Kenya Anna-Karin Borg Karlson, KTH Royal Institute of Technology Jenny Lindh, KTH Royal Institute of Technology

Presenter: Lynda Kirie Eneh, KTH Royal Institute of Technology

Anopheles gambiae sensu lacto is the principal vector of malaria in Africa. While the blood-feeding behavior of these species have been well studied, very few studies have been performed on the oviposition behavior. We studied the colonization by wild *An. gambiae* s.l. of artificial oviposition sites (ponds) in an open field setting in Mbita, Western Kenya between April and May 2013. The oviposition medium was soil mixed with water that had aged for 4, 8 or 21 days when opened for colonization. The age groups were based on previous studies. Chemical and physical parameters were measured in each pond and water samples were collected for bacterial and volatile profile analysis.

The 4 day old ponds had on average two times more larvae (49±25, mean ±95% CI) than the older age groups (20±12, 24±12, for 8 and 21 days respectively, p = 0.048, ANOVA). Furthermore, the ponds of different age differed in their bacterial and chemical profiles. Further analysis will be needed to determine if these differences can be used to identify oviposition semiochemicals that can be utilized to develop or improve on novel monitoring tools in mosquito attract and kill strategies.

## P39 Attraction of the redbay ambrosia beetle, *Xyleborus glabratus*, to key fungal and host plant odors

Emily Kuhns, Yolani Tribuiani, Xavier Martini, Monique Coy, Jorge Pena, Jiri Hulcr, and Lukasz Stelinski, University of Florida

#### Presenter: Emily Kuhns, University of Florida

The redbay ambrosia beetle, *Xyleborus glabratus*, is an invasive beetle that has become established in the southeastern United States and transmits a fungus, *Raffaelea lauricola*, that causes lethal laurel wilt. Among the susceptible Lauraceae hosts are redbay, *Persea borbonia* and avocado, *Persea americana*. We analyzed the volatile emissions of *R. lauricola* and redbay wood and tested synthetic odor blends as a potential attractants in a natural redbay forest infested with *X. glabratus*. In initial trials, the synthetic *Raffaelea* odor blend was not attractive to the beetles by itself; however, traps baited with the *Raffaelea* odor paired with manuka oil lures captured 56% more beetles on average than manuka lures

alone. Redbay odors consisted significantly of eucalyptol, p-cymene, and  $\alpha$ -pinene. We conducted trapping experiments using subtractive blends and found that eucalyptol was necessary for capturing *X. glabratus*. High release of eucalyptol caught similar numbers of *X. glabratus* when compared to trap capture baited with manuka.

#### P40 Can a plant modify its flowers to reduce risk of herbivory?

Brian M. Worthington and Robert A. Raguso, Cornell University

Presenter: Brian M. Worthington, Cornell University

Conflict may arise within a plant-pollinator mutualism when the pollinator species also plays the role of herbivore at an earlier life stage. In one such system, the hawkmoth Manduca sexta will often oviposit on the leaves of tobacco plants, Nicotiana spp., while foraging. M. sexta caterpillars feed on Nicotiana leaves, incurring fitness costs to the plant. To avoid the linked cost of herbivory while attracting a pollinator, a plant may adjust its strategy to attract alternate pollinators such as hummingbirds. Hawkmoths and hummingbirds pollinate the wild tobacco species Nicotiana sylvestris and Nicotiana langsdorffii respectively, but while hornworm caterpillars are major herbivores, hummingbird pollination is associated with no such cost. In a comparison of these two Nicotiana species, we used oviposition behavior by *M. sexta* to gain insight into the potential role that risk of herbivory plays in the divergence of floral traits, predicting that access to nectar increases oviposition risk. In the case of the hawkmoth pollinated N. sylvestris, oviposition preference was dependent on the life stage of the host plant, with preference shown towards the flowering life stage. However, when given the choice for oviposition on either host species, *M. sexta* show preference for oviposition on flowering *N. langsdorffii*. This was unexpected, but although floral signals play a significant role in pollinator attraction, female *M. sexta* preference may reflect other aspects of nectar reward, or other potentially deterrent phytochemical signals. Preferential oviposition on N. langsdorffii may help us to better understand why divergence in pollination strategy has occurred for this species.

### P41 The use of human sweat metabolites as bait for monitoring vectors of onchocerciasis in West Africa and Latin America

Ryan M. Young, University of South Florida Nathan Burkett-Cadena, University of South Florida Tommy W. McGaha Jr., University of Georgia Bill J. Baker, University of South Florida Thomas R. Unnasch, University of South Florida Andrew J. Shilling, University of South Florida Javier Garza-Hernández, Instituto Politécnico Nacional Mario A. Rodríguez-Pérez, Instituto Politécnico Nacional Laurent Toé, World Health Organization

#### Presenter: Ryan Young, University of South Florida

Onchocerciasis or river blindness is a parasitic disease caused by infection from the nematode *Onchocerca volvulus*. The parasite is transmitted to humans by black fly vectors of the genus *Simulium*. Most of the infections occur in central Africa, with significant incidence also in Central and South America. The World Health Organization (WHO) estimates 18 million people suffer from onchocerciasis. However, it is difficult to accurately access infection due to a prolonged period from infection to symptoms appearing in hosts. The current method for monitoring the spread employs human bait, which is neither optimal nor ethically sound. The need for a new monitoring method is crucial. It was noticed that anthropophilic gravid flies are attracted to human scent. This study will describe our

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efforts to identify key primary metabolites in human sweat. These compounds were taken onsite to breeding areas of *S. ochraceum* and *S. damnosum* in Southern Mexico and West Africa to identify which compounds attracted these vectors of onchocerciasis. This was accomplished through the use of electroantennography and attraction was determined in an olfactometer. The identified compounds will be developed as bait for a field trap for monitoring vector pressure in both Latin America and Africa. In this study we will describe the identification of key human sweat components via GC-MS, the identification of attractive metabolites to the two major species of *Simulium* as well as trap development and bait formulation for the continued monitoring of this important disease vector.

### **Chemical Ecology of Pollination**

#### P42 Effects of root herbivory by Acalymma vittatum on floral volatile emissions in cucumber

Nicholas A. Barber, Northern Illinois University Vanessa Bartolo, Elms College Nelson J. Milano, University of Massachusetts-Amherst Lynn S. Adler, University of Massachusetts-Amherst Nina Theis, Elms College

#### Presenter: Nicholas A. Barber, Northern Illinois University

Insect herbivores can affect plant fitness both the directly through the physiological costs of tissue loss and indirectly by altering plant interactions with other community members. Changes in the release of volatile organic compounds (VOCs) following herbivore damage are well-studied, but considerably less is known about changes in floral VOCs in response to herbivory root herbivory, even though VOCs can mediate pollinator attraction. Here we tested the effects of root herbivory by striped cucumber beetles (Acalymma vittatum) on the fragrance production of flowers from the host cucumber plant (*Cucumis sativus*) and consequences for plant fitness. We manipulated root herbivory using beetle larvae and collected floral VOCs using dynamic headspace sampling. We also measured plant growth, flower and fruit production, pollinator visitation, and leaf herbivory. Root damage reduced plant growth, fruit production, and seed production, but this seems to be due to direct effects rather than changes in pollinator attraction. Although root damage decreased pollinator visitation, this was likely caused by reduced flower production, as there was no significant effect on floral volatile emissions. While root damage treatments did not affect either leaf herbivory or fragrance, leaf damage was positively correlated with fragrance production of benzyl alcohol, the dominant component of the floral volatile blend. Since benzyl alcohol has antimicrobial properties, these data suggest that this compound may be up-regulated during times of herbivory or damage. These aboveground-belowground interactions are significant because they reveal multiple potential pathways by which organisms can influence plant growth and fitness.

# P43 Antennal electrophysiological responses of florivorous flies of the genus *Beebeomyia* (Richardiidae) to floral VOCs of aroids (Araceae)

Artur Campos D. Maia, Universidade Federal de Pernambuco Daniela Maria do Amaral Ferraz Navarro, Universidade Federal de Pernambuco Paulo Milet-Pinheiro, University of Ulm Eduardo Gomes Gonçalves, Universidade Católica de Brasília

Geanne Karla Novais Santos, Universidade Federal de Pernambuco Marcelo Felipe Rodrigues da Silva, Universidade Federal de Pernambuco

#### Presenter: Artur Campos D Maia, Universidade Federal de Pernambuco

Fly larvae of the genus Beebeomyia (Richardiidae) have been observed as avid specialized flower predators of night-blooming aroids (Araceae) pollinated by scent-oriented cyclocephaline scarabs (Scarabaeidae, Cyclocephalini). Over-infested inflorescences of Taccarum ulei and Gearum brasiliense may host hundreds of feeding larvae that lead to reproductive hindrance. Olfactory cues are believed to be used by adult female flies for selecting oviposition sites, however, there is no experimental evidence supporting this assumption. In a comparative approach, we performed electrophysiological analyses with the antennae of two undescribed species of Beebeomyia and floral volatile organic compounds (VOCs) of their preferred hosts. Analyses of gas chromatography coupled with electroantennographic detection (GC-EAD) revealed that the main floral VOCs emitted by inflorescences of T. ulei (2) and G. brasiliense (5) are perceptible to both female and male Beebeomyia flies. Electroantennography (EAG) analysis evidenced detectable responses elicited by floral headspace samples at concentrations as low as 10-11 g. Although the role of the floral VOCs of *T. ulei* and *G. brasiliense* in the attraction of *Beebeomyia* spp. is yet untested in bioassays, our study presents initial evidence that both pollinators and flower predators may detect the same specific olfactory signals and use them to find their preferred hosts, and that given floral VOCs may act as both synomones and kairomones. Already investigated, the dominant compounds in the floral scent of T. ulei, (S)-2-hydroxy-5-methyl-3-hexanone and dihydro- $\beta$ -ionone, are attractive to cyclocephaline scarabs and elicit strong electrophysiological antennal responses of florivorous *Beebeomyia* flies.

#### P44 Chemical ecology of a pollinator-vectored plant pathogen

Scott McArt, University of Massachusetts Cesar Rodriguez-Saona, Rutgers University Matthew Grieshop, Michigan State University Lynn Adler, University of Massachusetts

#### Presenter: Cesar Rodriguez-Saona, Rutgers University

At least 26 plant pathogens are transmitted at flowers by pollinators. Despite the ecological and economic importance of pollinator-vectored plant pathogens, we know surprisingly little regarding how floral traits influence disease transmission. Furthermore, there is an almost complete lack of knowledge regarding plant volatiles that mediate the attraction of vectors in hosts. This lack of information is surprising given the importance of volatile chemistry in attracting pollinators to flowers. Here, we investigate how volatiles of blueberry (Vaccinium corymbosum) influences patterns of transmission of mummy berry disease (Monilinia vaccinii-corymbosi), an economically important pollinator-vectored fungal pathogen. Specifically, we examine how volatile chemistry of infected leaves mimic floral scent. Because pollinators acquire conidia (the infectious phase of the pathogen) at infected leaves and vector this phase to flowers, attraction to infected leaves is an integral component of disease transmission. Our results showed evidence of floral mimicry in the volatiles produced by leaves infected with mummy berry disease. Three volatiles unique to blueberry flowers were produced by infected leaves, and two unique fungal odors (octenone isomers) were produced by infected leaves. The three 'floral' volatiles produced by infected leaves are known to be attractive to blueberry pollinators such as

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honey bees and bumble bees, and octenones are known to be attractive to insects in other fungal systems. We found that flies are the dominant visitors to infected leaves, while bees are the dominant visitors to flowers. These results suggest that blueberry leaves are chemically manipulated by mummy berry in order to increase disease transmission.

# P45 Electrophysiological responses of *Erioscelis emarginata* (Cyclocephalini, Dynastinae) to floral scent volatiles of *Philodendron mello-barretoanum* (Araceae)

G.K.N. Santos, Universidade Federal de Pernambuco A.C.D. Maia, Universidade Federal de Pernambuco M.F.R. Silva, Universidade Federal de Pernambuco P. Milet-Pinheiro, University of Ulm D.M.A.F.N. Navarro, Universidade Federal de Pernambuco

Presenter: Geanne Karla Novais Santos, Universidade Federal de Pernambuco

Philodendron mello-barretoanum (Araceae) is a night-blooming Neotropical aroid pollinated by cyclocephaline scarab beetles of *Erioscelis emarginata* (Scarabaeidae, Cyclocephalini). Like many other beetle-pollinated angiosperms, P. mello-barretoanum exhibits thermogenesis associated with strong odor emission, sheltered floral chambers and sterile nutritive flower tissues used as food resources by the pollinators. Thermogenesis and odor emission are synchronized to pollinator activity, characterizing a highly specialized association. In this work we studied the electrophysiological responses of antennae of E. emarginata to volatiles emitted by inflorescences of P.mello-barretoanum, using gas chromatography combined with flame ionization and electroantennographic detection (GC-EAD). Floral volatiles of P. mello-barretoanum were collected in situ for 20 minutes using dynamic headspace extraction during the pistillate (female) phase of anthesis. GC-MS analysis showed that the bouquet is composed mainly by (Z)-jasmone (62.4%), (Z)-pent-2-en-1-yl-acetate (18.7%), methyl salicylate (3.8%), pent-1-en-3-yl-acetate (2.3%) and yet unidentified compound (11.7%). The lamellate scarab antennas were mounted on glass capillaries containing Ringer solution. Small amounts of paraffin were used to keep the lamellas opened during GC-EAD analysis. (Z)-Pent-2-en-1-yl-acetate and pent-1-en-3-ylacetate elicited strong electrophysiological response from *E. emarginata* antennae. The sensitivity of the antennae of E. emarginata to the compounds present in the floral scent of *P. mello-barretoanum* indicates that these compounds might be used by the pollinator beetles in the identification of host plants. Bioassays are needed to confirm the behavioral activity of these compounds and determine the exact role they play in this interaction.

### **Chemical Ecology of Social Behavior**

#### P46 Sex difference in spatial response to intruder male chemical signals in Sceloporus undulatus

Stephanie M. Campos, Christian King, and Emilia P. Martins, Indiana University, Bloomington

Presenter: Stephanie M. Campos, Indiana University, Bloomington

In many animals, territorial residents have a considerable advantage in aggressive encounters, and in gaining access to mates and other resources. Here we use male *Sceloporus undulatus* lizards to ask whether intruder chemical cues influence a resident male's use of territorial space. Male chemical cues were collected and placed in different male territories repeatedly over several days. We recorded the presence and absence of lizards in each territory, sex and identity of lizards present, and distance of present lizards from the placed chemical cue. Males and females collectively, males only, and resident males only were analyzed in subsets, and in all cases were active more often in territories with intruder chemical cues than in territories to which a control was introduced. Lizards

tended to keep in closer proximity to the chemical cues than to a blank control. This suggests that a resident male is attracted to an area of its territory that has been chemically marked by a male intruder. Our results indicate the chemical modality of communication assists in shaping spatial distributions of territorial individuals, and influences conspecific encroachment on territorial space.

# P47 Can dog appeasing pheromones (D.A.P.<sup>°</sup>) influence the reproductive attractiveness of a female dog?

Michał Dzięcioł, Wroclaw University of Environmental and Life Sciences Theresa L. DePorter, Oakland Veterinary Referral Services Wojciech NIżański, Wroclaw University of Environmental and Life Sciences

Presenter: Michał Dzięcioł, Wroclaw University of Environmental and Life Sciences

Appeasing pheromones are produced in the sebaceous glands of mammary tissue and provide anxiolytic effect on juveniles and adults.1 The aim of the study was to assess if dog appeasing pheromones (D.A.P.) may influence libido and investigative breeding behavior of the stud dog. During this ongoing experiment 20 client-owned breeding animals were assessed (10 female, 10 male). Bitches were not intended to be bred and oestrus was monitored by serum progesterone levels. From oestrus onset, the female wore a treatment collar. Breeders were blinded to the identity of the collar: each female was provided a D.A.P. collar (Adaptil<sup>®</sup>, CEVA) and a similar looking anti-parasitic collar to be used on subsequent cycles. The experienced owner/breeder assessed the stud's reaction to the bitches by observation during daily direct contact. Approaching, sniffing and licking the anovaginal region and mounting attempts were recorded. When the bitches were wearing a D.A.P. collar, seven out of ten males displayed reduced interest and did not try to mount the bitches. In trials with the non-D.A.P. collar, all males were highly interested in mating. The effect may be mediated by simple aversion, reduced attractiveness of the bitches emitting signals associated with nursing, obscuring of semiochemicals of oestrus, or discordant semiochemical signals. Future studies are indicated to explain the mechanism of the males reduced interest in breeding bitches influenced by D.A.P.

<sup>1</sup>Siracusa et al. Effect of a synthetic appeasing pheromone on behavioral, neuroendocrine, immune, and acute-phase perioperative stress responses in dogs. J Am Vet Med Assoc. 2010 (6):673-81

# P48 Pair-specific signals in African wild dogs, *Lycaon pictus*: An example of a potential method to identify signals within complex chemical mixtures

Neil Jordan, Botswana Predator Conservation Trust

Peter J. Apps, Paul G. Allen Family Foundation Laboratory for Wildlife Chemistry, Botswana Predator Conservation Trust

Krystyna Golabek, Botswana Predator Conservation Trust

J. Weldon McNutt, Botswana Predator Conservation Trust

Presenter: Peter J. Apps, Paul G. Allen Family Foundation Laboratory for Wildlife Chemistry, Botswana Predator Conservation Trust

Discriminant function analyses have shown that the tandem over-marks of dominant African wild dogs—where one member of a pair places its urine on top of the urine of its partner—have pair-specific chemical qualities. However, identifying the specific signalling compounds within complex mixtures is a common problem in chemical ecology, and it is not yet known which compounds code for pair in African wild dogs. Here we present an example using data on pair-specificity in wild dog scents, but suggest (and invite you to contest) the following method to determine signature compounds from complex

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mixtures generally. Organic volatiles from 24 dominant urine tandem-marks (four from each of six free-ranging packs in Botswana) were extracted with methanol and separated by gas chromatography on a RtxWax column. Single quadrupole mass spectral data were fed through deconvolution software. To remove intra-pair variance and retain inter-pair variance, log-transformed percentage abundances from one sample from each of the six pairs (n=6) were subjected to a principal component analysis. This analysis was repeated 64 times (each sample being uniquely combined with each other from every pair), and the resulting loadings of each chemical to the principal components were used to determine likely signals of pair-specificity. From initial samples containing hundreds of compounds, this method identified <20 compounds potentially contributing to a pair-specific signal. Synthetic mixtures of subsets of these compounds can now be bioassayed in the field. Are these statistical methods wide of the mark, or a recipe for success?

### P49 The role of chemical cues in reproductive isolation in the house mouse superspecies complex Mus musculus s.lato

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Presenter: Vera Vosnessenskaya, A.N. Severtzov Institute of Ecology and Evolution RAS

Our study aimed to investigate the role of chemical cues in precopulatory reproductive isolation in closely related Mus species. Test subjects were two sympatric species which do not hybridise under natural conditions: house mouse *M. musculus* and mound-building mouse M.spicilegus and mice of M. domesticus group. To monitor plasma testosterone and corticosterone we used ELISA. To visualise activated neurons in vomeronasal organ receptor epithelium (VNO-RE) and in olfactory bulb in response to stimulation, Fosimmunohistochemistry was used. To assess behavior we used: standard two and four preference tests and habituation-dishabituation tests. In all tests individuals of sympatric and allopatric species discriminated con- and heterospecific odors. Both males and females investigated significantly (p<0.01) longer opposite sex urine of conspecifics versus heterospecifics. Males responded to exposure to oestrous conspecific female samples with elevated testosterone level (p<0.01). However, we did not observe plasma testosterone response in males when heterospecific female urine was used. In males of different species, Fos-immunoreactivity was recorded in main olfactory bulb, accessory olfactory bulb and in VNO RE in response to stimulation with urine from receptive females. In M. musculus, in response to stimulation with conspecific receptive female urine we observed Fos-immunoreactivity in apical and bazal zone of VNO. Using the very same design of experiments in *M. spicilegus*, we observed Fos-immunoreactivity in VNO apical zone only. There were no activated cells in VNO-RE in response to stimulation with receptive heterospecific female urine. Our data support the hypothesis that chemical cues play a critical role in reproductive isolation of closely related Mus species.

### Chemical Signals—Analysis and Synthesis

### P50 Toward defining the scope and limitations of the tandem enamine-enal cycloaddition/ pyridine formation approach to the synthesis of the cyclopenta[C]pyridine substructure

Nat Fox, Camelia Milnes, and John Hofferberth, Kenyon College

Presenter: Nat Fox and Camelia Milnes, Kenyon College

Members of a small family of monoterpene alkaloids containing the cyclopenta[C] pyridine substructure have been identified as natural products from a variety of plant, animal and bacterial sources. Actinidine, the first representative natural product of this family to be described (1) is produced by several plant species and is present in the semiochemical repertoire of several insects including stick insects, ants, and beetles. As many of the natural products in this family display interesting biological activities, we were motivated to develop a general synthesis for molecules containing the cyclopenta[C] pyridine substructure. Our method employs easily-prepared linear 1,8-enedial precursors that undergo a tandem enamine-enal cycloaddition / pyridine reaction to produce the target molecules (2). We describe here our progress in defining the scope and limitations of this methodology.

1. Bulletin of the Chemical Society of Japan 1959, 32, 315-316

2. Organic Letters 2010, 12(7), 1408-1411

#### P51 Sex pheromone of the Japanese owl moth, Brahmaea japonica (Bombycoidea: Brahmaeidae)

Akiko Kumanotani, Tokyo University of Agriculture and Technology

Hideshi Naka, Tottori University, Japan

Tetsu Ando, Tokyo University of Agriculture and Technology

Presenter: Akiko Kumanotani, Tokyo University of Agriculture and Technology

Bombycoidea is divided into 10 families, such as Saturniidae, Sphigidae, and Brahmaeidae. Brahmaeidae consists of seven genera and about 40 species in the world, but only one species, Brahmaea japonica, inhabits Japan. The larva is a defoliator of Oleaceae trees and the large adult (about 10 cm) with huge eyespots in wings is called the Japanese owl moth. We were interested in speciation of this species, and compared the sex pheromone with those of other Bombycoidea females. A pheromone gland extract was prepared with three virgin females. GC-EAD analysis with an aliquot of the extract (0.5 female equivalent, FE) found three EAG-active components, I–III, approximately in a ratio of 6:1:3. GC-MS analysis with the extract (0.5 FE) showed characteristic ions of I at m/z 238 (M+) and 220 ([M-18]+), indicating a C16 monoenyl aldehyde. The mass spectra of II and III showed diagnostic ions of C1611,13-dienyl aldehydes at m/z 236 (M+), 95, and 82. With reference to GC-MS data of synthetic compounds, we determined their structures as follows; I = (Z)-11-hexadecenal, II = (11Z,13E)-11,13-hexadecadienal, III = (11Z,13Z)-isomer (Z11,Z13-16:Ald). Synthetic lures were evaluated in the field during late spring in 2013 and 2014. Interestingly, traps baited with only Z11,Z13-16:Ald, one of the minor components, could successfully attract the males. Other two components showed no synergistic effect on the attraction and their roles in matting communication were unclear. Z11,Z13-16:Ald has been identified from some species in Pyraloidea and Noctuoidea but from no species in Bombycoidea, while the (11E,13E)-isomer is a pheromone component of Agrius convolvuli in Sphigidae.

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#### P52 Harmonine-the effective defensive alkaloid of the invasive lady beetle Harmonia axyridis

Nadja Nagel, Max Planck Institute for Chemical Ecology

#### Presenter: Nadja Nagel, Max Planck Institute for Chemical Ecology

The lady beetle family (Coccinellidae) comprises more than 5200 species worldwide which occupy diverse biological habitats. They display a great variety of food preferences, but most of these beetles are carnivorous demonstrating an enormous appetite for aphids, coccids and mites. Therefore lady beetle species like the Asian lady beetle Harmonia axyridis have been used as biological control agent for many decades, but have become an invasive species threatening the biodiversity and altering the native lady beetle assemblage dramatically. The enormous invasive success is most likely to derive from its resistance against miscellaneous pathogens. Recent studies have shown that harmonine ((17-R, 9-Z)-1,17-diaminooctadec-9-ene)-the main defensive compound of H. axyridis-displays antibacterial activity against fast-growing mycobacteria, Mycobacterium tuberculosis and *Plasmodium falciparum.* It also demonstrates multi-stage antimalarial activity (1). With ongoing research more and more properties of harmonine are discovered which raise the interest in easily accessible, synthetic harmonine. Therefore we developed a highly flexible synthesis providing access to racemic harmonine via reductive olefination of a macrocyclic lactone derived from cyclooctanone. By enantioselective saponification of the lactone both enantiomers of harmonine will become available in good yield. Through minor changes of the synthesis, closely related derivatives of harmonine become also available. To better understand the mode of action of lady beetle alkaloids assays with these derivatives are expected to deliver new insights into structure-activity relationships.

1.Roehrich, C. R. et al., Biol. Lett. 2012, 8(2), 308

# P53 Search for electrophysiological test to reveal cereal contamination by microfungi based on EAG of *Plodia interpunctella*

Rita Butkienė, Irena Nedveckytė, Dalė Pečiulytė, Violeta Apšegaitė, Laima Blažytė-Čereškienė, and Vincas Būda, Nature Research Centre

Presenter: Vincas Būda, Nature Research Centre

To ensure food quality, various analyses is used, including those for evaluation of cereal infestation by standard methods for mycotoxin identification. The latter are quite timeconsuming and expensive. Biosensor technologies have recently emerged as valuable candidates for food quality control due to their simplicity of use, low cost, rapidity (Falasconi et al., 2011). Our aim was to establish if antennae of stored product pest *Plodia interpunctella* (Lepidoptera, Pyradilae) could be used as biosensor. It is known, volatiles emitted by cereal attract the moths. Compounds responsible for gravid female attraction for egg laying were revealed (Uechi et al., 2007). However, if the antennae contain sufficient number of olfactory sensilla to evoke EAG amplitudes suitable for registration, remains to be analyzed. As far as we know, there are no data on *P. interpunctella* EAG reactions to emissions from cereal contaminated by fungi.

At the initial step of analyses, we established 4 VOC in grain emissions which evoke EAG responses of *P. interpunctella*. Application of headspace SPME GS-MS revealed those as hexanol, nonanal and (E)-2-decenal, and 1 compound wasn't identified. Data on EAG reactions to cereal contaminated by microfungi from genus *Aspergillus, Fusarium, Penicilium, Alternaria* will be presented.

Uechi K. et al, 2007. J Stored Prod Res 43: 193-201.

Falasconi M. et al., 2011. Int J Electrochem, 1-12.

# P54 Identification of oviposition semiochemicals of the black flies *Simulium vittatum* and *S. damnosum* (Simuliidae: Diptera)

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#### Presenter: Ryan Young, University of South Florida

Many black fly species of the genus *Simulium* exhibit a communal oviposition response, where multiple females deposit eggs on a single substrate and generate a large egg mass. In order to dissect the nature of this response and to identify the semiochemicals responsible, the communal oviposition response of *Simulium vittatum* was investigated using a variety of behavioral bioassays. Freshly laid eggs from colony or blood-fed flies were extracted in hexane and analyzed by GC-MS. The oviposition stimulating factors were identified as 1-pentadecene, 1-tridecene, and cis-9-tetradecen-1-ol in *S. vittatum* and oleyl alcohol and 1-heptadecacene in *S. damnosum*. Compounds identified in the lipophilic extract were purchased or synthesized and evaluated for their ability to induce oviposition. It was determined that contact with eggs or extracts was required to induce a quicker oviposition reponse. This study demonstrates that communal oviposition for gravid *S. vittatum* is induced by non-polar compounds present on the conspecific eggs.

### Forest Insect Chemical Ecology

# P55 Determining the genetic underpinnings of phytochemistry and associated arthropod communities

Hilary Bultman, University of Wisconsin-Madison Liza Holeski, Northern Arizona University Pär Ingvarsson, Umeå Plant Science Center Richard L. Lindroth, University of Wisconsin-Madison

### Presenter: Hilary Bultman, University of Wisconsin-Madison

Community genetics research has shown that different genotypes of plants harbor analogously different arthropod communities. While this pattern has been revealed in multiple plant systems, the genes underlying the pattern have not been identified. To address this void, we have established a genetic mapping population ("WisAsp") of 515 trembling aspen (*Populus tremuloides*) replicated genotypes to identify the genes associated with phenotypic traits (particularly secondary metabolites) that influence arthropods and arthropod community composition. Thus far, we have found substantial genotypic variation in both condensed tannins and phenolic glycosides, two major groups of plant secondary compounds in *Populus* that are known to influence insects. We have also found considerable variation in arthropod community composition across aspen genotypes at

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a nearby common garden with a subset of nine genotypes from WisAsp (PerMANOVA F = 2.02, P < 0.002). Foliar condensed tannin and phenolic glycoside levels helped explain the variation in arthropod communities (PerMANOVA F = 2.22, P < 0.04; F = 1.87, P = 0.069, respectively), indicating that plant secondary metabolites can indeed serve to structure arthropod communities. In the future we will use genome-wide association mapping with 40,000 markers (SNPs) to identify significant associations between aspen genetic regions and condensed tannins, phenolic glycosides, and arthropod communities. This work will help advance the field of community genetics by linking plant genes to dependent arthropod communities, while also examining a possible mechanism for this link, i.e., plant secondary metabolites.

# P56 Evidence of contact pheromones mediating the mating behavior of *Aphrodisium sauteri* (Coleoptera: Cerambycidae)

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Presenter: Longwa Zhang, Agricultural University, China

Aphrodisium sauteri (Coleoptera: Cerambycidae) is one of the most destructive forest pests in China. Currently, large-scale outbreaks of A. sauteri have resulted in unprecedented ecological and economic losses, especially in Mount Huangshan, Mount Sanqingshan, and other famous scenic sites. Here, we provide the first evidence for contact sex pheromones in the mating behavior of A. sauteri. First, we serendipitously observed a male of A. sauteri exhibited mating behavior to human fingers after handling female beetles. We then wiped polypropylene micro-centrifuge tubes over the cuticles of females, and found that males exhibited mating behaviors similar to those observed with contaminated fingers. As expected, male A. sauteri attempted to mate with females killed by freezing, but ignored dead females which had been extracted with hexane. However, treatment of extracted females with the extract restored the activity, and males displayed mating behavior towards polypropylene micro-centrifuge tubes treated with the extract of female elytra. Preliminary gas chromatography-mass spectrometry analysis of cuticular extracts indicated that although the cuticular lipid profiles were similar between the two sexes, at least six hydrocarbons were consistently more abundant in samples from females than in those from males. One or more of these compounds are likely to be components of the female's contact pheromones in A. sauteri.

### **Invasive Species**

# P57 Separation, quantification and attractiveness testing of putative pheromones released by reproductive male round gobies

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Stephen Loeb, University of Windsor Alexander Scott, Centre for Environment, Fisheries and Aquaculture, Weymouth Barbara Zielinski, University of Windsor

#### Presenter: Michelle Farwell, University of Windsor

The round goby (Neogobius melanstomus), native to the Ponto-Caspian region of Eastern Europe, is an invasive species within the Laurentian Great Lakes exhibiting rapid seasonal range expansion throughout several Great Lakes tributaries. Pheromone application is currently being investigated as a potential method of population management for the round goby. Steroids released by reproductive male round gobies may function as pheromones based on odor potency recorded by electro-olfactogram and increased reproductive female activity toward water that previously contained these males. The reproductive males release steroids, including 11-oxo-etiocholanolone (11-O-ETIO), 11-O-ETIO-3-sulfate and 11-O-ETIO-3-glucuronide. Following separation by high-performance liquid chromatography, and enzyme-linked immunosorbent assay, we found that the conditioned water contained levels of 11-O-ETIO and 11-O-ETIO-3-sulfate previously demonstrated to elicit olfactory responses. Non-reproductive females were previously found to be attracted to isolated 11-O-ETIO while reproductive females were not. Subsequent preliminary laboratory experiments examining female attraction to 11-O-ETIO-3-sulfate has been inconclusive, likely due to the use of both reproductive and non-reproductive females and the delivery of 11-O-ETIO-3-sulfate concentrations at or below electro-olfactogram threshold. Further laboratory and field testing are required to determine whether 11-O-ETIO-3-sulfate is a candidate for pheromone trap development.

# P58 Cuticular lipids and mate attraction in the avian parasite *Philornis downsi* (Diptera: Muscidae)

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K. Boroczky, SUNY-ESF

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#### Presenter: S. A. Teale, SUNY-ESF

*Philornis downsi* is a neotropical parasite of bird hatchlings, has been introduced to the Galapagos Archipelago, and is causing high levels of mortality of species of Darwin's finches and other passerines. In particular, *P. downsi*-related mortality has contributed substantially to the IUCN critically endangered status of the mangrove finch and medium tree finch. There is an urgent need to develop environmentally benign management strategies to prevent the extinction of these iconic species. The objective of our research is to identify chemical attractants that can be used to manage *P. downsi*. Given the importance of cuticular lipids in other muscoids, we conducted a GC-MS analysis of cuticular lipids in *P. downsi*. Females and immature males produce at least 50 cuticular lipids with chain lengths in the range of C25-33, are of relatively low volatility, and are mostly saturated hydrocarbons including many mono- and di-methyl branched hydrocarbons. By 5-6 days post-eclosion, males produce at least 64 cuticular lipids in the range of C19-27 including

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many oxygenated compounds. Field assays in the Galapagos using McPhail traps indicate that males are the attractive sex and that the presence of food odors is necessary for attraction. Ongoing work includes isolating food odor components, identifying the source of male attraction, and isolating nest odors (i.e., oviposition attractants).

### Molecular Mechanisms of Semiochemical Perception

# P59 Antennal transcriptome analysis of odorant receptor genes in the red turpentine beetle (RTB), *Dendroctonus valens*

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Presenter: Longwa Zhang, Anhui Agricultural University

The red turpentine beetle (RTB), Dendroctonus valens LeConte (Coleoptera: Curculionidae, Scolytinae), is a destructive invasive coniferous pest and has become the second most important forest pest nationwide in China. D. valens is known to use aggregation pheromones and host volatiles, as well as non-host volatiles, in its host location and in the modulation of mass-attacks on trees. To date, there is no information on the genes underlying olfaction in D. valens. Here, we report the antennal transcriptomes of D. valens from next-generation sequencing, with the goal of identifying the olfactory gene repertoire that is involved in odor detection and odor processing by D. valens. We obtained 51 million reads that were assembled into 61,889 genes, including 39,831 contigs and 22,058 unigenes. In total, we identified 68 novel putative odorant reception genes including 21 transcripts encoding for putative odorant binding proteins (OBP), six chemosensory proteins (CSP), four sensory neuron membrane proteins (SNMP), 22 odorant receptors (OR), four gustatory receptors (GR), three ionotropic receptors (IR), and eight ionotropic glutamate receptors. Predicted protein sequences were compared with counterparts from the beetles Tribolium castaneum, Megacyllene caryae, Ips typographus, Dendroctonus ponderosae, and Agrilus planipennis. The antennal transcriptome described here represents the first study of the repertoire of odor processing genes involved in D. valens. The genes reported here provide a significant addition to the pool of identified olfactory genes in Coleoptera, which might represent novel targets for insect management. The results from our study will also assist with evolutionary analyses of coleopteran olfaction.

#### **Plasticity of Plant Defenses**

#### P60 Corn biological warfare against insects: defenses, communication and growth

Lina Castano-Duque, and Dawn S. Luthe, The Pennsylvania State University

Presenter: Lina Castano-Duque, The Pennsylvania State University

Corn responds to attack by insect herbivores with a suite of natural defenses that vary depending on the organ attacked and the type of insect feeding on the organ. At the onset of insect herbivory in aboveground organs (shoots), belowground organs (roots) are able to produce several messages that can trigger direct defenses like the production of the cysteine protease MIR1-CP, which accumulates in leaves and degrades the peritropic matrix of the attacking caterpillar's midgut. We are studying the response of two corn inbred lines that differ in herbivore susceptibility to aboveground feeding by fall

armyworm (FAW, *Spodoptera frugiperda*) and belowground infestation with the western corn rootworm (WCR, *Diabrotica virgifera virgifera*) using biomechanical, proteomics and gene expression analyses. In the insect resistant genotype Mp708, we found that Mir1-CP transcripts increase in abundance during WCR infestation and the transcripts are at least 50% more abundant in roots than shoots. However, Mir1-CP transcript abundance does not significantly change in either the roots or shoots during FAW feeding. In preliminary experiments with the susceptible genotype Tx601, we observed that FAW feeding decreased the number of fine roots and WCR infestation significantly depleted secondary and nodal roots. In Mp708 WCR caused only minor root damage. In addition, analysis of root and shoot dry biomass showed that Mp708 had lower losses during WCR and FAW infestations compared to Tx601. During WCR feeding of roots, Mp708 lost cortical cells whereas Tx601 lost both cortical and vascular tissues.

# P61 MAPKs modulate chemical defenses against stink bugs (*Nezara viridula*) in developing seeds of soybean

Romina Giacometti and Jorge A. Zavala, INBA CONICET

#### Presenter: Jorge A. Zavala, INBA CONICET

Stink bugs (Nezara viridula) are a key pest in soybean (Glycine max), which attack decreases yield crop. Plant responses to insect attack begin with the recognition of cell injury and oral secretions, triggering mitogen activated protein kinases (MAPK) pathway and inducing defenses against herbivores. Soybean MAPK's role in mediating responses to stink bugs attack remains largely unexplored. In this work we examined early and late MAPKs signaling responses in developing seeds under attack of stink bugs and its involvement in defense regulation. We found that MAPK3, MAPK4 and MAPK6 as well as a MAPKK (MAPKK1) are differentially transcribed in time after insect perception. We analyzed the phosphorylation status and identified soybean MAPK6 as an herbivore and wound-induced kinase, while MAPK3 and MAPK4 were found to be activated after SA and JA treatments. Although two critical genes involved in flavonoid synthesis in seeds, Phe-ammonia lyase (PAL2) and chalcone synthase (CHS7) were up-regulated under all treatments, only stink bug attack induced isoflavone synthase (IFS), an enzyme responsible for the synthesis of daidzein and genistein, which are the main chemical defenses against these insects. These results suggest that stink bugs feeding on developing seeds triggers JA and SA-mediated defenses through a tightly regulated induction of MAPKs transcription and also phosphorylation of a particular MAPK module in a time-dependent fashion.

# P62 Southern green stink bug (*Nezara viridula*) attack alters soybean gene expression related with cell wall proteins in developing seeds

Natalia Ilina, Jesica A. Barneto, and Jorge A. Zavala, INBA CONICET

Presenter: Jorge A. Zavala, INBA CONICET

Plant cell walls are complex and dynamic structures with several functions, mainly being a physical barrier against pathogens and herbivores. Southern green stink bug (*Nezara viridula*) is one of the most important pests of soybean (*Glycine max*) in South America. Plants are constantly challenged by insect attack, thus they have evolved an array of defense mechanisms against herbivores. Cell wall can be an important barrier against insects, such as stink bugs. However seeds cell wall-insect interaction is an unexplored field. Here we studied gene expression of cell wall associated proteins involved in the modification of the cell wall soybean seeds. We proposed that the stink bug attack cause a rearrangement of the cell wall. These changes may be regulated by hormones related to plant defenses such as jasmonic acid (JA) and salicylic acid (SA). To address this question we apply to

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developing seeds JA or SA, allowed stink bugs to feed on seed and analyzed the cell wall transcriptional response. We found that 72h after stink bugs attack increased expansine (EXP), xyloglucan endo-transferase (EXT), pectate lyase (PL) y polygalacturonase (PG) expression. Interestingly, when SA was applied we detected a strong induction for PG, PL, xyloglucan fucosyltransferase (XFT) and xyloglucan endo-transglycosylase (XET) transcripts; while with JA treatment only EXP and XFT were up-regulated. Our results suggest that stink bug attack increases expression of cell wall genes transcription that produce chemical rearrangements regulated by both JA and SA.

# P63 Smelling tropical tree diversity: a survey of constitutive foliar VOCs in two neotropical forests

Gerald F. Schneider, The University of Utah

#### Presenter: Gerald F. Schneider, The University of Utah

A growing number of studies in temperate ecosystems have demonstrated that speciesspecific signaling or cuing is commonplace in trophic interactions mediated by plantproduced volatile organic chemicals (VOCs). In tropical forests, studies have shown strong relationships between foliar secondary metabolite diversity and insect herbivore species richness. However, no studies thus far have examined the constitutive volatile component of this secondary metabolite diversity, which may be crucial in mediating sensory contact and thus determining trajectories of interactions between herbivores and plants in such forests. Using dynamic headspace collection, I sampled the constitutive emissions of 12 species of woody plants at Barro Colorado Island (moist forest) and Parque Nacional San Lorenzo (wet forest), Panama. I sampled both expanding and mature leaves from both adults in the canopy and saplings in the understory. All species produced measurable constitutive emissions, with a trend towards higher emissions in expanding vs. mature leaves and in the canopy vs. the understory. I found considerable intraspecific as well as interspecific diversity in secondary metabolite emissions. These results support the idea that VOCs play a role in mediating the plant-herbivore co-evolutionary arms race in tropical forests.

# P64 The unsuccessful adaptation of *Spodoptera litura* larvae against the defense substance of *Gardenia jasminoides*

M. Shino, H. Naito, T. Aboshi, N. Yoshinaga, R. Nishida, and N. Mori, Kyoto University

#### Presenter: M. Shino, Kyoto University, Japan

The leaves of *Gardenia jasminoides* contain gardenoside, an iridoid glycoside, which could be a defense chemical compound against herbivorous insects. In this study, the detailed defense mechanism of *G. jasminoides* was explored by using *Spodoptera litura* larvae, which are extremely polyphagous but do not feed on *G. jasminoides* leaves under natural conditions. When *S. litura* larvae fed on fresh leaves of *G. jasminoides*, their growth was clearly inhibited, while on preheated leaves they grew as normal. Feeding on an artificial diet enriched with gardenoside aglycones caused similar growth inhibition, suggesting the activity of  $\beta$ -glucosidase in fresh leaves released toxic gardenoside aglycones. We found that  $6\beta$ -hydroxygenipin, one of gardenoside aglycones, decreased when incubated with crude protein extracts from larval midgut tissues. Also the fact that a digestive enzyme activity in *S. litura* larvae fed on leaves significantly decreased suggested the binding activity of the gardenoside aglycone against larval proteins. On the other hand, *S. litura* larvae attempted to overcome the gardenoside aglycone effect

by secreting large amount of  $\beta$ -alanine that can couple and detoxify the gardenoside aglycone. When gardenoside aglycones were incubated with  $\beta$ -alanine, a conjugate was detected at m/z 294 by LCMS analysis. This compound was also contained in frass of *S. litura* larvae fed on leaves, suggesting that  $\beta$ -alanine is actively induced to detoxify and excrete gardenoside aglycones. The efficiency of this detoxifying strategy was addressed by the growth rate of *S. litura* larvae.

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# **Author Index**

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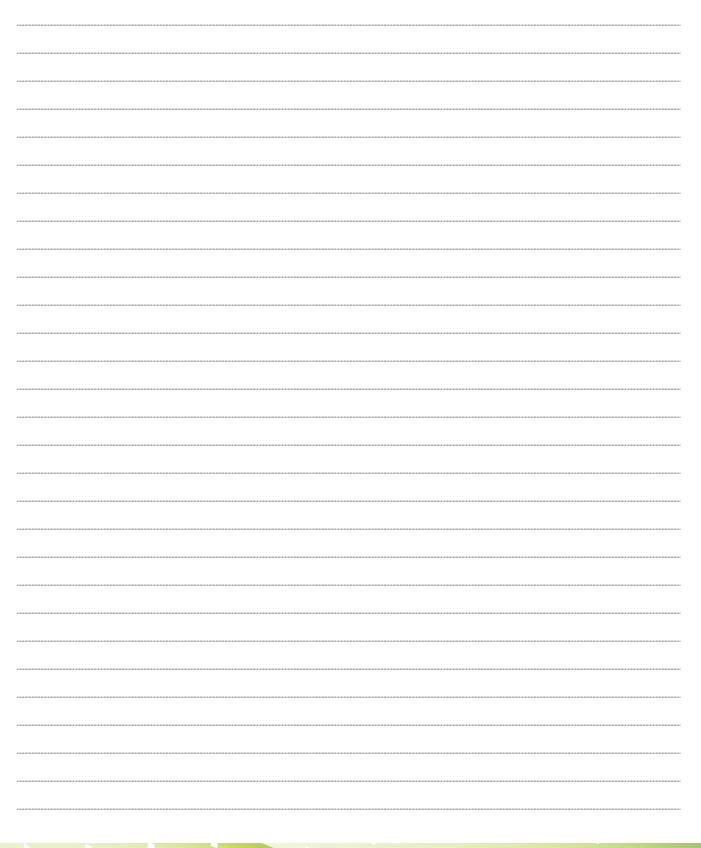
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# Notes

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# Notes



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