

1860
150 ans d'excellence
au service de notre Terre
2010



Gembloux Agro-Bio Tech
Université de Liège



Identification et caractérisation des allergènes d'insectes

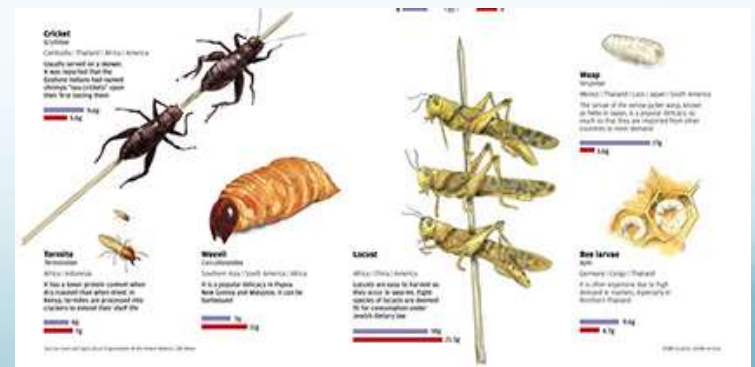
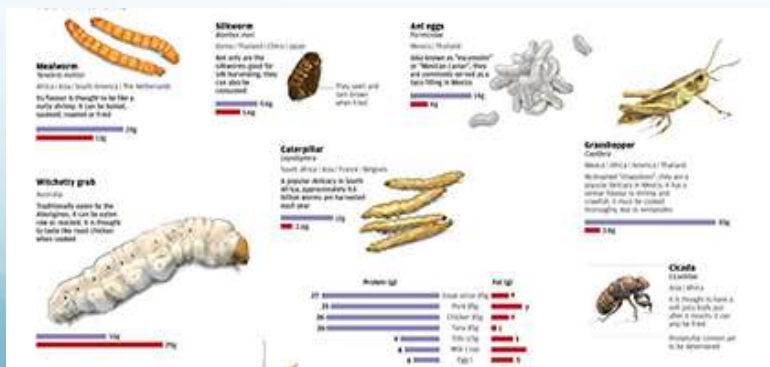
Frédéric FRANCIS

*Gembloux Ago-Bio Tech – Université de Liège,
Entomologie Fonctionnelle et Evolutive*

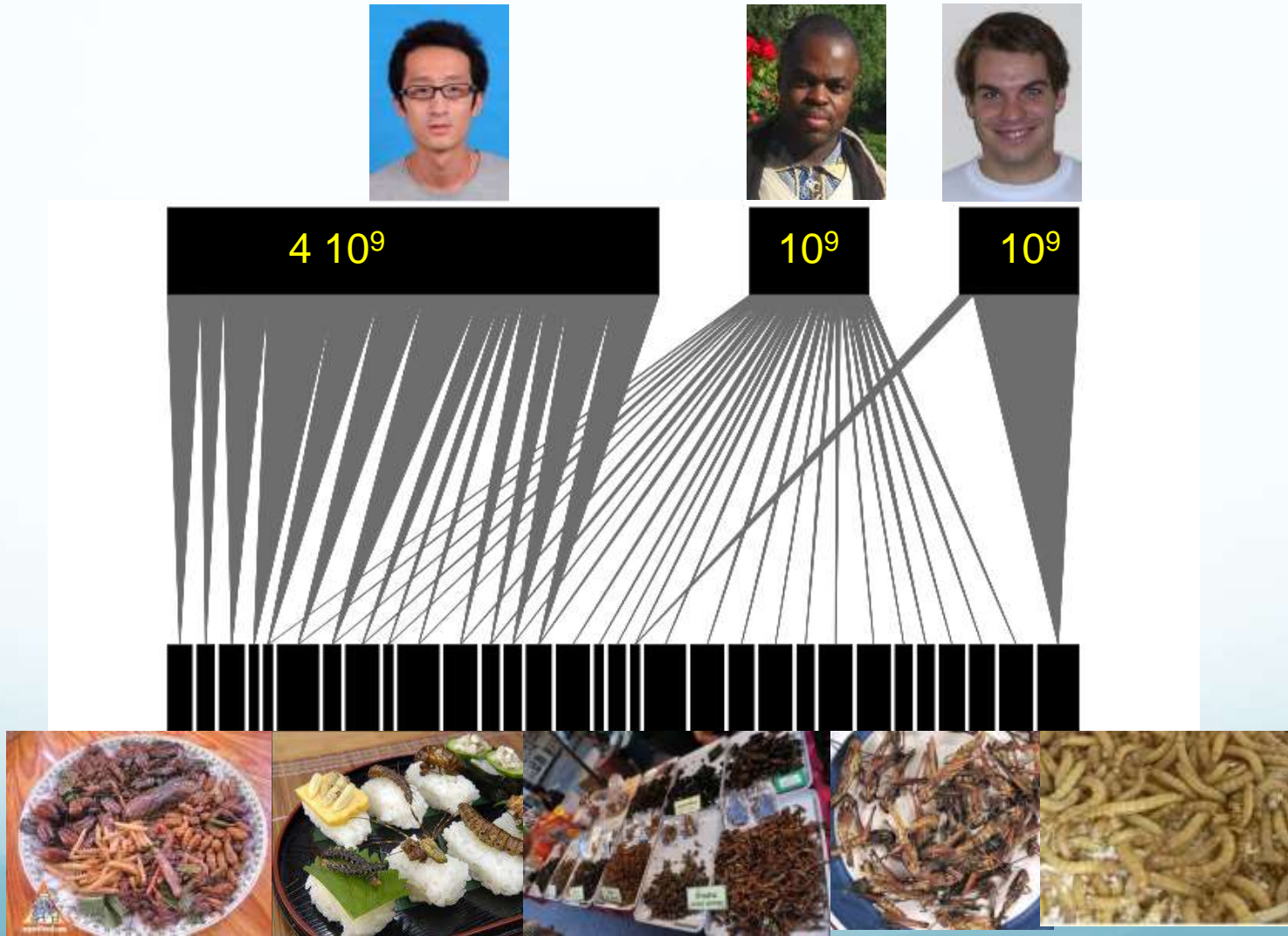
Situation mondiale



Environ 2000 espèces comestibles, large diversité en Afrique et Asie



Foodweb insectes-homme





Edible insects acceptance of Belgian consumer ?

Journal of Sensory Studies



Journal of Sensory Studies ISSN 0887-8250

EDIBLE INSECTS ACCEPTANCE BY BELGIAN CONSUMERS: PROMISING ATTITUDE FOR ENTOMOPHAGY DEVELOPMENT

RUDY CAPARROS MEGIDO^{1,4,5}, LUDOVIC SABLON¹, MÉLODIE GEUENS¹, YVES BROSTAU²,
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FRÉDÉRIC FRANCIS^{1,4}

- 384 visitors were recorded
 - 189 people participated to this study
- 49.2 % of the people!



8 Preparations



Boiled 6.5 min - Baked for 7 min (200°C) -
Baked + Vanilla - Baked + Paprika -
Baked + Chocolate



Boiled 8 min - Baked for 15 min (200°C)



Edible insects acceptance of Belgian consumer ?

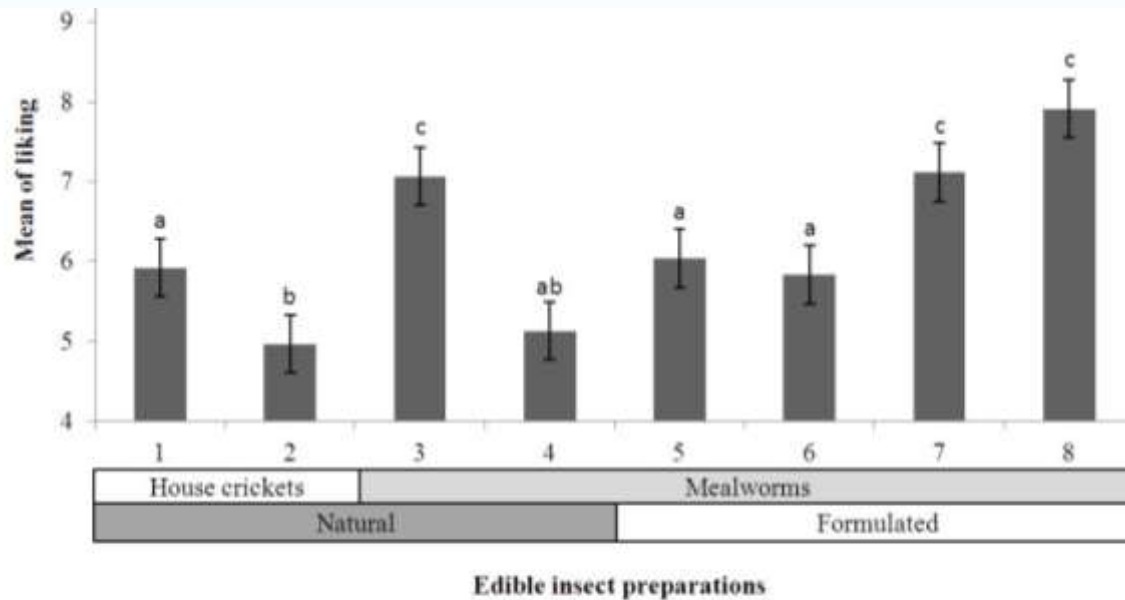


Figure 1: Liking of different meal preparations: (1) house crickets baked; (2) house crickets boiled; (3) mealworms baked; (4) mealworms boiled; (5) crushed mix of both species; (6) mealworms with vanilla; (7) mealworms with paprika; (8) mealworms with chocolate. Different letters show a significant difference for meal preparations at $P < 0.05$ (pairwise comparisons by Tukey's test).

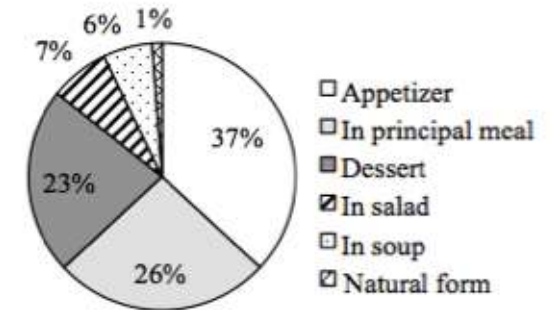


Figure 3: Responses ($n = 97$) to the question: "If yes, in which form would you cook insects?"

Situation en Belgique - 2016

Insectes : feed to food

Du laboratoire à l'échelle industrielle de production : Sixlegs sa



Situation en Europe - 2015

Traditional vs new companies

Insect growers											
Countries	1978	1995	2002	2004	2007	2009	2011	2012	2015	In development	Total
Belgium		1		1				1		2	6
Spain											2
France					1	1	2		1		7
Netherland	1		1			1					7
Total	1	1	1	1	1	2	2	1	1	1	22



Conventional companies (before 2010)

Feed & Food	Food only	Feed only	Total
5	1	1	7

→ **Slow switch to Food - diversification but low volumes**

New companies (after 2010)

Feed & Food	Food only	Feed only	Total
1	5	0	6

→ **Most direct orientation for Food – high perspectives but low certitude**

Situation en Belgique - 2016

Décembre 2013



Federal Agency for the Safety of the Food Chain



Circular concerning the breeding and marketing of insects and insect-based food for human consumption

House cricket	<i>Acheta domesticus</i>
African migratory locust	<i>Locusta migratoria migratorioides</i>
Giant mealworm	<i>Zophobas atratus morio</i>
Mealworm	<i>Tenebrio molitor</i>
Buffalo worm	<i>Alphitobius diaperinus</i>
Wax worm	<i>Galleria mellonella</i>
American desert locust	<i>Schistocerca americana gregaria</i>
Tropical house cricket/banded cricket	<i>Grylloides sigillatus</i>
Lesser Wax Moth Worm	<i>Achroia grisella</i>
Silkworm	<i>Bombyx mori</i>

Novel Food : 2012-2015 pour discussions &
pour application



2018

Situation in Belgium – 2016

4. Conditions for marketing

4.1. Registration of activities

The activity "breeding of insects" is inserted into the activity tree of the FASFC. The activity belongs to primary production; the direct sale of live animals by the producer is considered as an implicit activity of breeding. The operators, who breed insects, must be registered at the FASFC as mentioned under point 4.1.1. The sale of dead insects or the preparation and marketing of insect-based foodstuffs are activities which need to be registered or authorised by the FASFC. Depending on the exercised activities, registrations or authorisations associated with the codes-place-activity-product under points 4.1.2. to 4.1.4. apply. This is a non-exhaustive list with the most relevant activities. If insects are incorporated in special foodstuffs, other activities can apply. In this case, one refers to the activity tree of the FASFC.

4.2. Main principles of food legislation

For the activities 'breeding and placing on the market insects or insect-based foodstuff for human consumption', the general rules of food legislation are in force and inter alia, in particular the application of the good hygiene practices, the traceability, the obligatory notification, the labelling,... and the implementation of HACCP based self-checking system.

Situation in Belgium - 2015

4.3. Specific aspects linked to food safety which must be taken into account for the insects and the insect-based food for human consumption

- Catering waste, food leftovers and non processed animal **by-products cannot be used for feeding insects** (EC) No 1069/2009. .
 - A **heating step** may be needed as a germicidal treatment before the marketing of the product.
 - The products, which are placed on the market, must be tested periodically to detect **pathogenic agents** like *Salmonella*, *Listeria*
- It is necessary to take into account the fact that persons allergic to seafood and/or mite can have an **allergic reaction** after consuming insects.

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Allergens

All things are not allergens and immune response to a substance. People and pets can be allergic to almost any substance. However, there are common allergens that a high percentage of allergy sufferers react to when exposed.

According to published medical reports one out of every five Americans suffer from allergies. These range from mild and get-it-done allergies to pollen allergies and even allergic reactions to such common materials as latex.

According to the U.S. Department of Health & Human Services, millions of Americans also have allergic reactions to food allergens each year. The reactions can range from mild to severe and can even be life-threatening. Hence there are more tests for the type of allergy than the other 8 non-food allergens.

EMSL Analytical, Inc.
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Food Allergen Icons



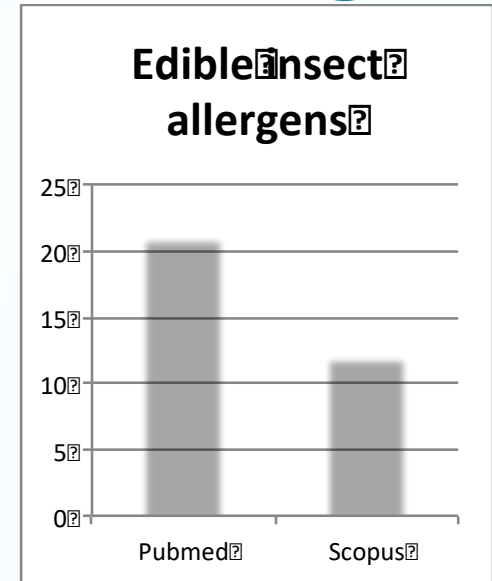
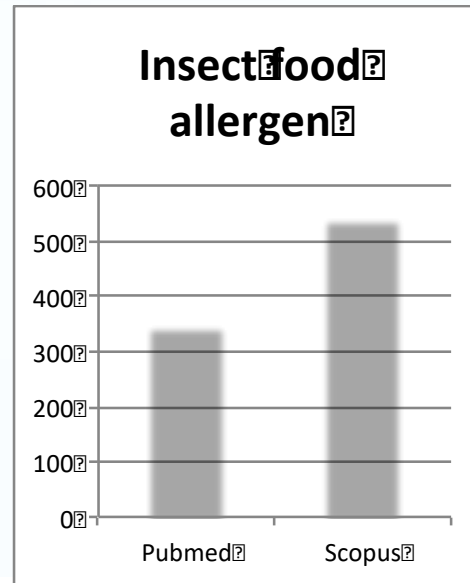
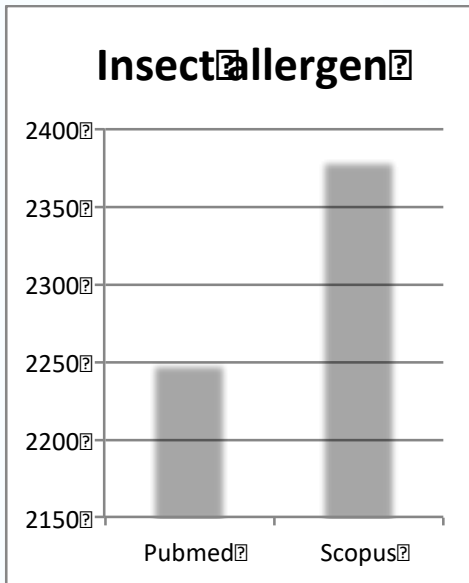
Download from www.dreamstime.com

Top 14 Allergens

All foods containing these allergens must be clearly labelled on menus or signs in your catering outlet



Insectes et allergènes



Annales de la Société entomologique de France (N.S.)
International Journal of Entomology



ISSN: 0037-9271 (Print) 2168-6351 (Online) Journal homepage: <http://www.tandfonline.com/loi/tase20>

Risques et valorisation des insectes dans l'alimentation humaine et animale

Rudy Caparros Megido, Taofic Alabi, Stéphane Larreché, Louxinger Alexandra, Éric Haubruge & Frédéric Francis

Insectes et allergènes

Annales de la Société entomologique de France (N.S.) 19

Tableau 9. Les principales espèces d'insectes causant des allergies par contact, inhalation ou ingestion.

Ordres	Espèces	Origine	Sources
Blattodea	<i>Blattella germanica</i>	Contact	Metz-Favre et al. (2009)
	<i>Periplaneta americana</i>	Contact	Metz-Favre et al. (2009)
Zygentoma	<i>Lepisma saccharina</i>	Contact	Metz-Favre et al. (2009)
Diptera	<i>Calliphora vomitoria</i>		Siracusa et al. (2003)
	<i>Chironomus kiiensis</i>	Contact	Metz-Favre et al. (2009)
	<i>Drosophila melanogaster</i>	Inhalation et Contact	Bernton and Brown (1967) cité dans Phillips and Burkholder (1995), Johnson et al. (1973)
	<i>Lucilia caesar</i>	Inhalation et Contact	Siracusa et al. (1994) cité dans Siracusa et al. (2003)
Lepidoptera	<i>Bombyx mori</i>	Ingestion	Liu et al. (2009); Zhou and Han (2006)
	<i>Chilecomadia moorei</i>	Inhalation et Contact	Siracusa et al. (2003)
	<i>Ephestia elutella</i>	Inhalation et Contact	Johnson et al. (1973)
	<i>Galleria mellonella</i>	Inhalation et Contact	Siracusa et al. (2003)
	<i>Plodia interpunctella</i>	Inhalation et Contact	Bernton and Brown (1967) cité dans Phillips and Burkholder (1995), Johnson et al. (1973)
Coleoptera	<i>Acanthoscelides obtectus</i>	Inhalation et Contact	Hutt (1999) cité dans Van der Brempt and Moneret-Vautrin (2013)
	<i>Rhyzopertha dominica</i>	Inhalation et Contact	Bernton and Brown (1967) cité dans Phillips and Burkholder (1995)
	<i>Sitophilus granarius</i>	Inhalation et Contact	Johnson et al. (1973), Hutt (1999) cité dans Van der Brempt and Moneret-Vautrin (2013)
	<i>Sitophilus oryzae</i>	Inhalation et Contact	Bernton and Brown (1967) cité dans Gorham (1979), Johnson et al. (1973)
	<i>Sitophilus zeamays</i>	Inhalation et Contact	Hutt (1999) cité dans Van der Brempt and Moneret-Vautrin (2013)
	<i>Tenebrio molitor</i>	Ingestion, Inhalation et Contact	Siracusa et al. (2003), Ledent and Mairesse (2006), Van der Brempt and Moneret-Vautrin (2013)
	<i>Tribolium castaneum</i>	Inhalation et Contact	Bernton and Brown (1967) cité dans Phillips and Burkholder (1995)
	<i>Tribolium confusum</i>	Inhalation et Contact	Bernton and Brown (1967) cité dans Phillips and Burkholder (1995), Johnson et al. (1973)
	<i>Zophobas atratus morio</i>	Ingestion, Inhalation et Contact	Freye et al. (1996)

Insectes comestibles et allergènes

Insectes impliqués dans allergies par ingestion

Venins de nymphes de Vespidae et Apidae
 Chrysalides d'*Hyblaea puera*
 Criquets
 Chenilles de *Gonimbrasia belina*
Bombyx mori
Tenebrio molitor et *Zophobas atratus morio*

Sources

Diekema & Reuter (2001)
 Lukiwati (2008)
 Auerswald and Lopata (2005)
 Okezie et al. (2010)
 Ji et al. (2008)
 Freye et al. (1996)

Allergènes identifiés

Espèces

Sources

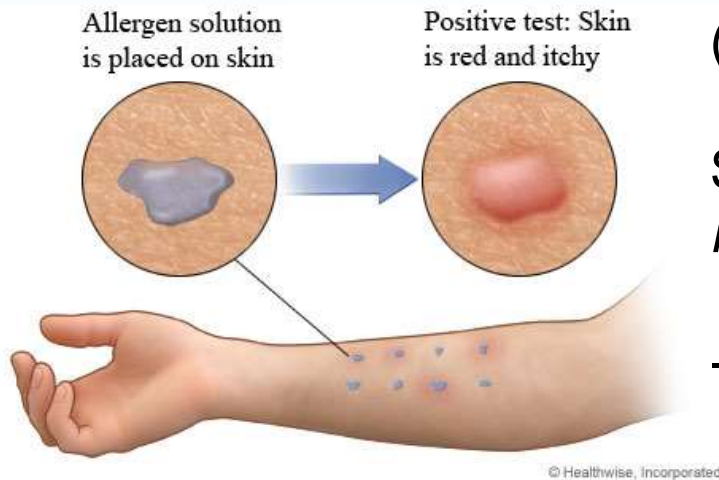
Allergènes identifiés	Espèces	Sources
chitine		Burton & Zaccone (2007), Brinchmann et al. (2011) Phillips & Burkholder (1995), Reese et al. (1999), Metz-Favre et al. (2009)
tropomyosine	Diverses	
alpha-amylase	<i>Tenebrio molitor</i> <i>Plodia interpunctella</i> , <i>Bombyx mori</i> , <i>Blattella germanica</i> , <i>Periplaneta americana</i>	Verhoeckx et al. (2013) Verhoeckx et al. (2013), Ji et al. (2009)
arginine kinase		
additif alimentaire E120	<i>Dactylopius occus</i>	Chung et al. (2001)
hexamerin 1B précurseur	<i>Tenebrio molitor</i> , <i>Zophobas atratus</i>	Van Broekhoven et al. (2015)
myosine musculaire	<i>Alphitobius diaperinus</i>	
hexamerin 1B précurseur	<i>Gryllus bimaculatus</i>	Srinroch et al. (2015)

Insectes comestibles et allergènes

Collaborateurs du laboratoire d'entomologie (GxABT – ULg) (n=31)

Skin prick tests (SPT) avec extraits de *T. molitor* (TM) et *A. domestica* (AD) :

- Poudres délipidées (80 % de protéines)
- Broyats d'insectes grillés (10 min à 200°)

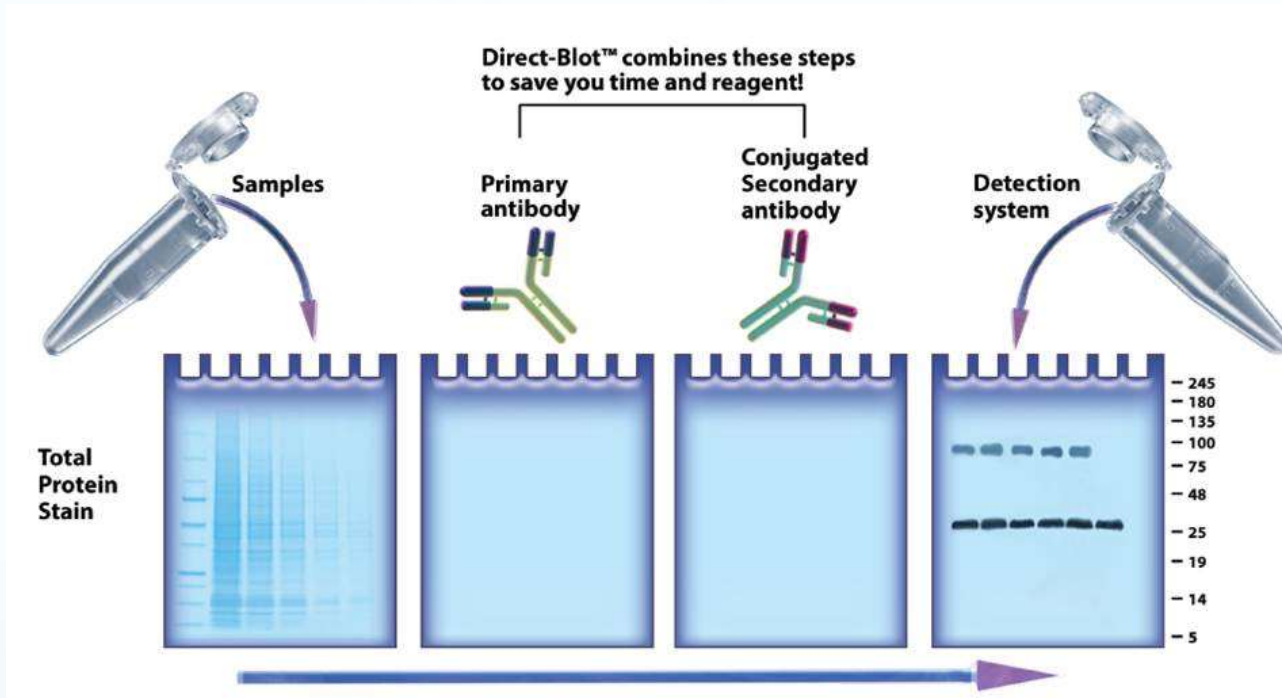


**6 personnes sur 31 (19%) sensibilisées
Equivalent pour poudres délipidées et
broyats – TM et AD**

**Personnes allergiques aux
crustacés/acariens : uniquement réactions
croisées**

European Academy of Allergy and Clinical Immunology
Congress 2016

Insectes comestibles et allergènes

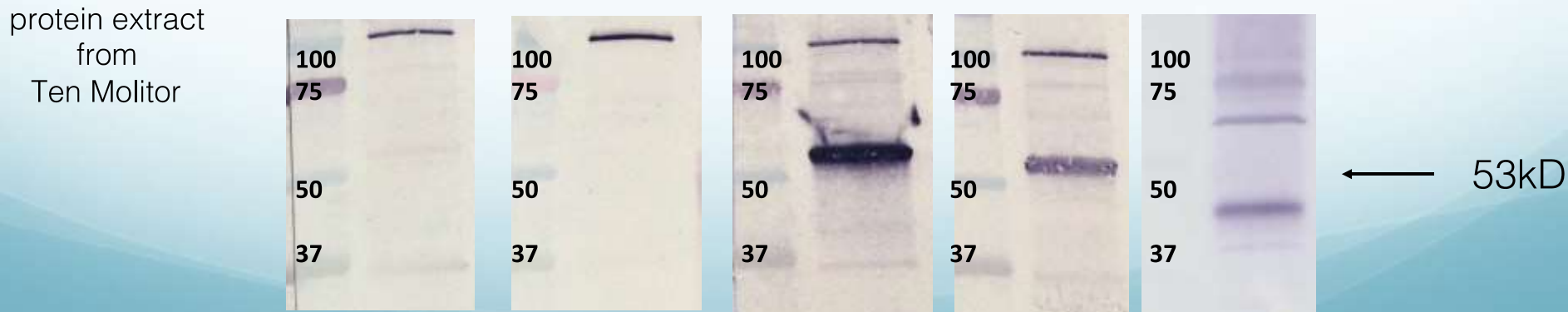
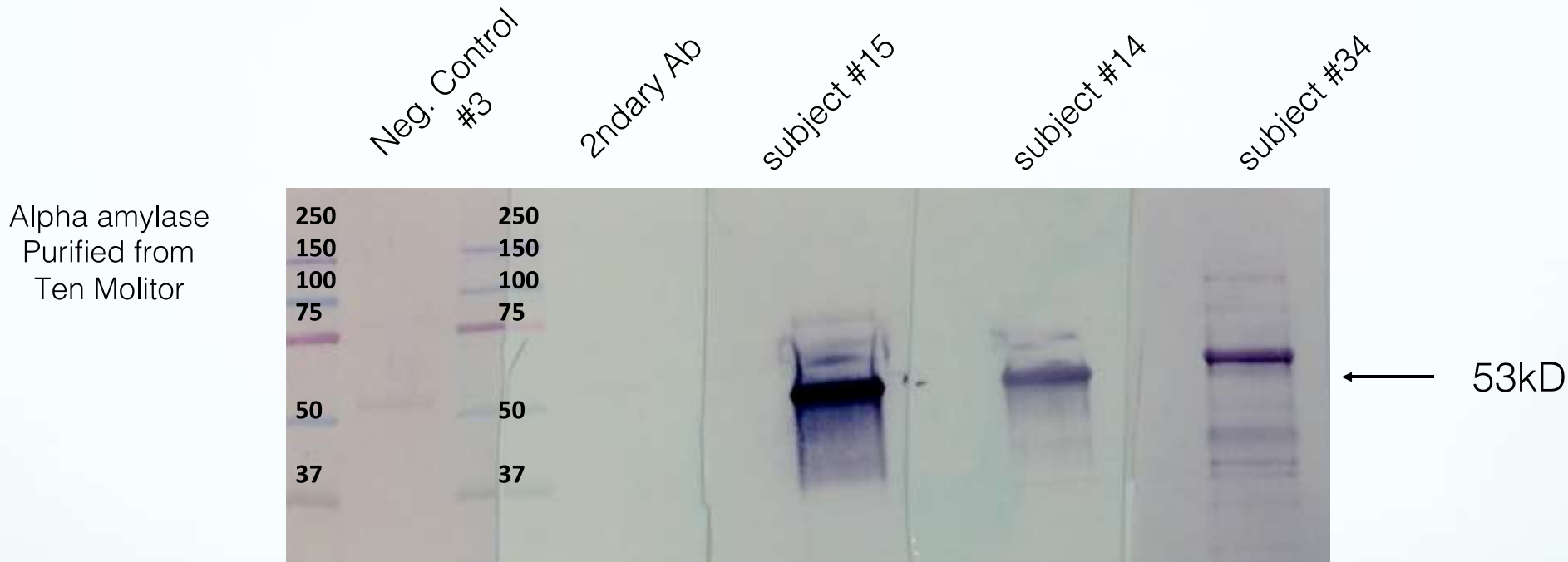


Sélection des séra des collaborateurs positifs et collection de patients CHU Brugman (n = 22)

Western blots avec extraits identiques de *T. molitor* (TM) et *A. domestica* (AD)

A.

Insectes comestibles et allergènes



Insectes comestibles et allergènes

#15

#41

#42

#44

#43

Extr Ten. M

Extr Ach. D

Amylase Ten M

Extr Ten. M

Extr Ach. D

Amylase Ten M

Extr Ten. M

Extr Ach. D

Amylase Ten M

Extr Ten. M

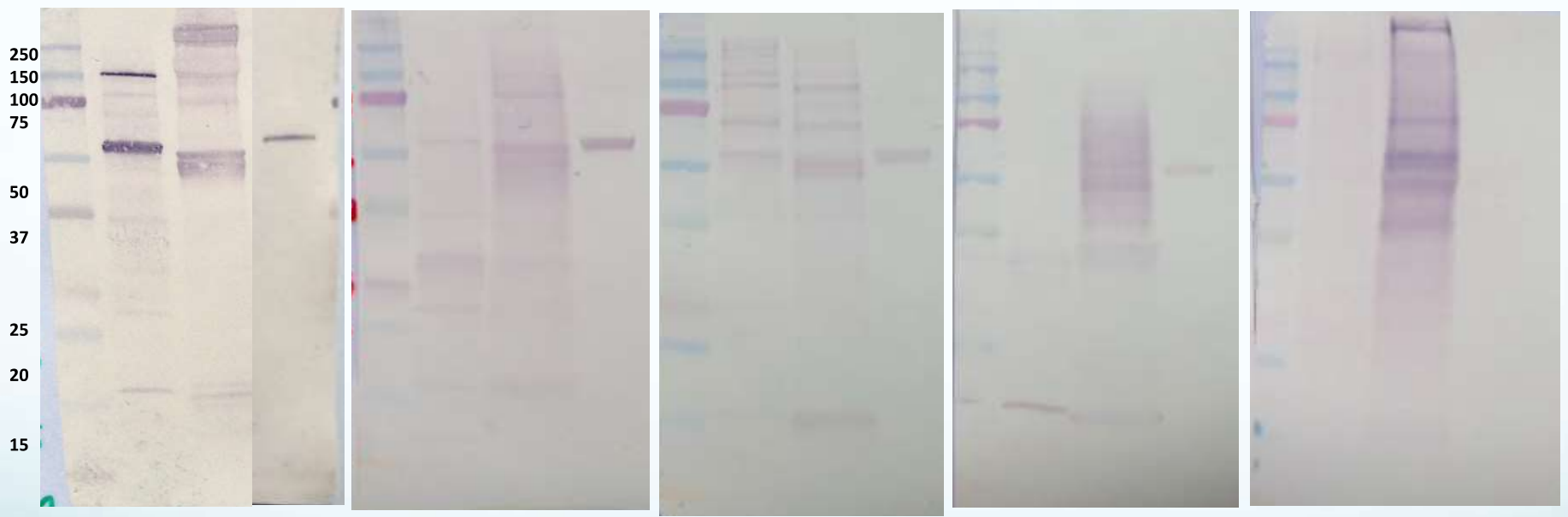
Extr Ach. D

Amylase Ten M

Extr Ten. M

Extr Ach. D

Amylase Ten M



SPT	5mm	4mm	Neg	Neg	Doubt
Clinical symptoms	++	++	+/-	+	+/-

Insectes comestibles et allergènes

Pat 6

Tm Ad

Pat 7

Tm Ad

Pat 8

Tm Ad

Pat 9

Tm Ad

Pat 10

Tm Ad

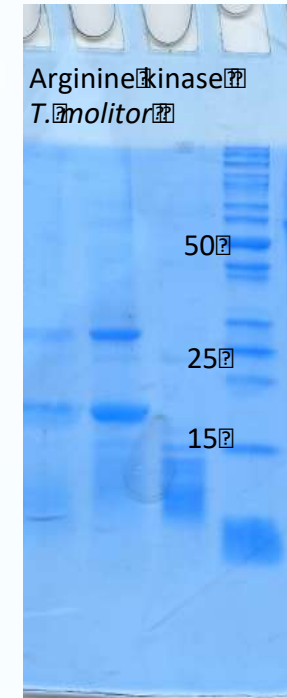
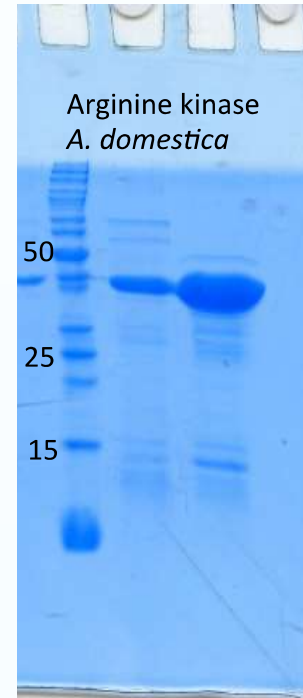


A. domestica : 50 kDa et 15kDa

Insectes comestibles et allergènes

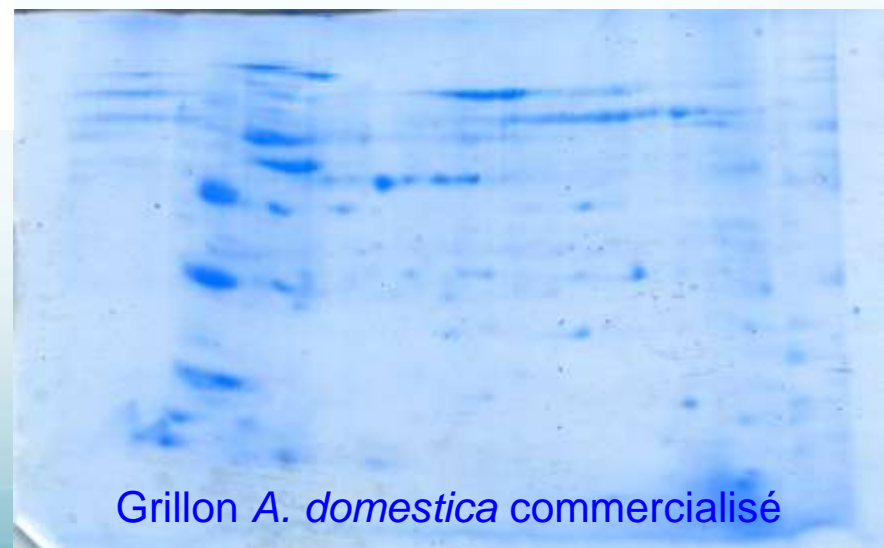
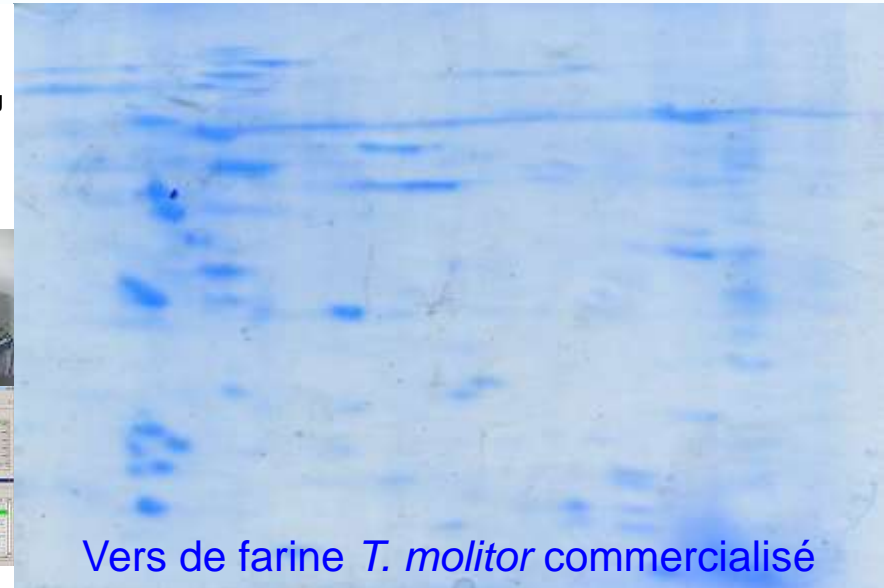
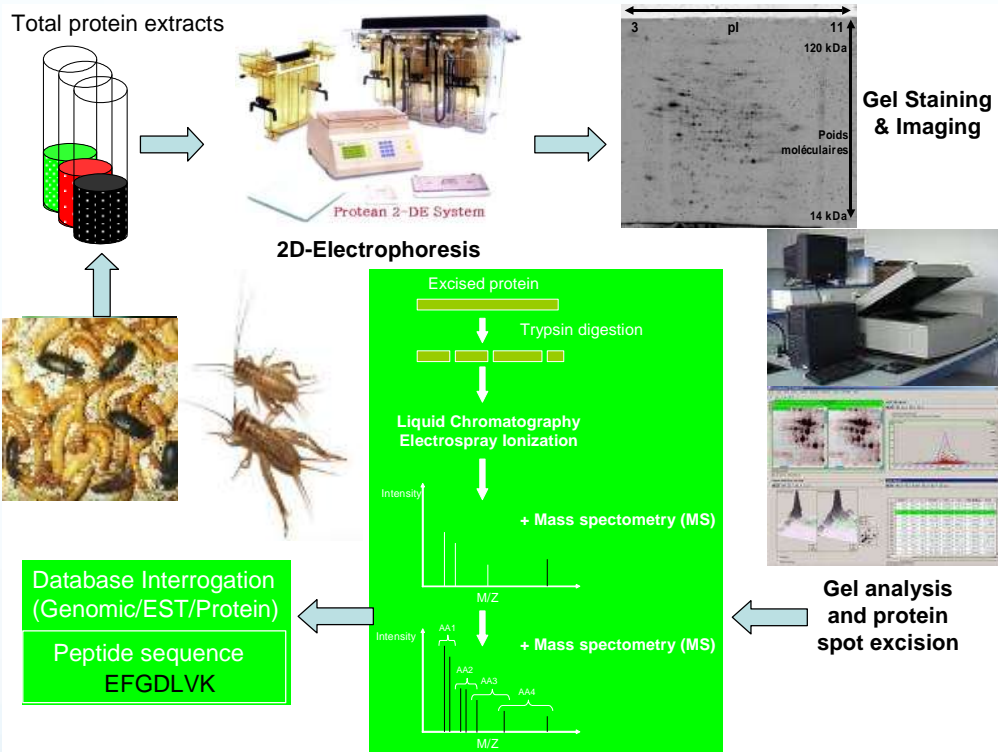


Purification des
arginines kinases
des deux
espèces modèles



Title	Organism	Accession number	Protein MW	pI-Value	Mowse score	MS coverage	Peptide numbers
arginine kinase	Calosoma scrutator	gi 189164042	28556	6.3	108	33	11
muscle M-line assembly protein	Papilio machaon	gi 943950024	186631	6.3	100	16	26
MPA13 allergen-like	Acyrtosiphon pisum	gi 240849091	15355	5.7	32	25	4
arginine kinase	Acromyrmex echinator	gi 332018357	40032	5.9	108	42	21
arginine kinase	Toxonotus cornutus	gi 228014792	18838	8.2	30	23	4
MAP/microtubule affinity-regulating kinase 14-3-3 zeta, partial	Tribolium castaneum	gi 1004401433	11138	9.7	36	45	5
28 kDa desiccation stress protein	Apis cerana	gi 315623762	11167	4.7	62	65	6
28 kDa desiccation stress protein	Tenebrio molitor	gi 1373404	25559	5.4	108	57	17
cockroach allergen-like protein	Tenebrio molitor	gi 32967475	65441	4.1	71	44	12
28 kDa desiccation stress protein	Tenebrio molitor	gi 1373404	25559	5.4	108	57	17
cockroach allergen-like protein	Tenebrio molitor	gi 32967475	65441	4.1	105	22	10

Insectes comestibles et allergènes



Picking des spots et identification des protéines en cours

Effet du fractionnement et processus de stabilisation en cours

Take home message ?

Méconnaissance des allergènes
d'insectes comestibles

Degré de résistance à la dénaturation
thermique et process industriels ?

Risques allergiques potentiels relatifs la
consommation d'insectes comestibles ?

Six pattes et si délicieux



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<http://www.gembloux.ulg.ac.be/entomologie-fonctionnelle-et-evolutive/>