Cheese as an Unconventional Mosquito Attractant

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Limburger Cheese as an Attractant for the Malaria Mosquito *Anopheles gambiae s.s.*

B.G.J. Knols and R. De Jong

It smells like feet!!!

TABLE 2

Numbers of West or East African *An. gambiae s.s* caught in traps baited with either headspace of (i.e. air blown over) Limburger cheese (30 or 500 mg, for Tanzanian and Liberian mosquitoes respectively) or odourless air (A); headspace of 220 µl of water or odourless air (B); headspace of Limburger cheese or headspace of 220 µl of water (C)

	Origin	Treatments		N	χ^2
(A)		Cheese	Control		
	Liberia	181	75	392	43.9***
	Tanzania	100	31	233	36.3***
(B)		Water	Control		
	Liberia	92	104	369	0.7ns
(C)		Cheese	Water		
	Liberia	142	85	383	14.3***

ns: not significant; ***: $P<0.0001$; N: total number of mosquitoes tested (not all of which reacted to either stimulus).

Behavioural and electrophysiological responses of the female malaria mosquito *Anopheles gambiae* (Diptera: Culicidae) to Limburger cheese volatiles

Bart G.J. Knols[1*], Joop J.A. van Loon[1], Alan Cork[2], Rosemary D. Robinson[2], Wim Adam[1], Jocelijn Meijerink[1], Ruurd De Jong[1] and Willem Takken[1]

Winner of the 2006 Ig-Noble prize!

The attraction of mosquitoes to human skin microbes

Journal of Chemical Ecology, Vol. 25, No. 3, 1999

INCUBATED HUMAN SWEAT BUT NOT FRESH SWEAT ATTRACTS THE MALARIA MOSQUITO *Anopheles gambiae* SENSU STRICTO

MARIETA A. H. BRAKS* and WILLEM TAKKEN

Differential Attraction of Malaria Mosquitoes to Volatile Blends Produced by Human Skin Bacteria

Niels O. Verhulst, Rob Andriessen, Ulrike Groenhagen, Gabriella Bukovinszkiné Kiss, Stefan Schulz, Willem Takken, Joop J. A. van Loon, Gosse Schraa, Renate C. Smallegange

Published: December 30, 2010 • https://doi.org/10.1371/journal.pone.0015829

nature › scientific reports › articles › article

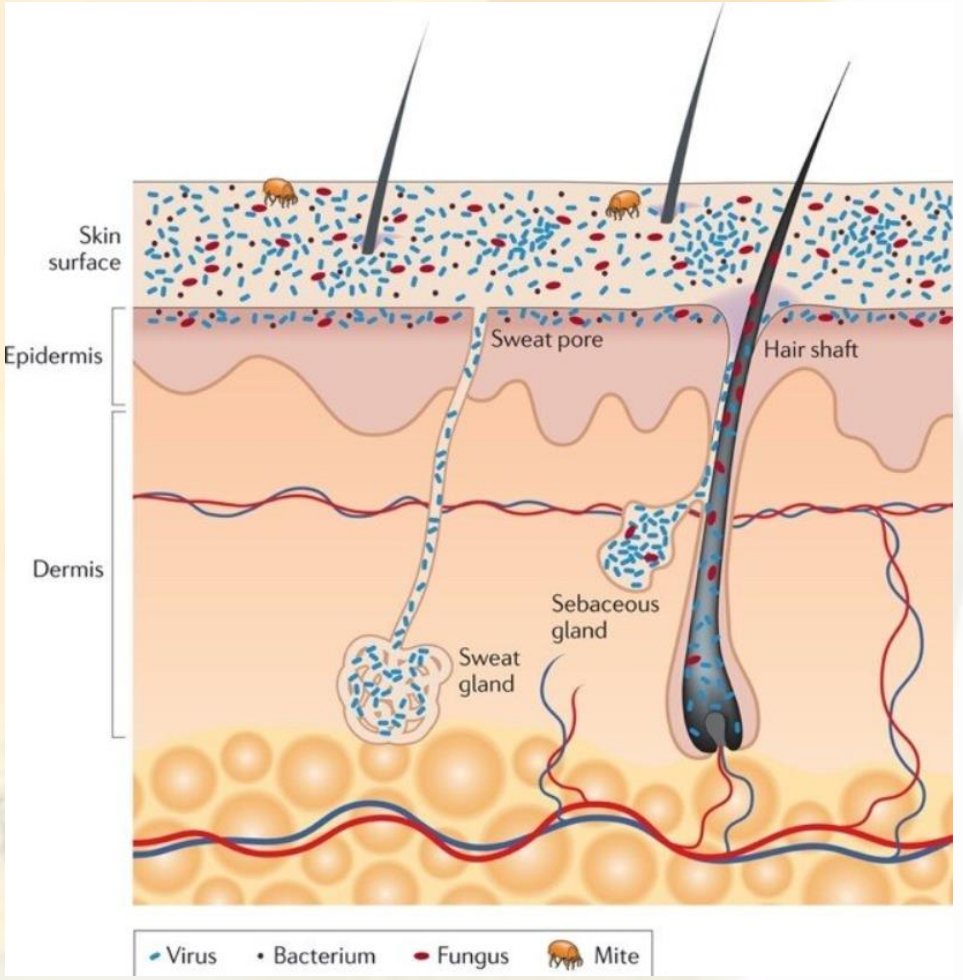
Article | Open access | Published: 18 January 2024

Identification of human skin microbiome odorants that manipulate mosquito landing behavior

Iliano V. Coutinho-Abreu, Omid Jamshidi, Robyn Raban, Katayoon Atabakhsh, Joseph A. Merriman & Omar S. Akbari

Evolutionary connection

- Skin microbes get their nutrition from several sources, including sweat and sebum/skin oils.
- Only mammals possess sweat glands.

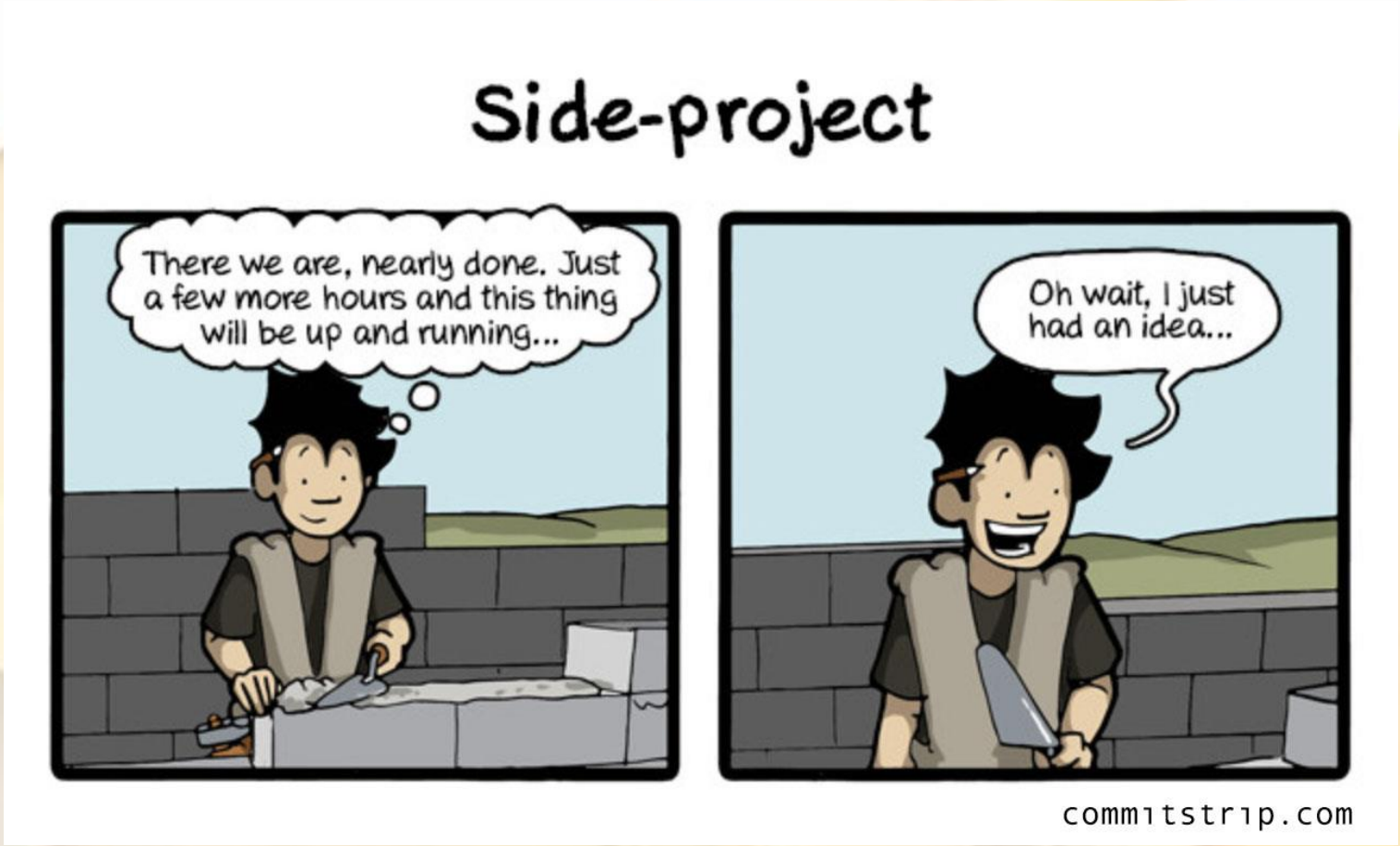


How is cheese made?

- Cheese-making dates to at least 5500 BCE.
- Cheese is essentially the byproduct of adding microbes to milk.
- Many of these microbes are also found on skin (or are strains thereof) or are closely related to skin microbes.
- The first step is to separate milk into solid curds and liquid whey. This is done by acidification, usually by adding rennet and starter microbes, but in some cases (usually soft cheeses or yoghurts) only starter microbes are used.
- Starter microbes convert milk sugars into **lactic acid**.

How is cheese made? – cont'd

- Many different microbes are available, and they each produce different qualities, flavors, and odors.
- Some soft cheeses are now essentially complete, for the rest the curd is cut into small cubes and liquid is drained.
- Cheese is then ripened in a variety of environmental conditions for a wide range of durations, salts or additional microbes can be added.
- At least 500 varieties of cheese are recognized.

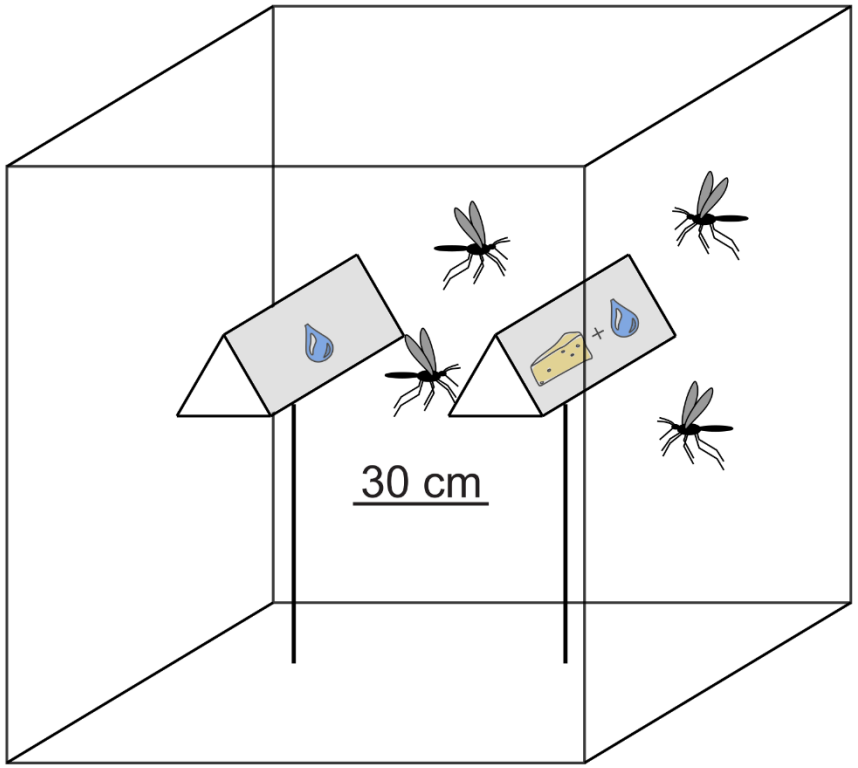


With the abundance of free time available during a PhD, I decided to start a side project investigating cheese as a mosquito attractant.

Hypothesis: Other types of cheese, in addition to limburger, are attractive to host-seeking mosquitoes.

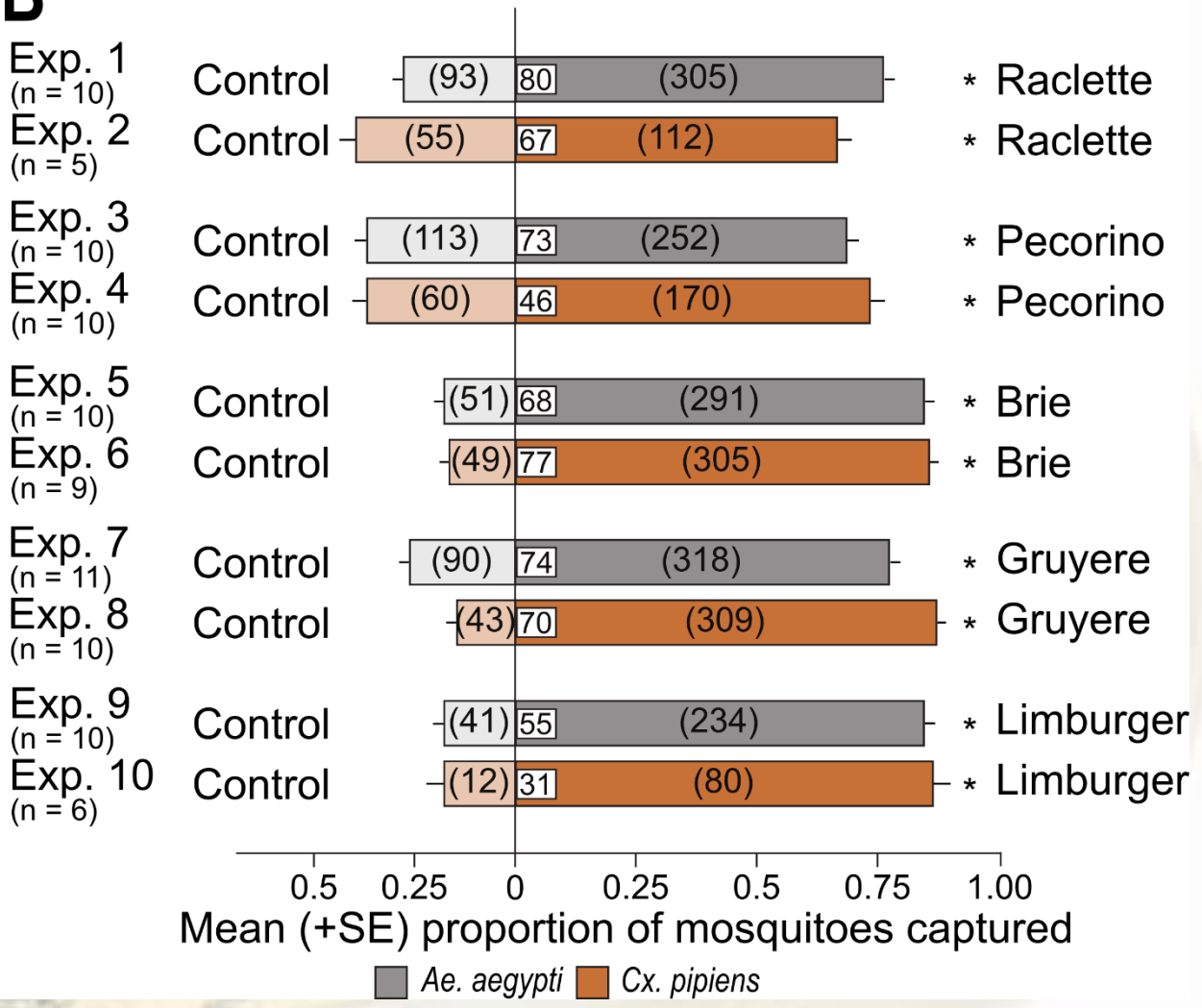
Host seeking tests

A



24 hours , 50 female mosquitoes
(*Culex pipiens* or *Aedes aegypti*)

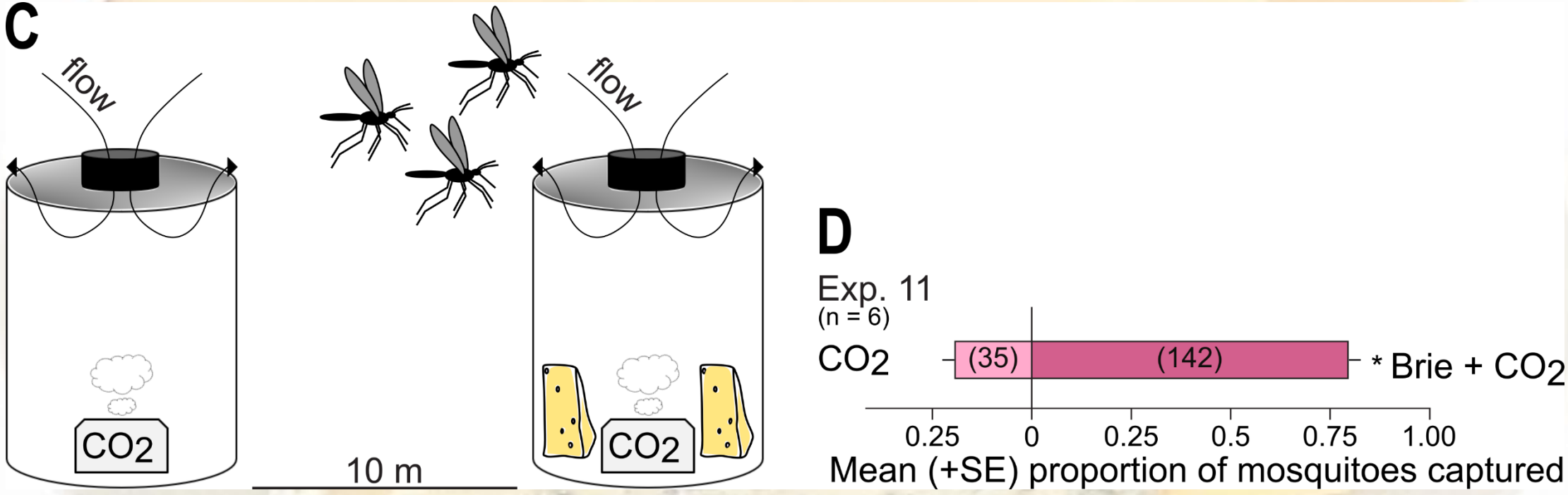
B



Conclusion: Cheese odors are attractive to female *Cx. pipiens* and *Ae. aegypti* mosquitoes.

There are no wild *Ae. aegypti* in British Columbia, so brie was selected for field tests.

Host-seeking field tests



20 g of brie + 2 lb dry ice for 90 minutes at dusk.

Primarily captured *Cx. pipiens* and *Culiseta incidens*, the most abundant local species.

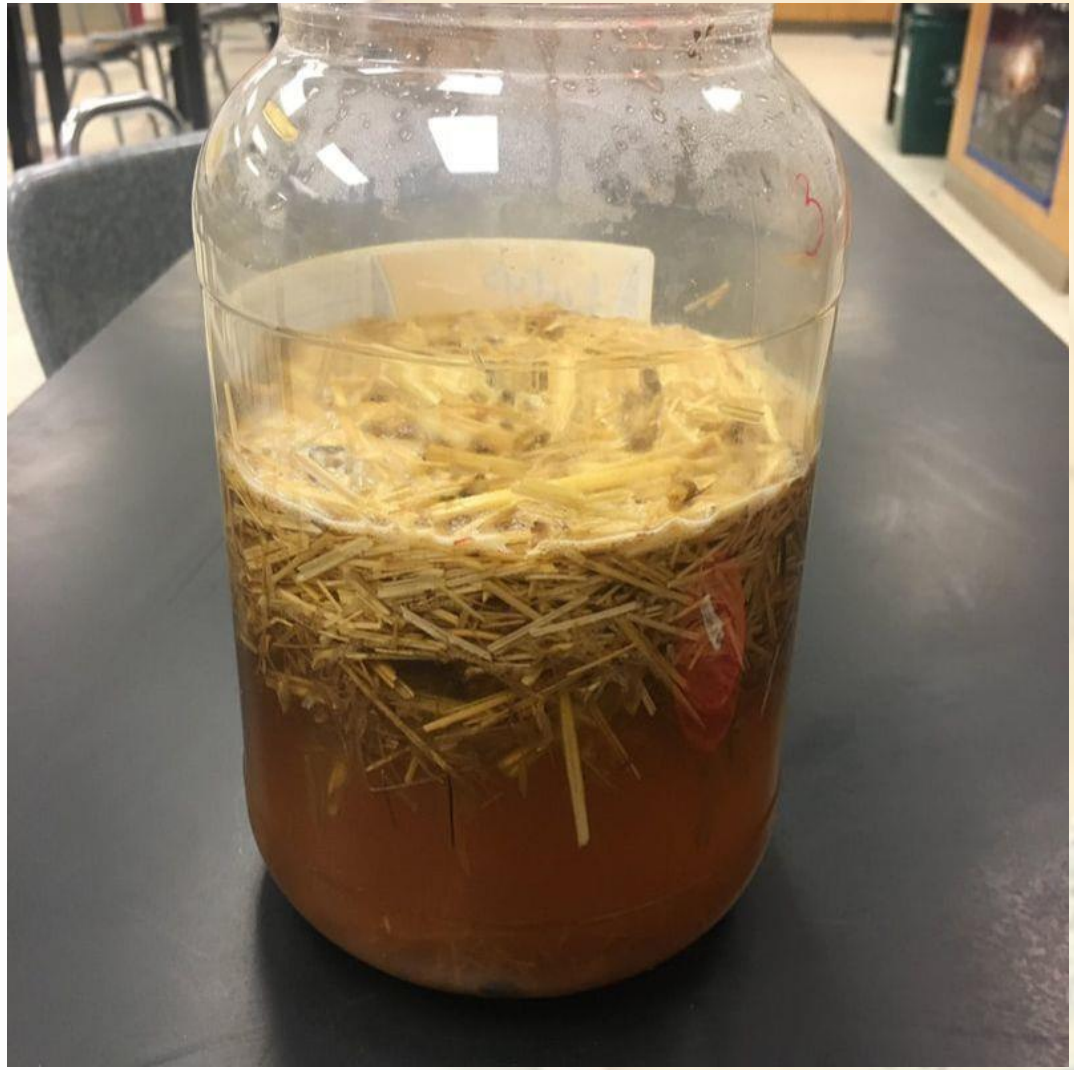
Conclusion: Cheese can function as an attractive odor lure for mosquitoes in the field, similar to BG lures.

What about Oviposition? E.g. Casu Martzu

- Several regional varieties of cheese are produced in Europe that involve fly oviposition and maggot fermentation.
- e.g. Sardinian casu martzu is made by fermenting sheep cheese in a peculiar way.
- The whole cheese is left outside to allow *Piophila casei* flies to oviposit on it (up to 500 eggs per females).
- The eggs hatch and the maggots begin to eat the cheese, excreting digested cheese and acids which further ferment the cheese, making is softer.
- Casu martzu is eaten with live maggots to avoid health issues associated with eating decomposing dead maggots.
- As if that wasn't enough, the maggots jump while the cheese is being eaten.

Hypothesis: Cheese acts as an oviposition attractant to mosquitoes.

Grass/hay infusions are mosquito oviposition attractants – why not emulate them?



Cheese infusion methodology

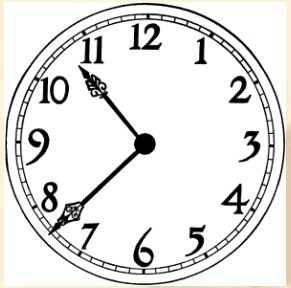
50g

+

5L

+

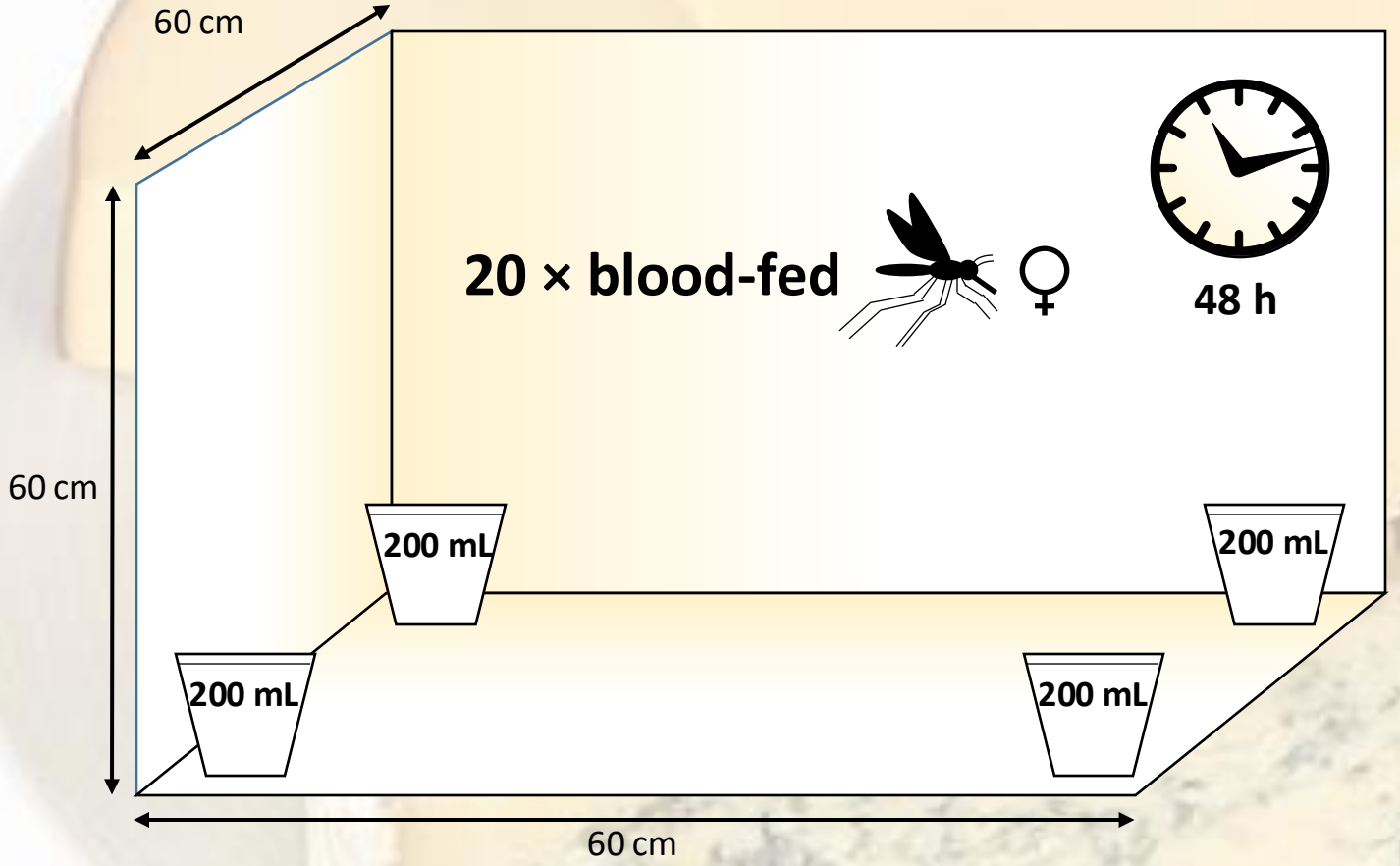
5 days*



=

*at room temperature, and also works after 2 days

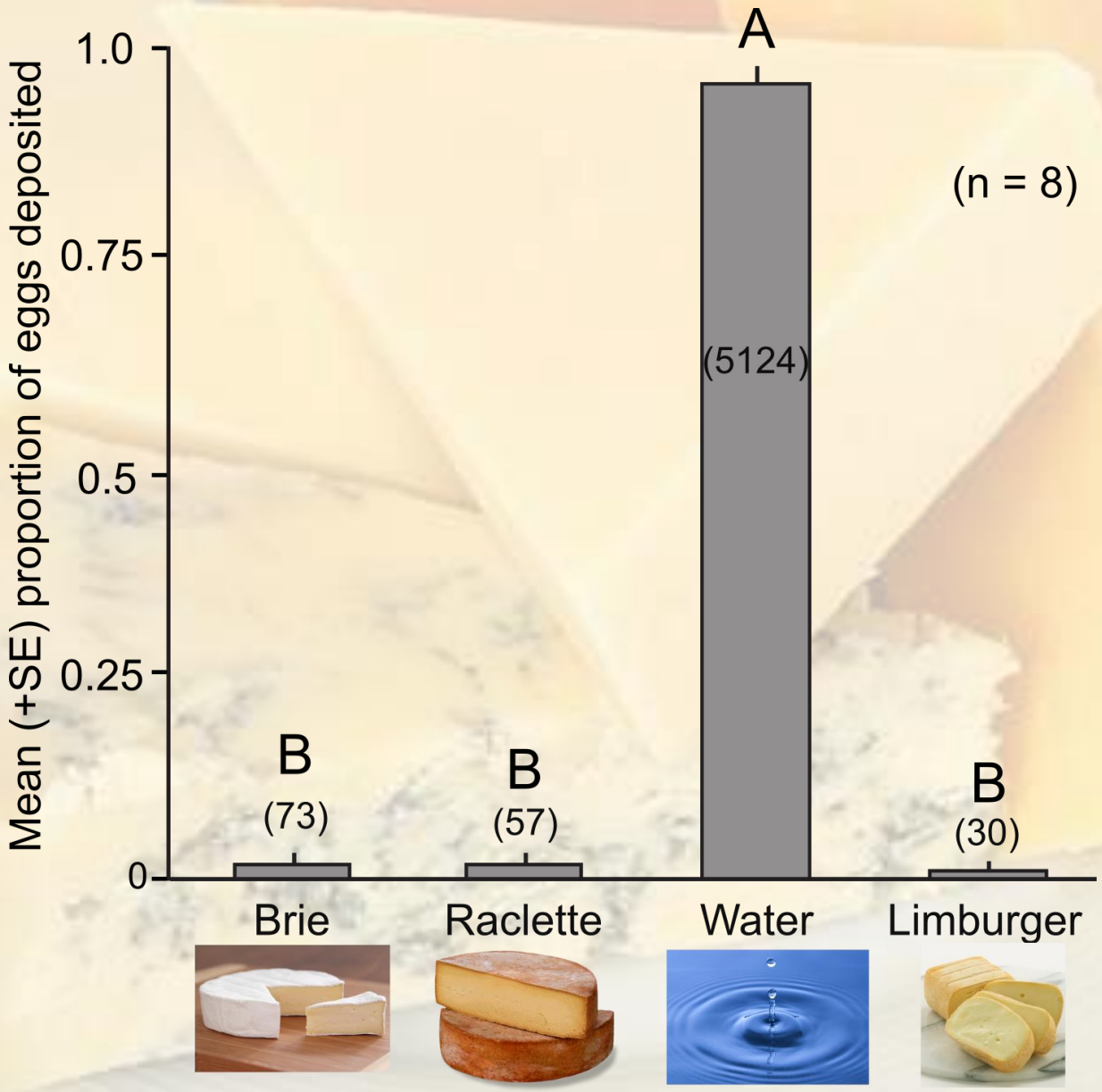
Oviposition methodology



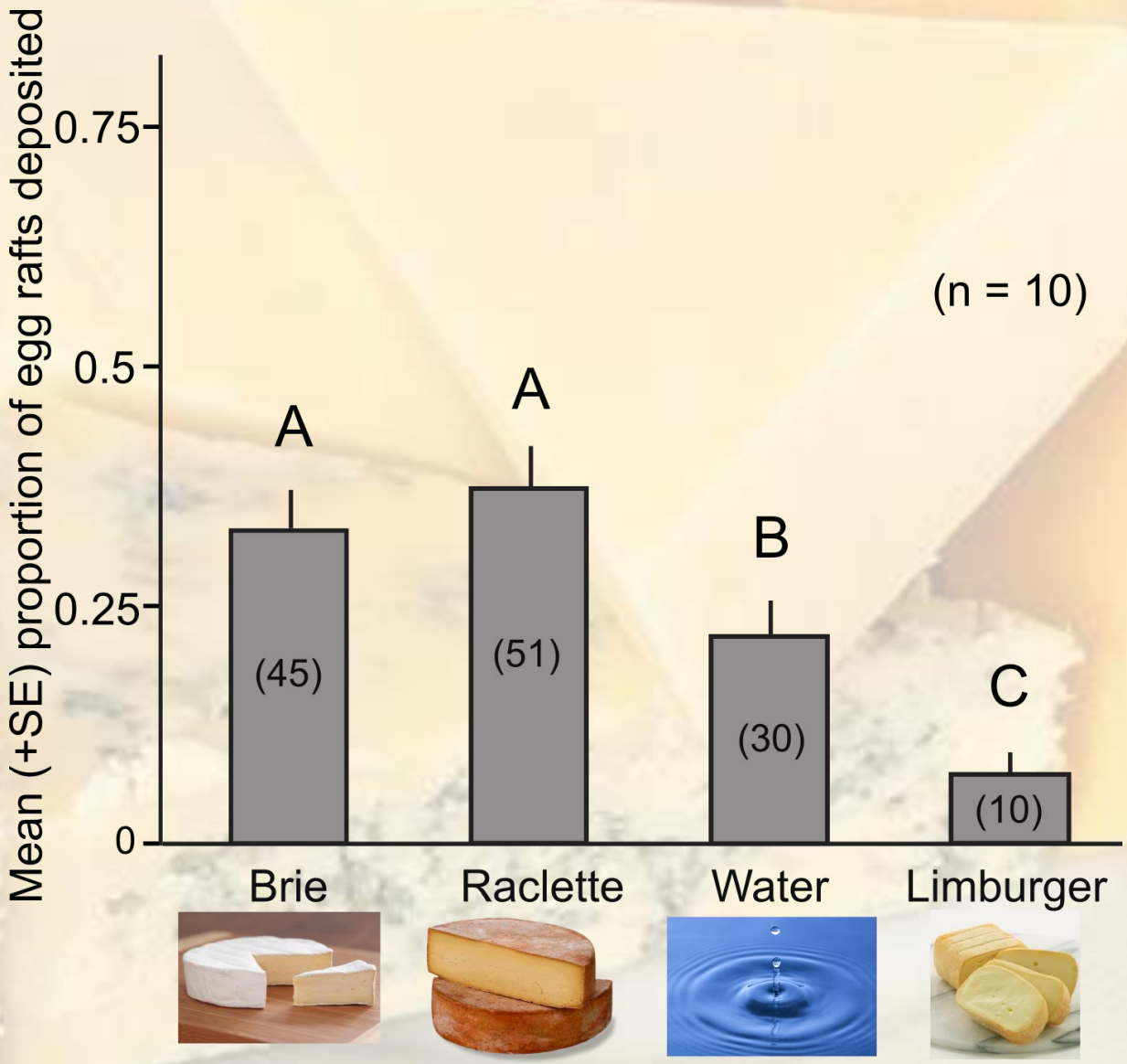
Treatments:

- Brie infusion
- Raclette infusion
- Limburger infusion
- Water

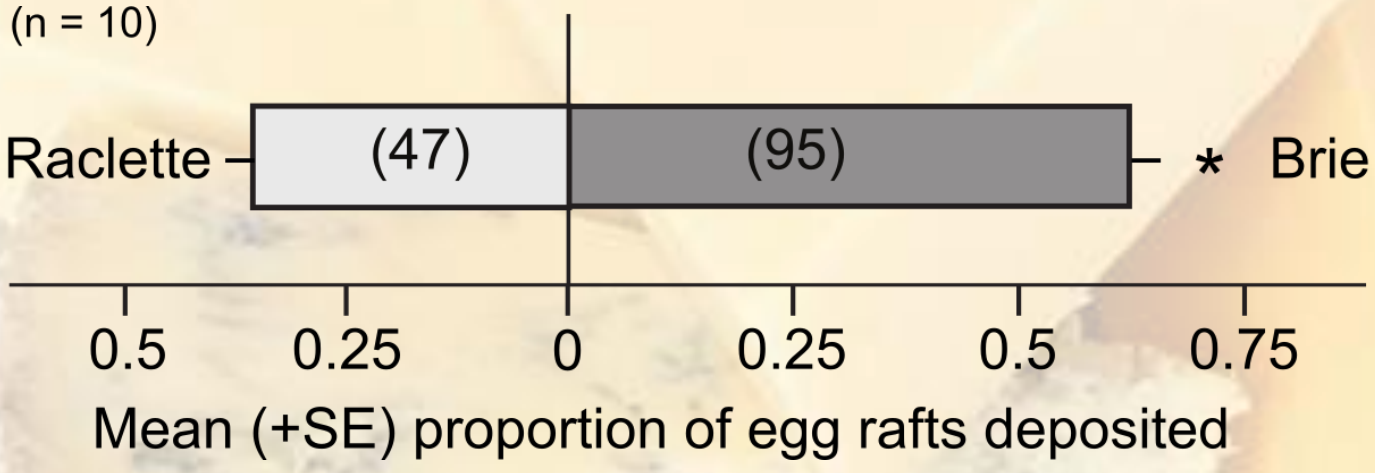
Oviposition tests – *Aedes aegypti* individual eggs



Oviposition tests – *Culex pipiens* egg rafts



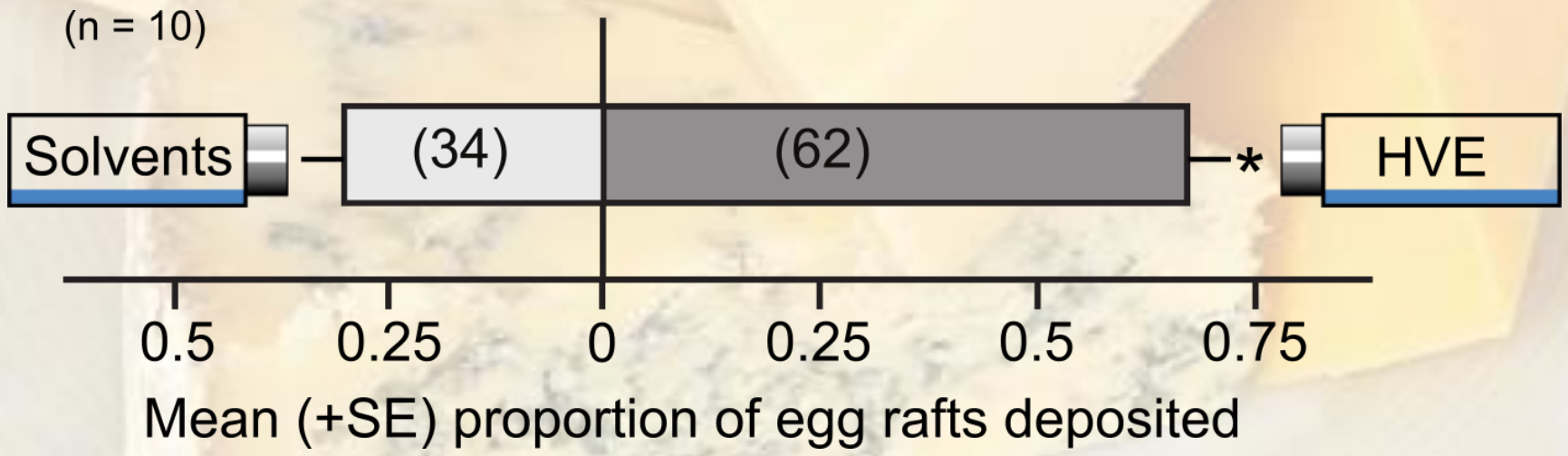
Oviposition tests – *Culex pipiens* egg rafts



Conclusion: brie infusions are oviposition/gravid attractants for *Cx. pipiens*.

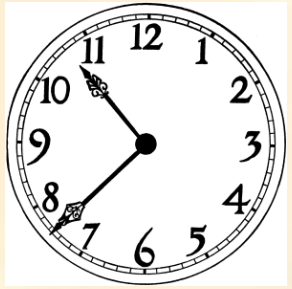
Volatile extraction methods

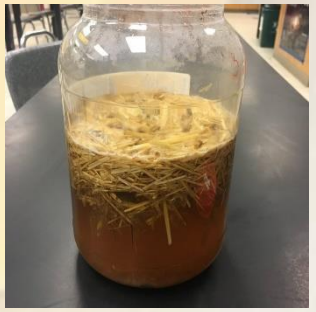
- 24 h of 200 mL
- Extracted into 1 mL
- 100 uL aliquots tested over 48 h (10 mL cheese infusion equivalent)

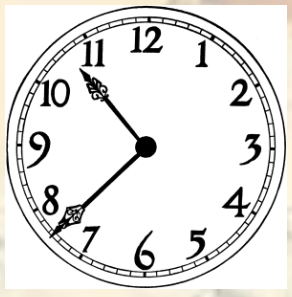


Field-test infusion methodology

50g + 5L + 5 days* = 



50g + 5L + 5 days = 

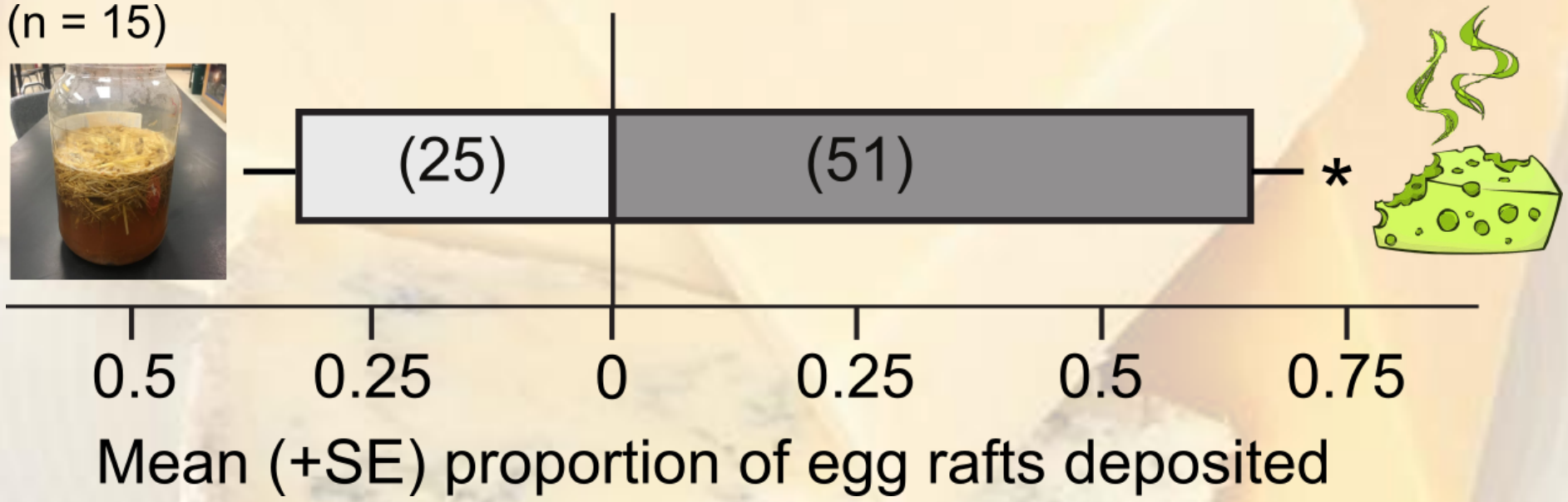


*also works after 2 days

Field test methods

- 2.5 L of brie cheese infusion or bluegrass (*Poa* sp.) infusion into black plastic tubs, treatments 3 m apart.
- Deployed on SFU campus for 48 hours.
- Eggs rafts removed, counted, and emerging larvae identified.

Field tests



Bluegrass infusion	
Species	Egg rafts
Culex pipiens	9 (35%)
Culiseta incidens	16 (65%)

Brie infusion	
Species	Egg rafts
Culex pipiens	19 (38%)
Culiseta incidens	32 (62%)

Conclusion: brie infusions work as oviposition/gravid attractants in the field.

Homemade cheese infusions

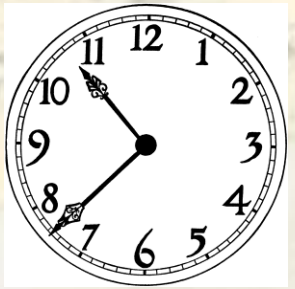
1g + 300 g + 900mL + 5 days =

Meso Aroma B

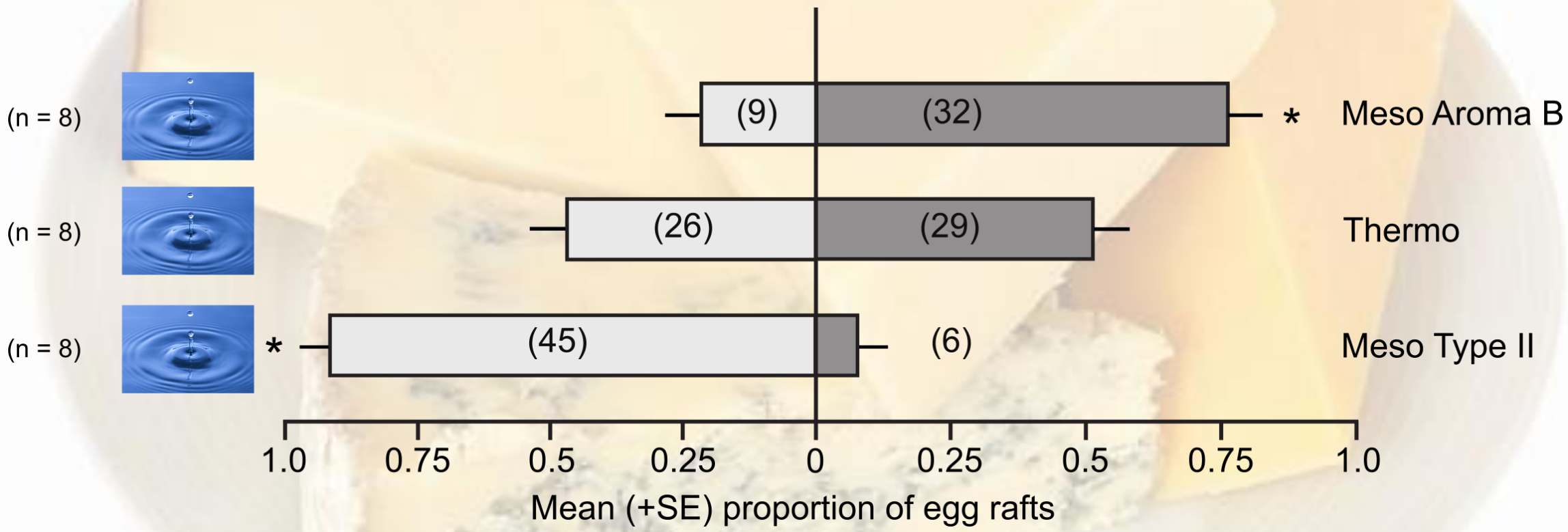
Meso Type II

Thermo

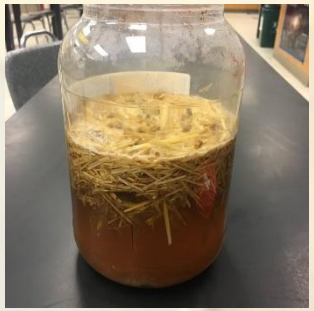
20g + 180mL + 5 days =



Homemade cheese

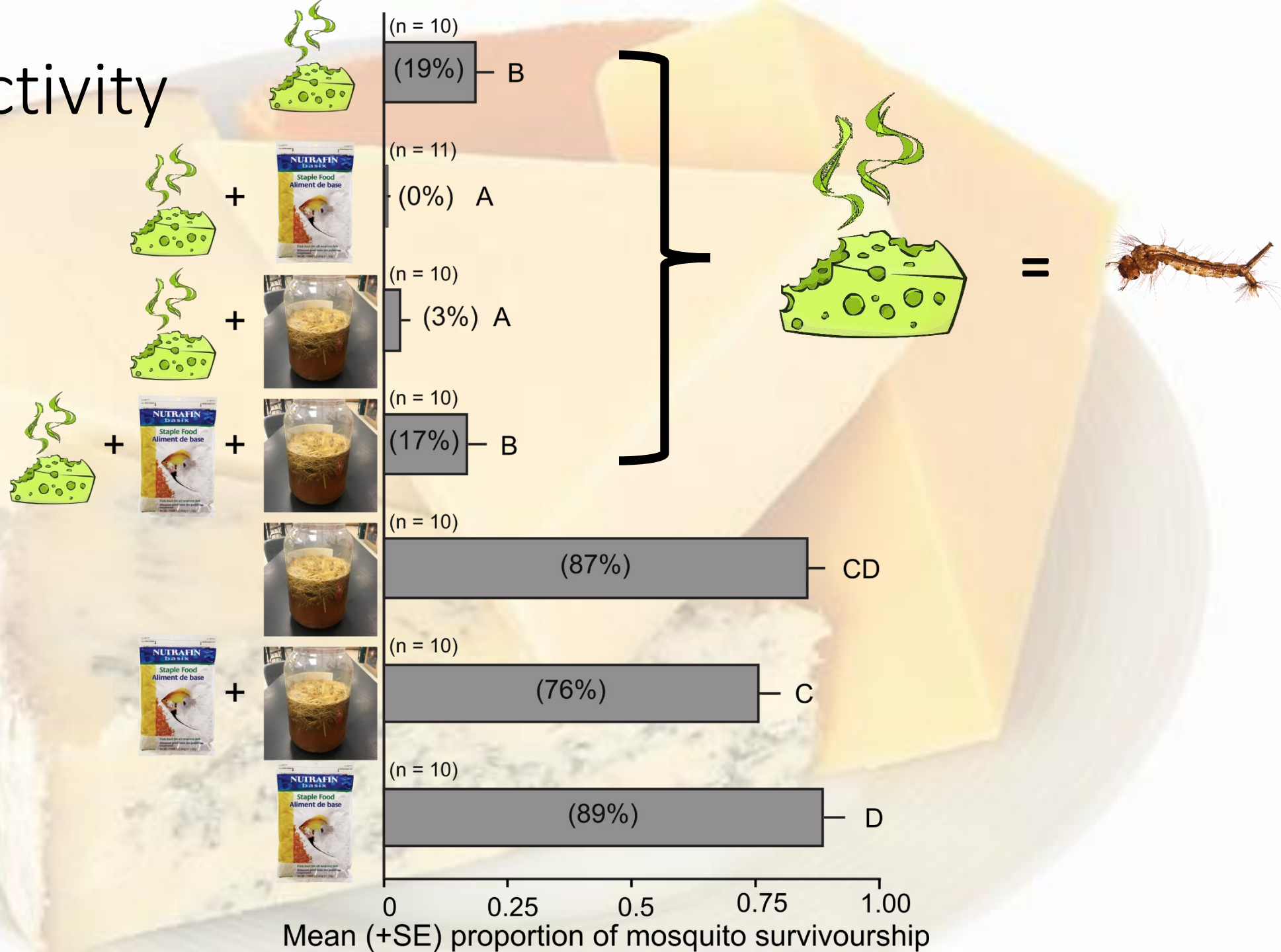


Larvicidal methods



Full factorial +

Larvicidal activity



Conclusions

- Cheese is a host-seeking attractant and cheese infusions are mosquito oviposition attractants.
- They both work in the field, and there may be larvicidal activity associated with cheese infusions (ecological trap).
- Cheese can be customized, so these attractants can be too.

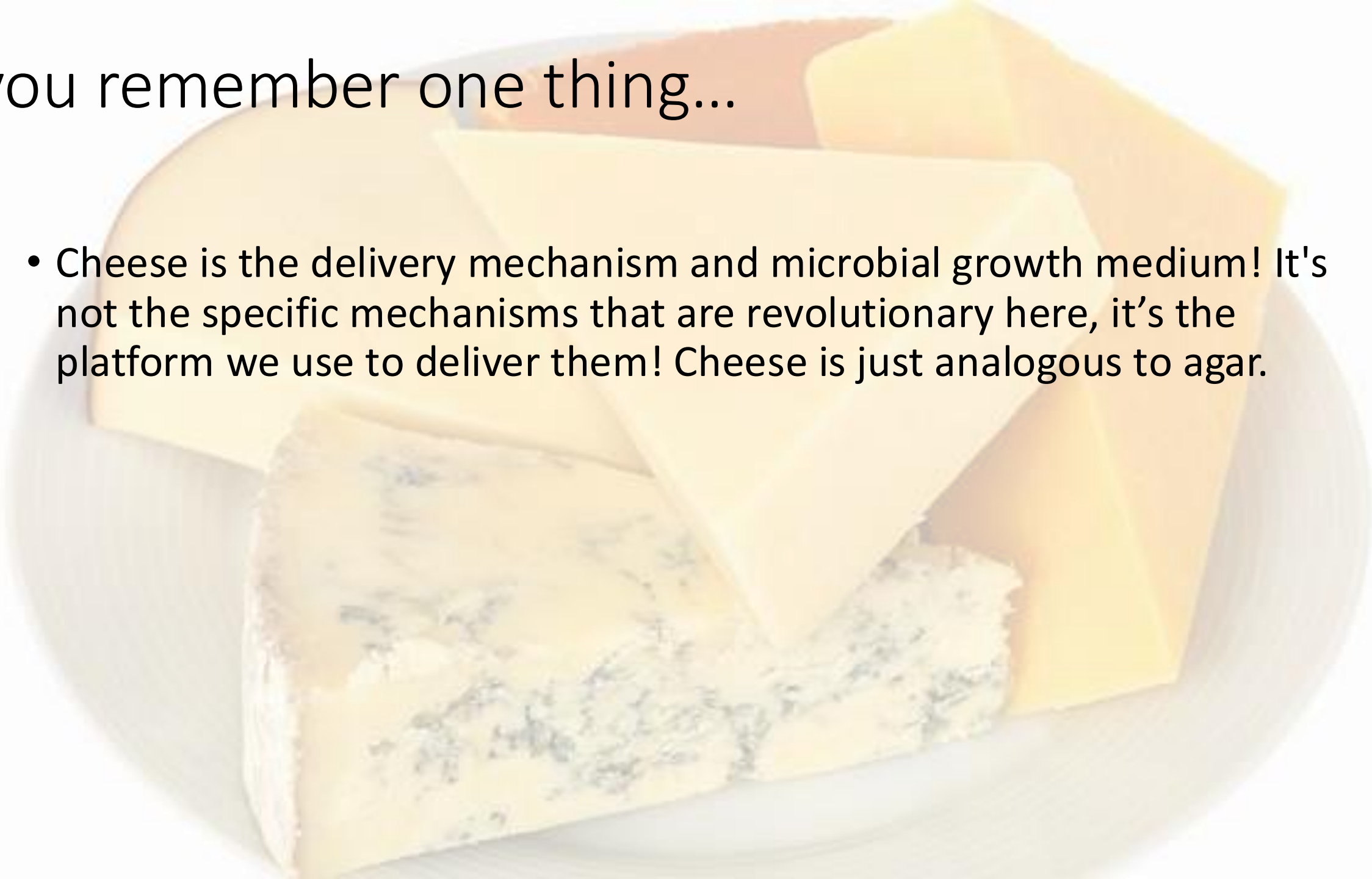
Next steps

- Customize and optimize attraction (designer cheese).

- Explore carbon dioxide production.

If you remember one thing…

- Cheese is the delivery mechanism and microbial growth medium! It's not the specific mechanisms that are revolutionary here, it’s the platform we use to deliver them! Cheese is just analogous to agar.



Acknowledgements

- All of the dairy cows and farmers involved
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