



Fighting against permethrin resistant and non-resistant strains of bed bugs (*Cimex lectularius*) with the use of a special fogger and a combination of H₂O₂ fluid and permethrin – a light at the end of the tunnel

Georg Gerhard Duscher¹, Adnan Hodžić¹, Elena Battisti², Stefanie Boigenzahn³, Thomas Schwan³, Patrick Jaeger³, Davul Ljuhar³

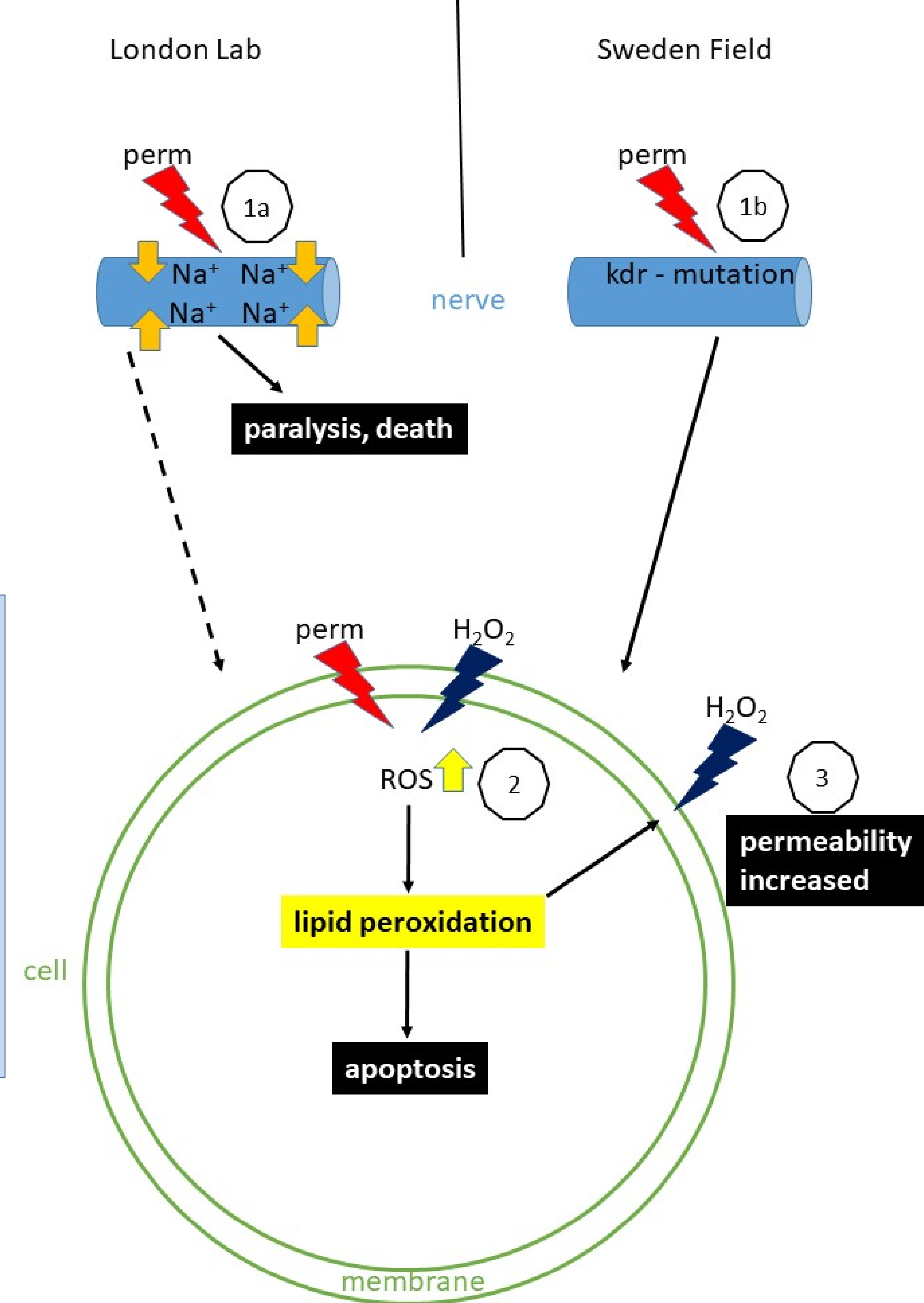
¹Institute of Parasitology, Department for Pathobiology, University of Veterinary Medicine Vienna, Vienna, Austria
²Università degli Studi di Torino, Turin, Italy
³Braincon Technologies, Austria

1) Bed bugs

- Insects in houses, hotel, hostels, cabins, hospitals etc.
- Distribution due to globalisation and traveling
- Nightly visits: wheals, redness, pruritus and mental impacts like insomnia and anxiety state
- Hidden lifestyle – hard to combat



Possible ways of intoxication: In the London lab strain (permethrin susceptible) the major mode of action of the permethrin (perm) is the opening of the (Na⁺) sodium channels (1a) which leads to paralysis and death. In the Sweden field strain (permethrin resistant) the kdr-mutation hampers the opening of the sodium channels (1b), therefore lacking depolarization of the nerve cells. The second mode of action gains importance, which is the shift to a higher oxidative level in the cells (2) to more radicals (ROS = reactive oxygen species) due to applying permethrin and hydrogen peroxide (H₂O₂). Those radicals oxidize mainly lipids, but also proteins and lead to apoptosis. Lipid peroxidation in the membrane induces a higher permeability of the membrane (3), which in turn is also affecting the survival of the cell.



Vector role of bed bugs:

- 40 pathogens experimentally proven
- *Trypanosoma cruzi* most likely:
 - Uptake by bed bugs from positive mice
 - Infection of mice with the bed bug faeces
 - Observation of defaecation of the bed bugs after feeding



https://www.pinterest.at/pin/440086194812061383/

- Selection for resistance (e.g. permethrin, knockdown resistant – kdr)

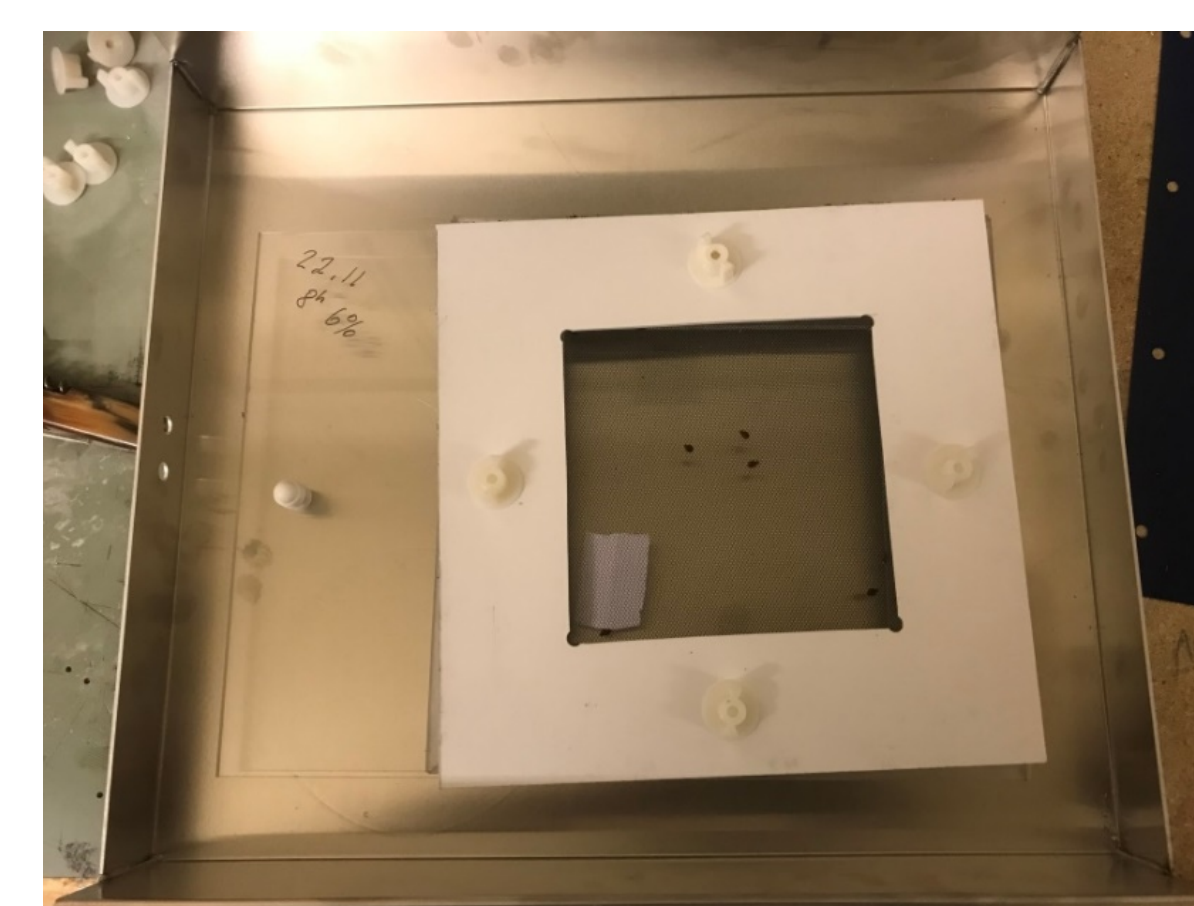
4) Discussion

- Synergistic effect on the **kdr – mutation (permethrin resistant) bed bugs**: the **lipid peroxidation** probably gains importance and becomes the dominant mode of action in the **resistant strain** (see blue box above)
- Other strains of bed bugs have to be tested
- Other target organisms (cockroaches, ticks, fleas, ...) have to be tested
- Fogger has to be adjusted depending on prevailing conditions (different treatment protocols)

2) The Study

- Permethrin susceptible strain (London lab strain)
- Permethrin resistant strain (Sweden field strain) kdr (knockdown resistant) mutation

- 15 bugs/group
 - Exposure to permethrin (3 %): 2 h, 4 h
 - Exposure to H₂O₂ (DXCF fluid): 2 h, 4 h
 - Exposure to permethrin/H₂O₂: 2 h, 4 h
 - Control groups

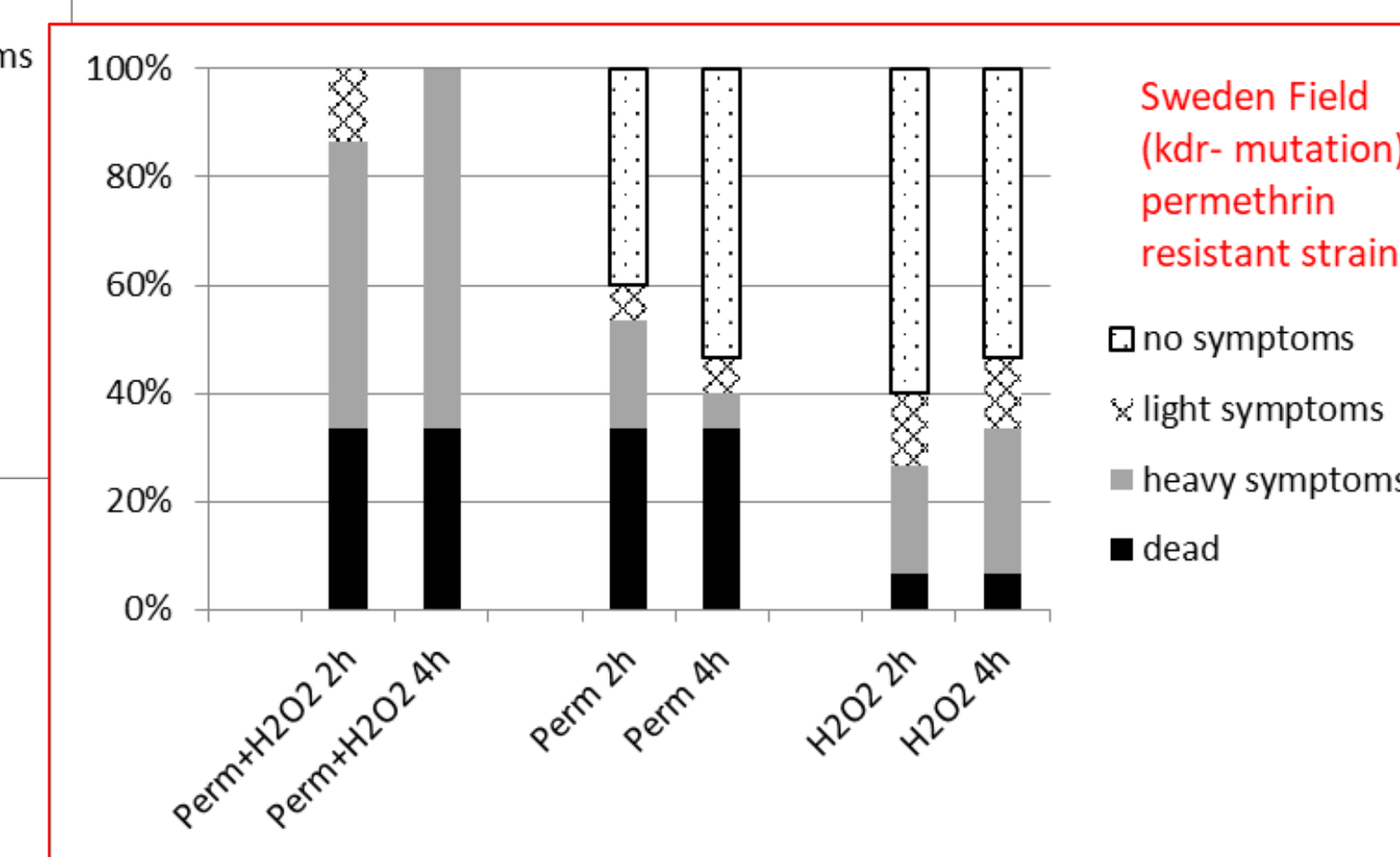
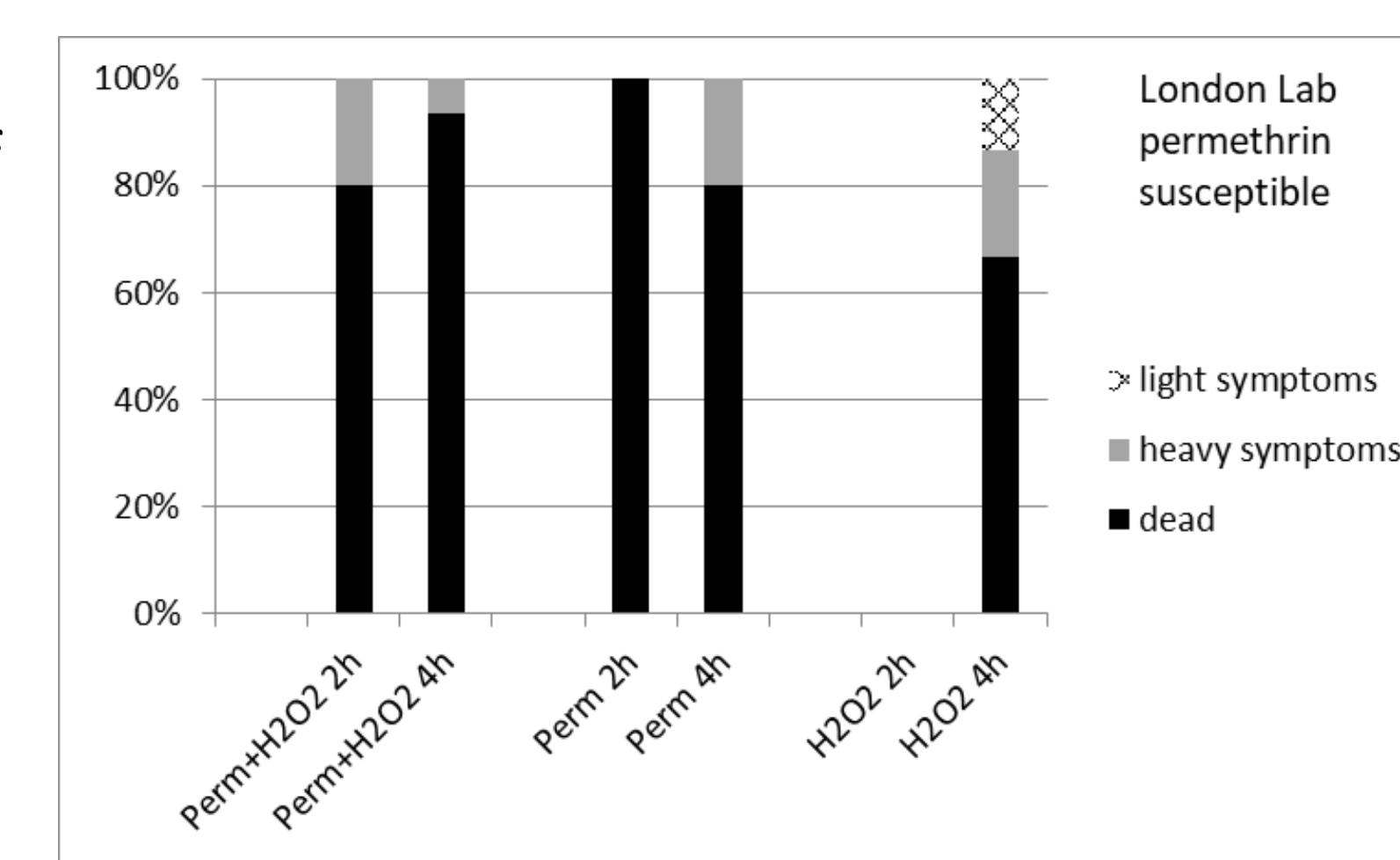


- Viability determination

- Freezing at -80°C, RNA extraction (Trizol)
 - Reverse transcriptase realtime PCR analysis on mRNA levels of detoxification enzymes (cytochrome P450 monooxygenases (P450), glutathione-S-transferases (GST) and carboxylesterases (CE))

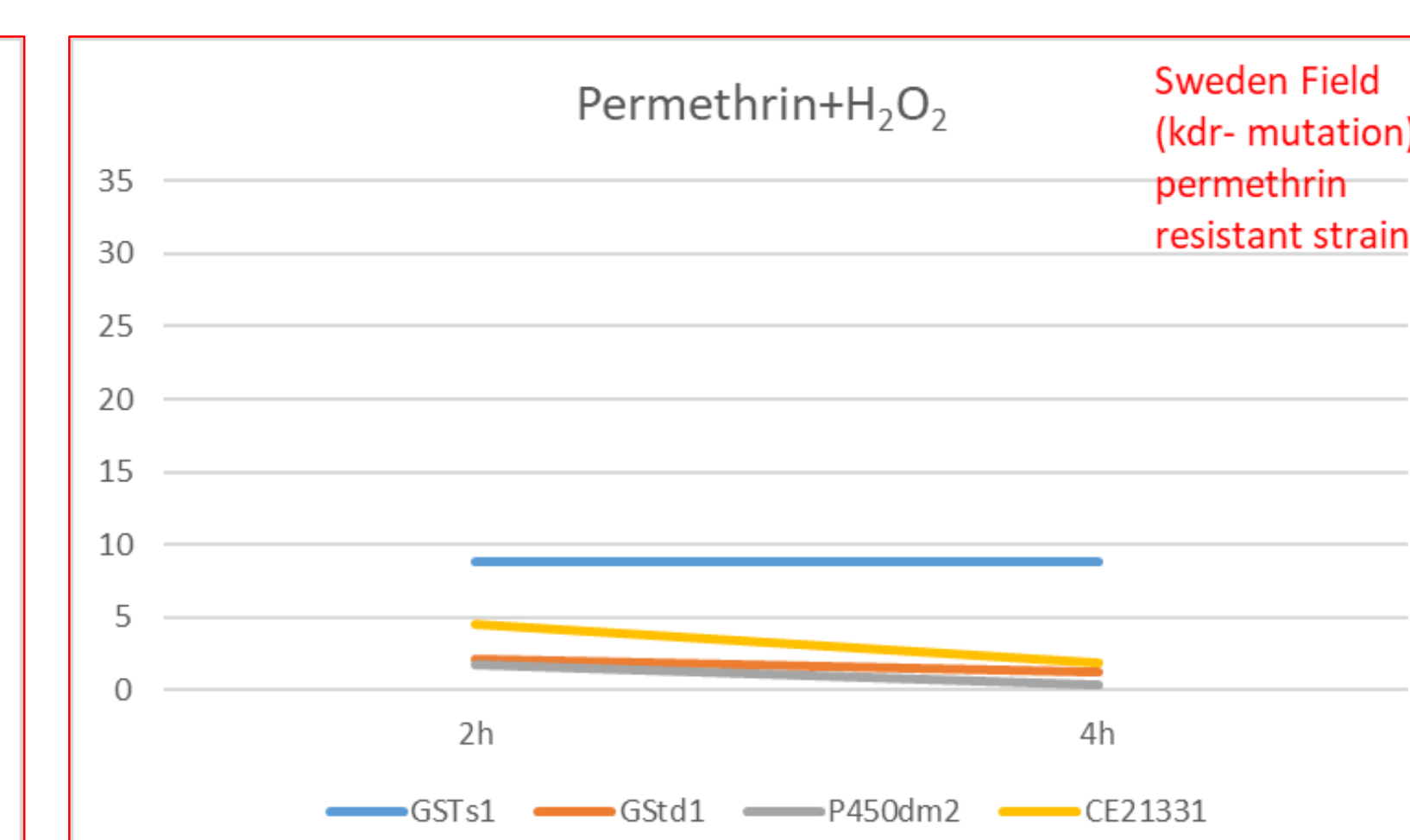
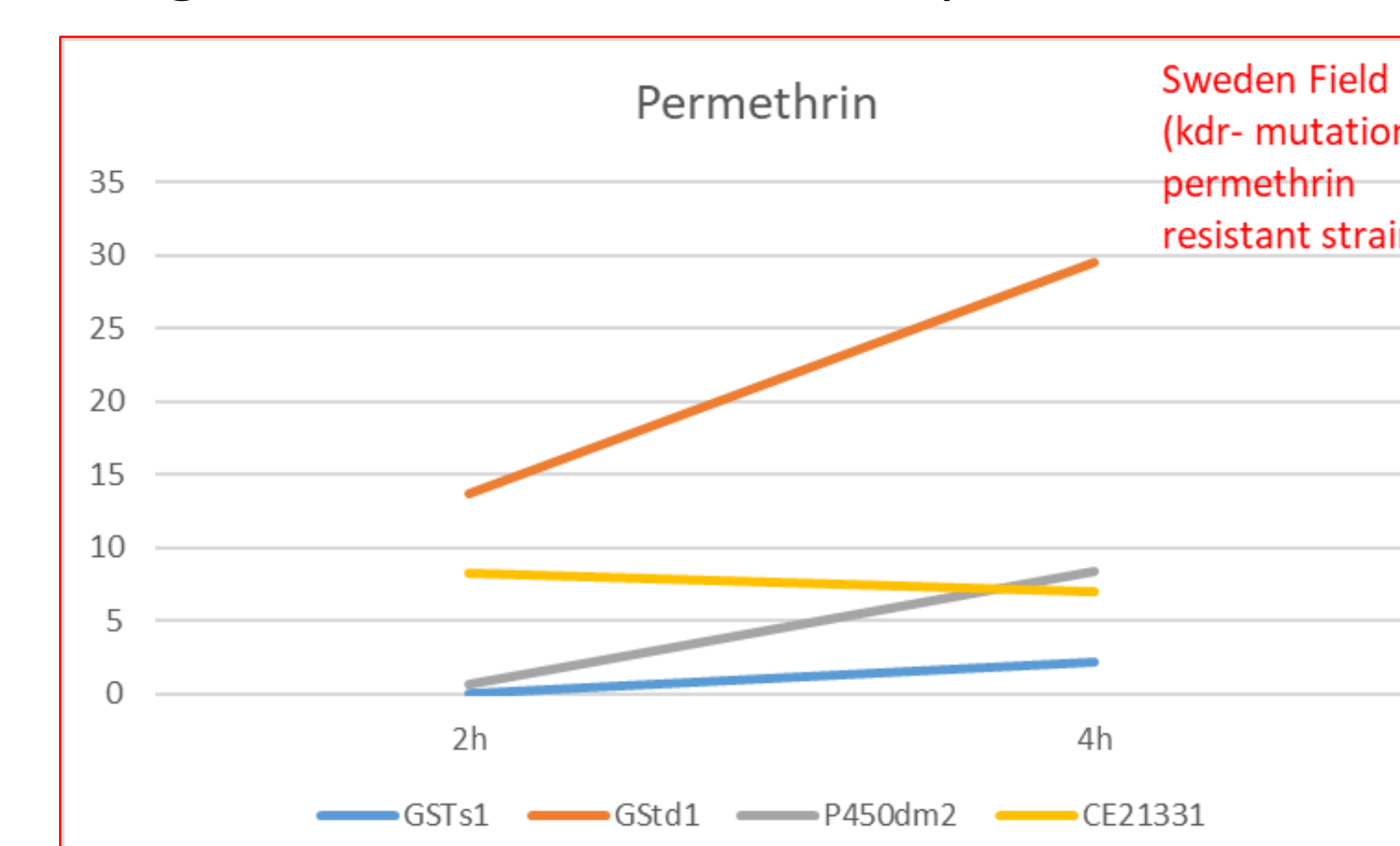
3) Results

The bed bugs from the London Lab strain (permethrin susceptible) showed heavy symptoms (dead or heavy) if treated with permethrin alone or in combination with the H₂O₂ (DXCF fluid), whereas the H₂O₂ (DXCF fluid) alone did not affect the bed bugs in the 4 h group. Unfortunately the 2 h group got lost due to technical reasons and therefore was excluded from the analysis. In the **resistant Sweden field strain (red frame)** the H₂O₂ (DXCF fluid) alone was not able to affect more than 40 % in the 2 h group and 50 % in the 4 h group.



The remaining unaffected bugs did not display any symptoms at all. The permethrin alone was effective in 60 % in the 2 h group and 50 % in the 4 h group, if including the light symptomatic bed bugs. The combination of permethrin and H₂O₂ affected all bed bugs in the 2 h group (light, heavy symptoms and dead) and in the 4 h (heavy symptoms, dead)

On the molecular level the **resistant strain** displayed an upregulation of some of the detoxification enzymes from 2 h to 4 h in the permethrin alone group, which can be seen as reaction onto the poisoning. In combination with H₂O₂ this increase of mRNA cannot be observed, indicating the lack of response against the xenobiotic compounds.



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