



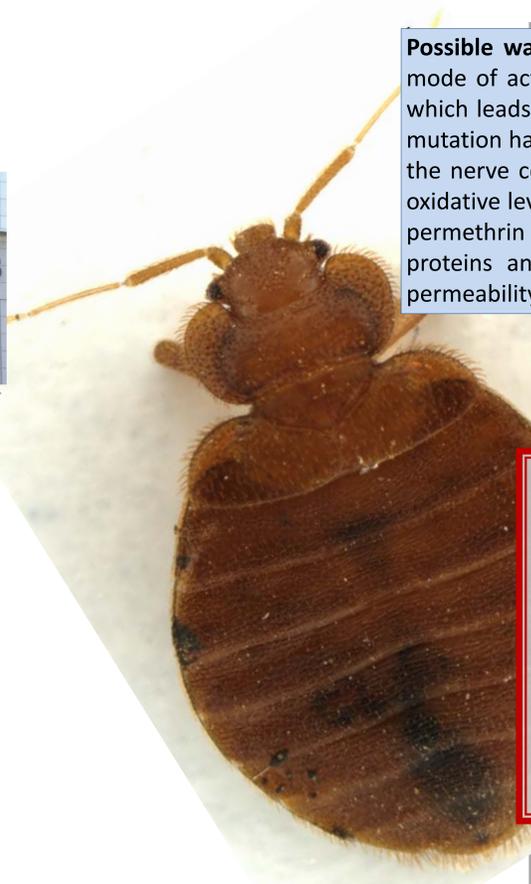
# Fighting against permethrin resistant and non-resistant strains of bed bugs (*Cimex lectularius*) with the use of a special fogger and a combination of H<sub>2</sub>O<sub>2</sub> fluid and permethrin – a light at the end of the tunnel

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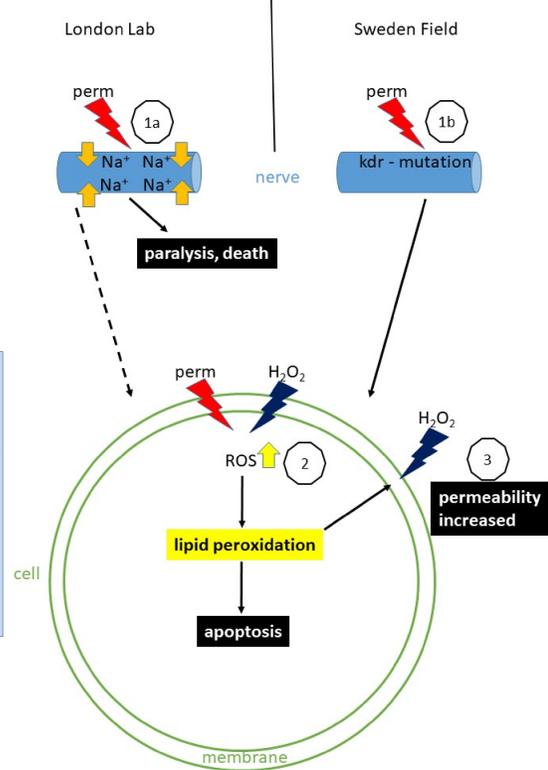
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## 1) Bed bugs

- 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

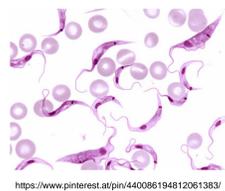


**Possible ways of intoxication:** In the London lab strain (permethrin susceptible) the major mode of action of the permethrin (perm) is the opening of the (Na<sup>+</sup>) sodium channels (1a) which leads to paralysis and death. In the Sweden field strain (permethrin resistant) the kdr-mutation hampers 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 (H<sub>2</sub>O<sub>2</sub>). 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 H<sub>2</sub>O<sub>2</sub> (DXCF fluid): 2 h, 4 h
  - Exposure to permethrin/H<sub>2</sub>O<sub>2</sub>: 2 h, 4 h
  - Control groups

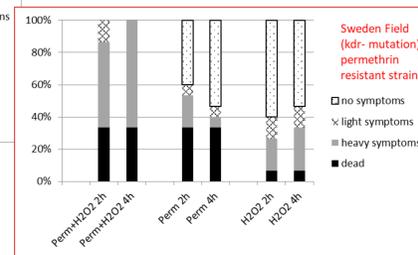
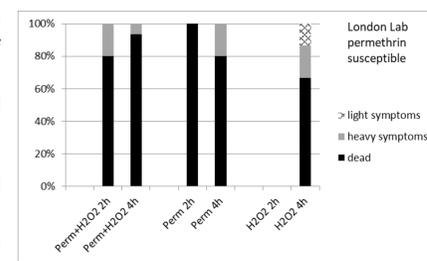


- Viability determination

- 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))

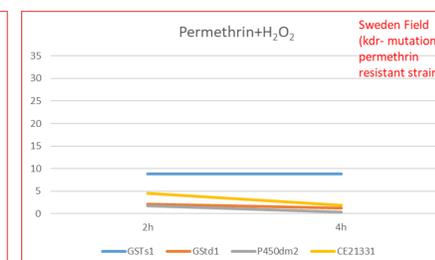
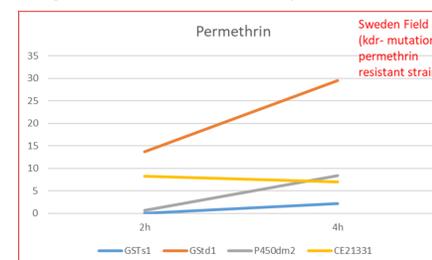
## 3) Results

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 H<sub>2</sub>O<sub>2</sub> (DXCF fluid), whereas the H<sub>2</sub>O<sub>2</sub> (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 H<sub>2</sub>O<sub>2</sub> (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 H<sub>2</sub>O<sub>2</sub> 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 H<sub>2</sub>O<sub>2</sub> this increase of mRNA cannot be observed, indicating the lack of response against the xenobiotic compounds.



Special thanks to: Walpurga Wille-Piazzai

