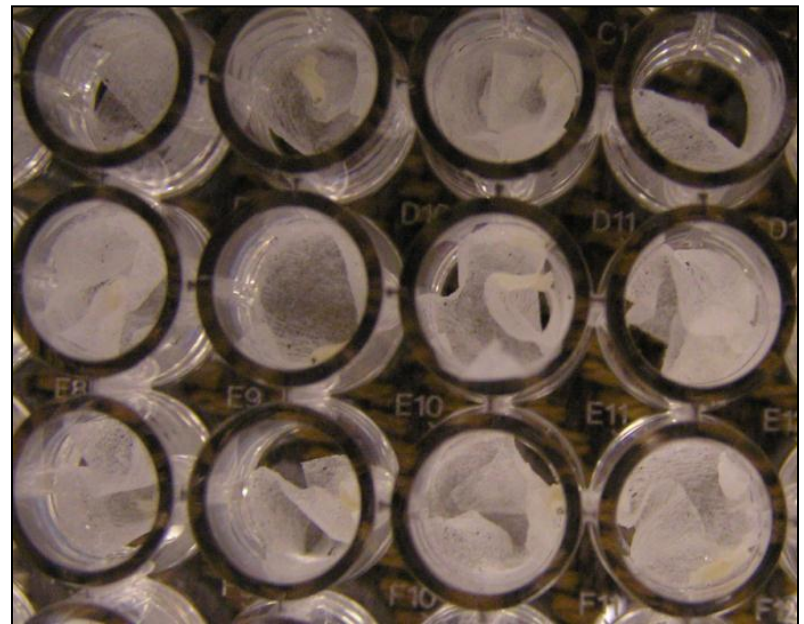
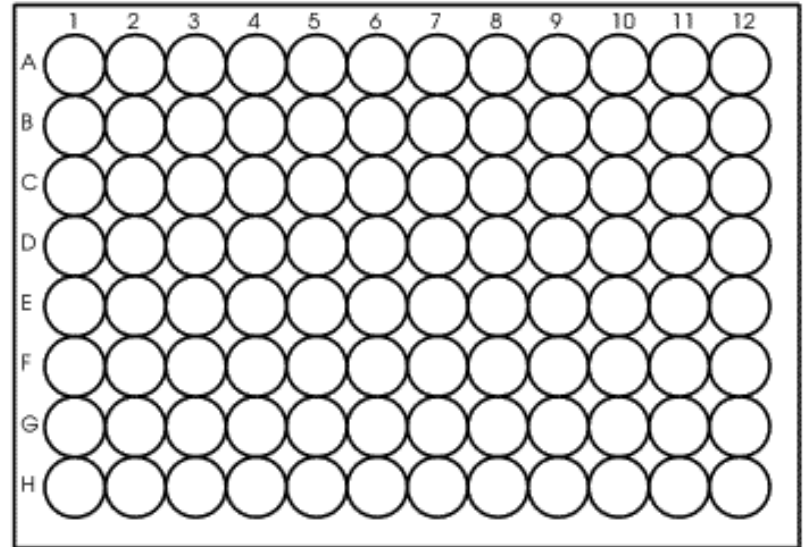


Nematode assay

(modified from Hallem et al. 2007)

96 well plate setting:

- tissue paper approx. 1cm²
- 25 IJs in 10µl PBS/ well / 29°C
- 100 IJs in 10µl PBS / well / 25°C
- 100 IJs in 10µl PBS / well / 22°C
- *Drosophila* larva in each well
- mortality counting after 24, 48 hours
- 48 larvae / group, in triplicates



Counting of mortality

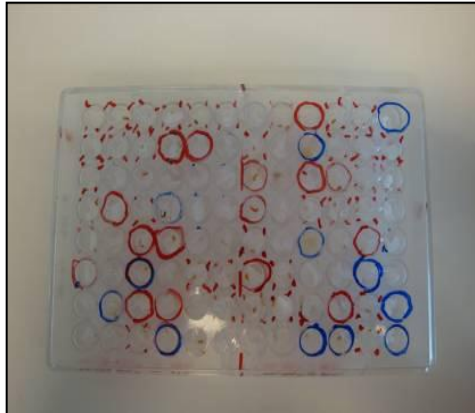


Plate after counting



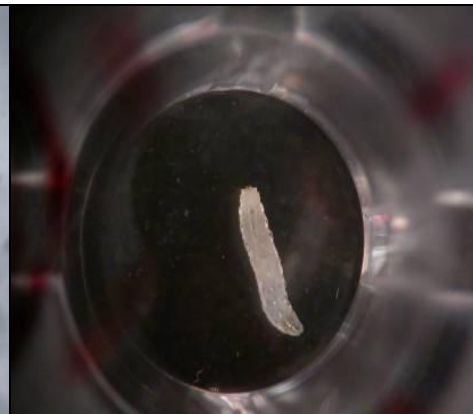
Infected larva



Infected pupa



Live pupa

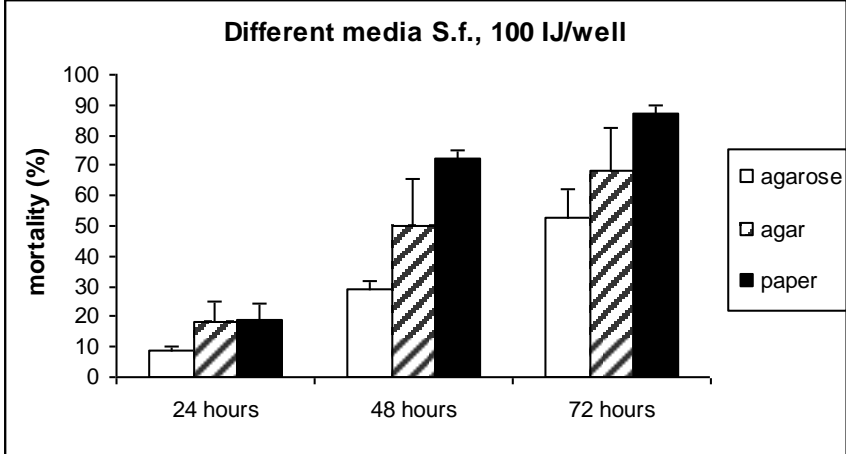
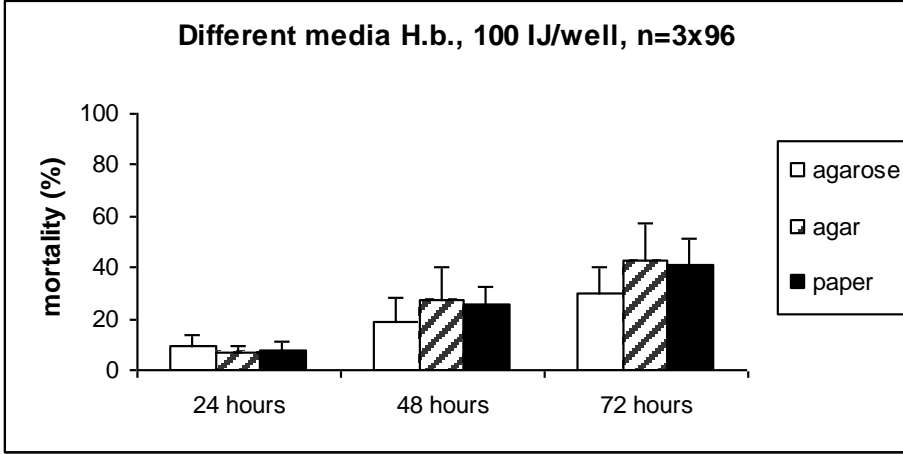


Live larva

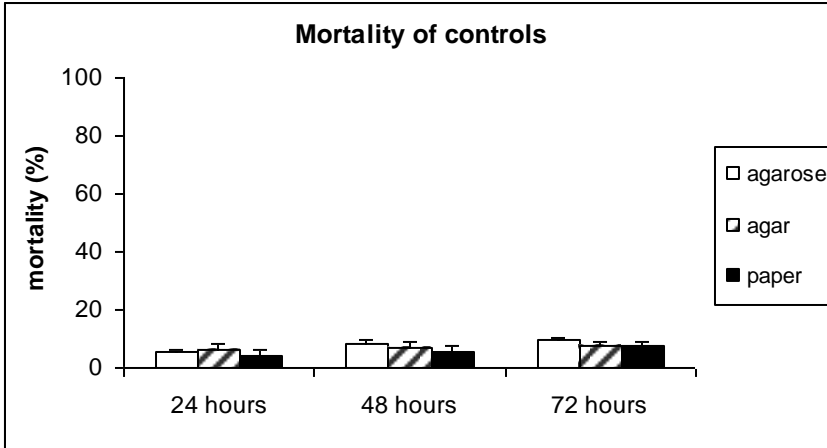


Mishandled larva (melanization)

Optimization - media (w^{1118} , lab. temp. 100 IJ/w):

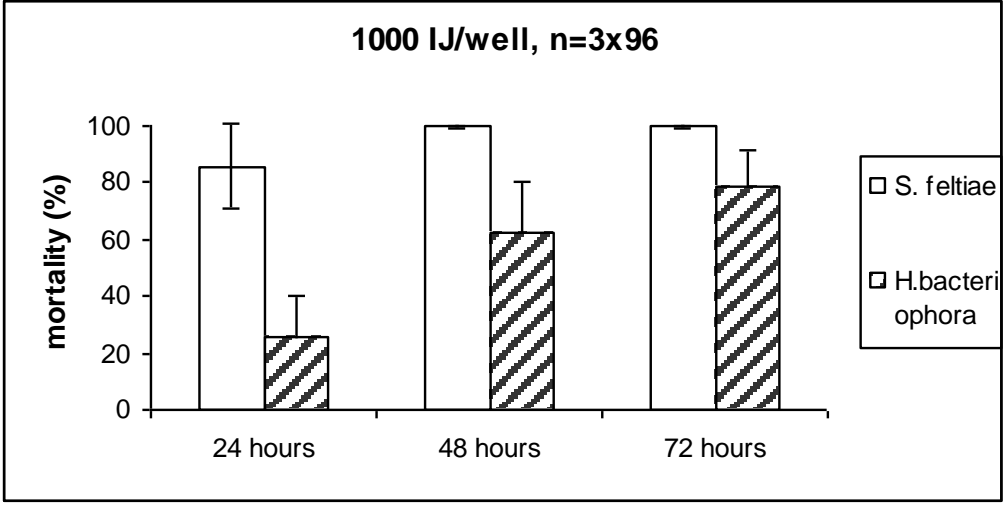


Mortality of *D. melanogaster* larvae after invasion of *H. bacteriophora* or *S. feltiae* in different media: agarose, agar or moistured tissue paper. The dose used is 100 IJs/1 larva (mean ± S.D.).

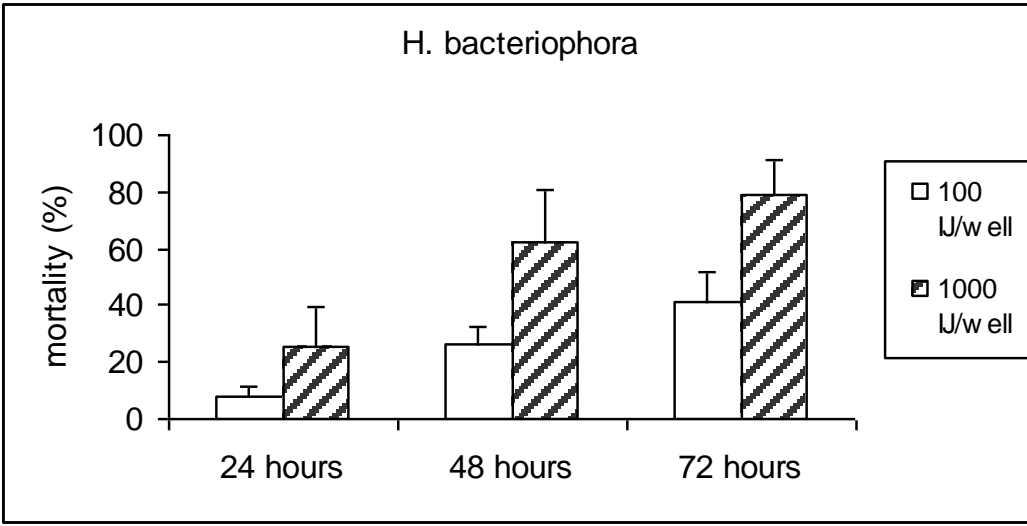


Mortality of control *D. melanogaster* larvae in different media: agarose, agar or moistured tissue paper (mean ± S.D.).

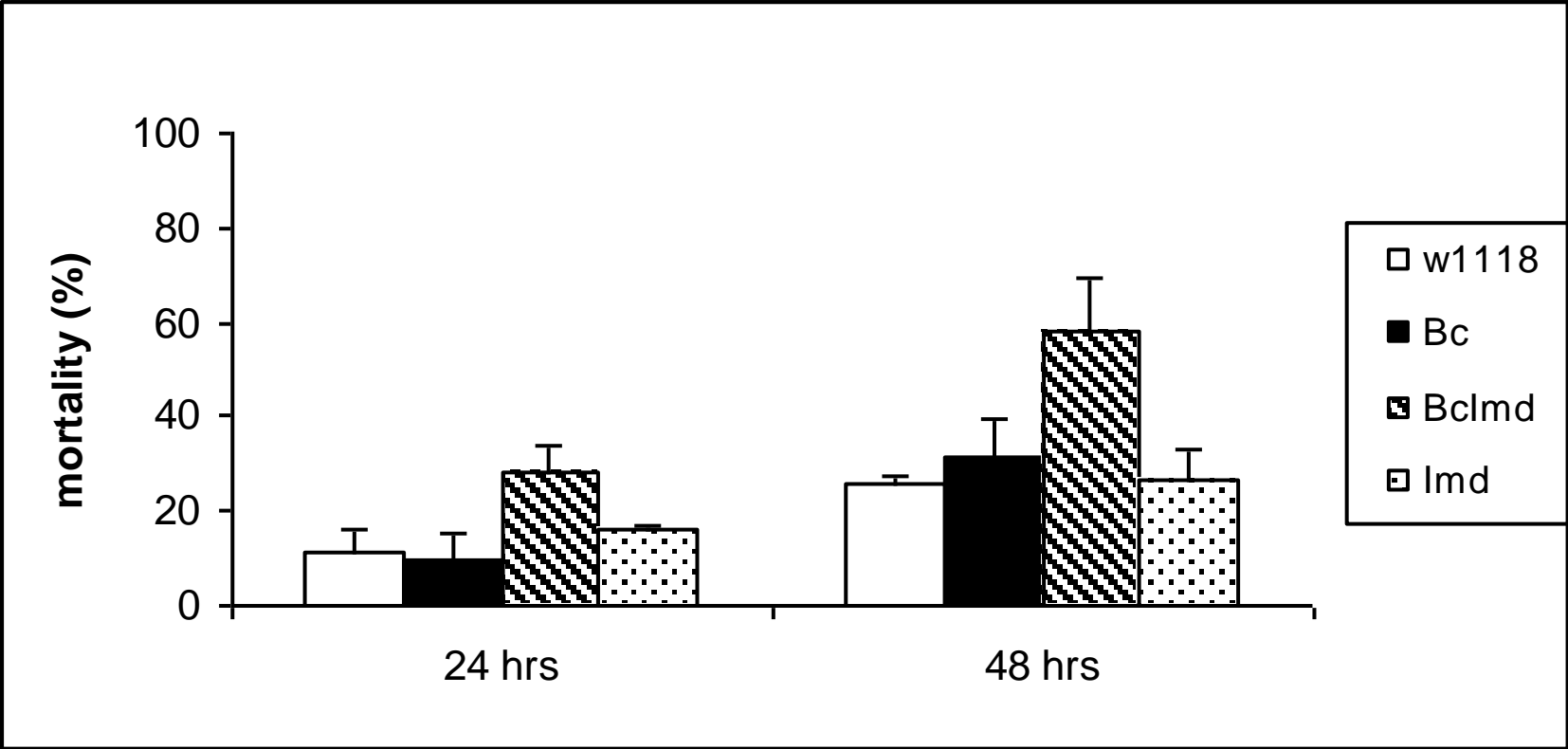
Optimization – dose (w^{1118} , lab. temp. 100 or 1000 IJ/w):



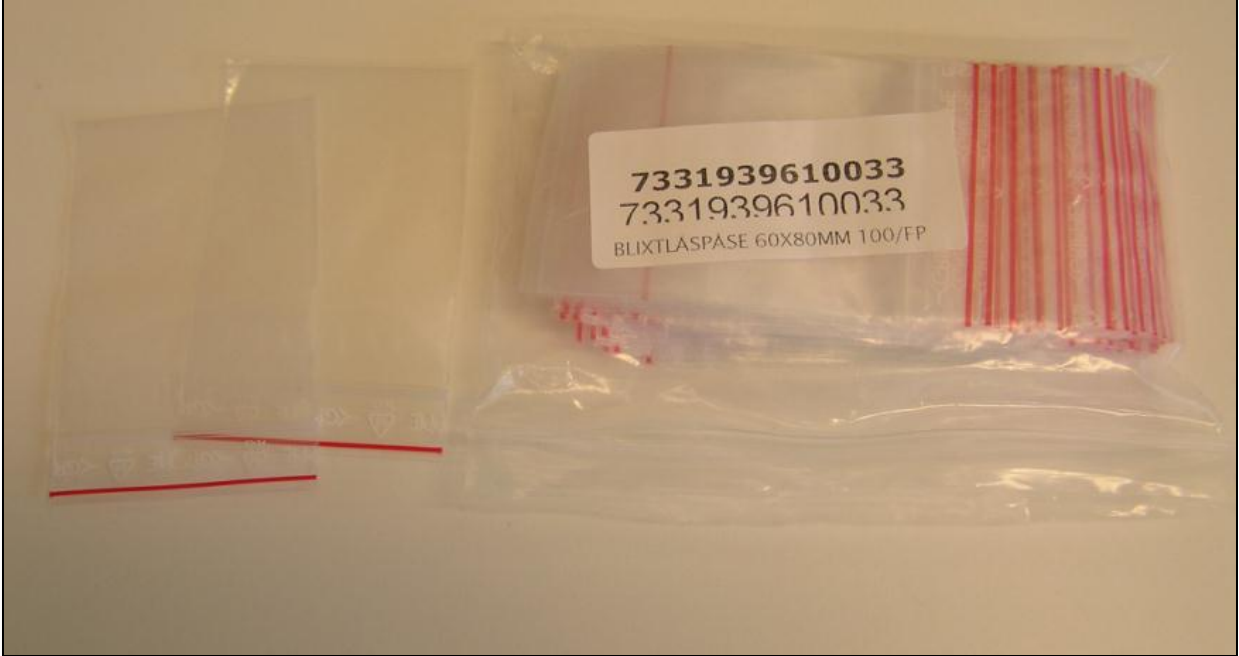
Mortality of *D. melanogaster* larvae after invasion of *S. feltiae* or *H. bacteriophora* in tissue paper using the dose 100 or 1000 IJs per larva (mean \pm S.D.).



(25°C, 100 IJ/w):

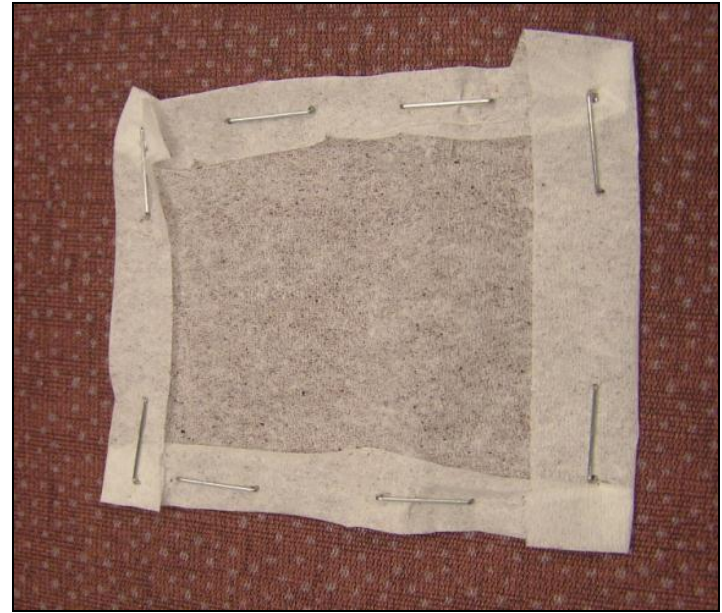


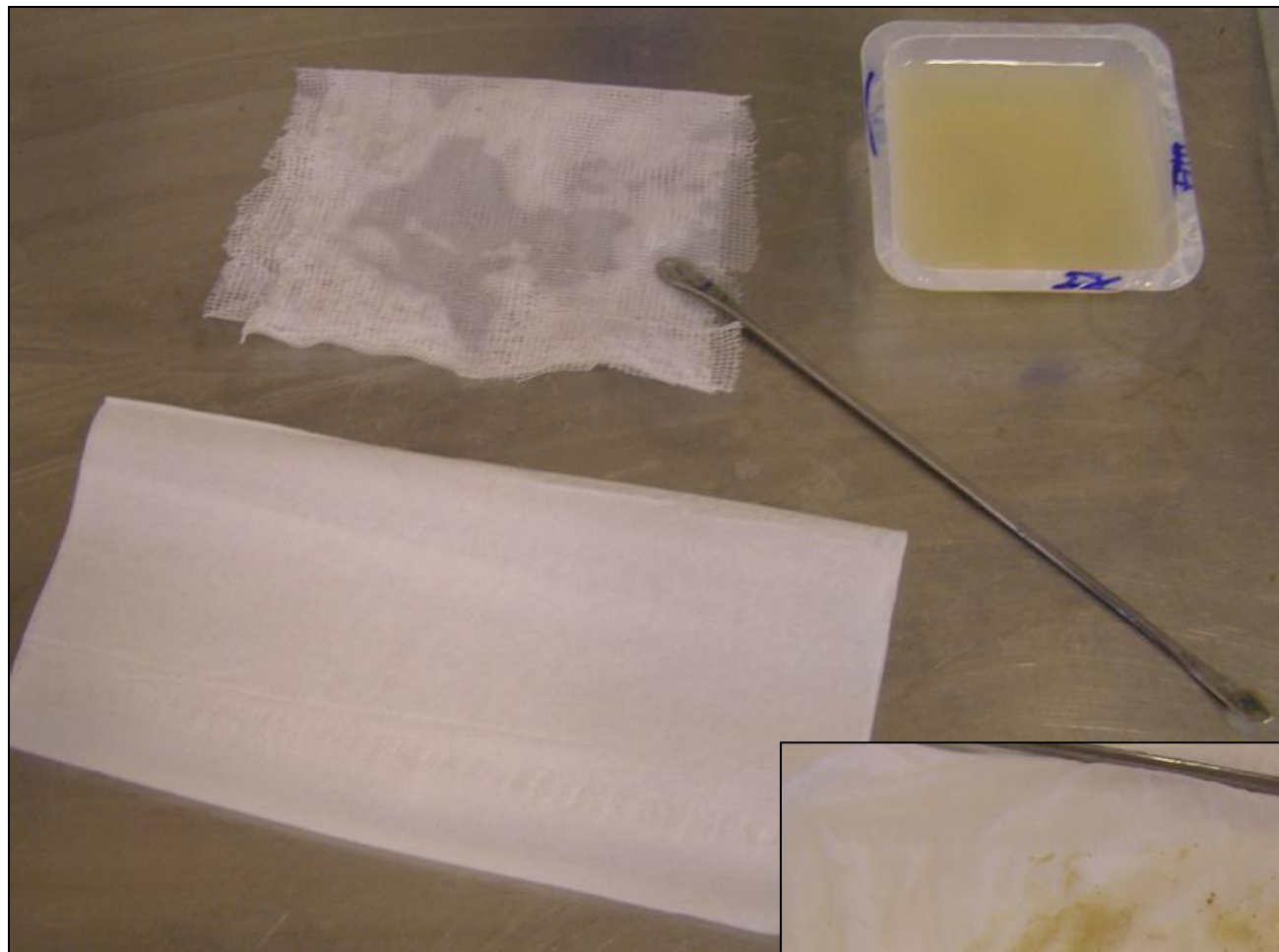
Scale down of nematode assay using plastic bags





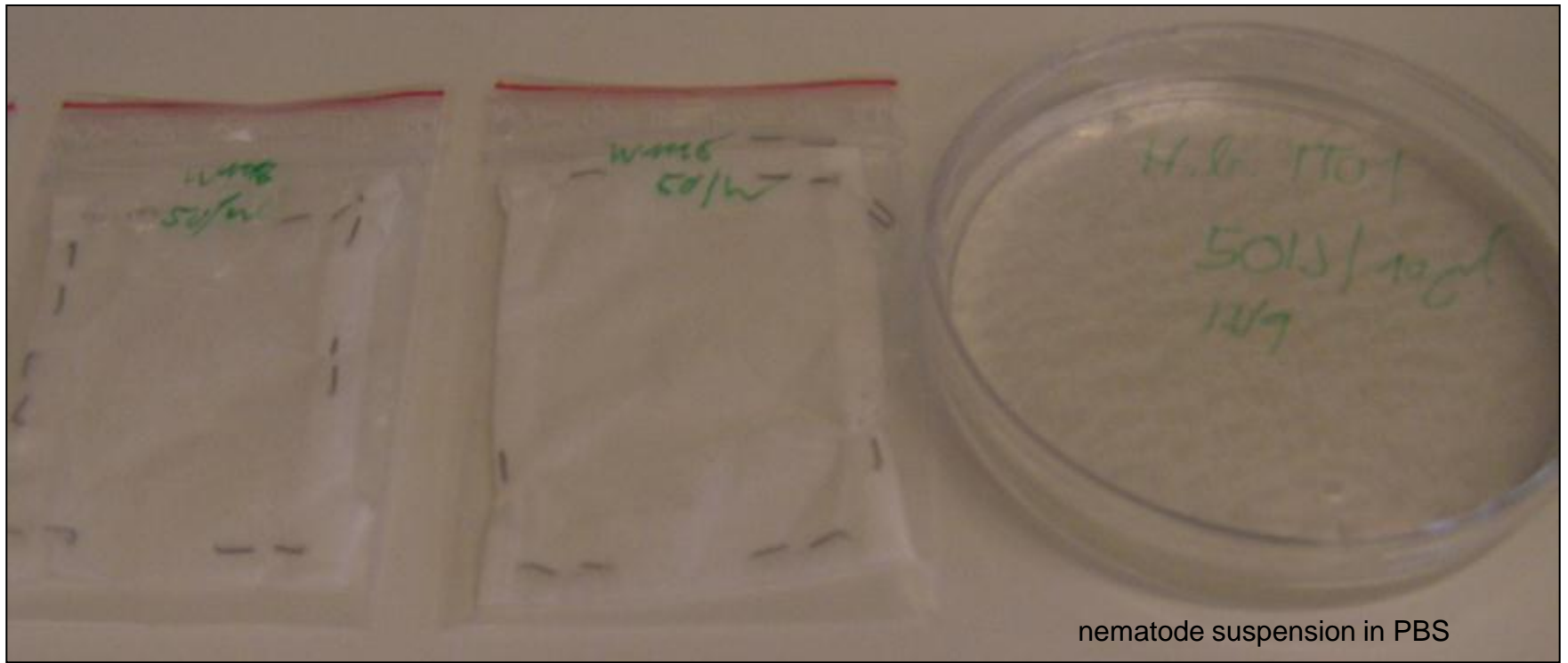
only one layer of tissue paper should be used...



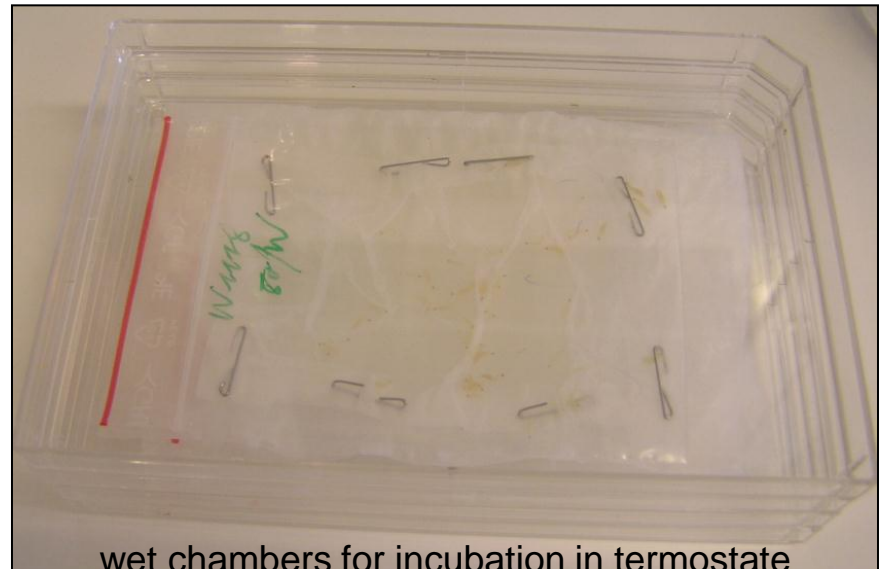


D.m. larvae should be washed out from the flyfood (using a sieve) and then by soft forceps transported into the bags





nematode suspension in PBS

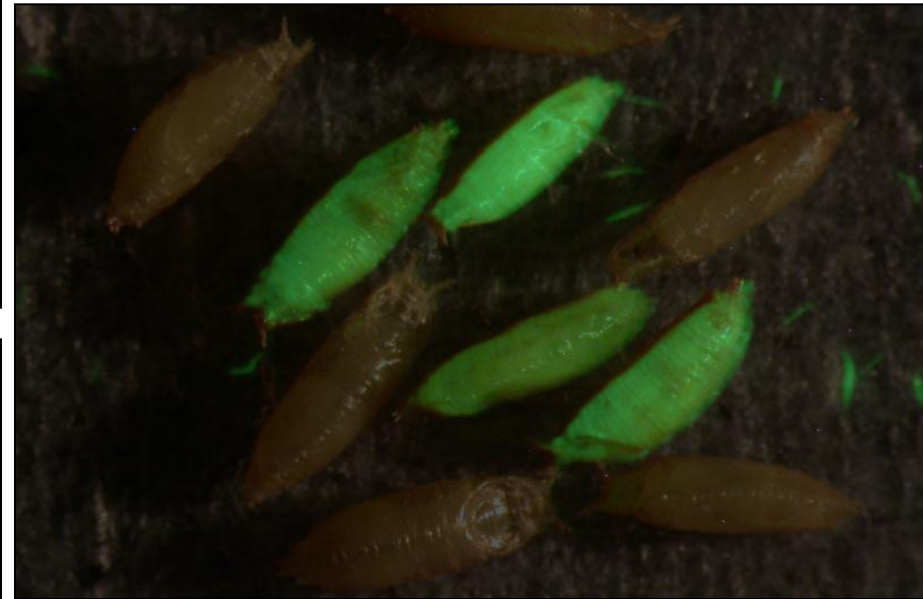
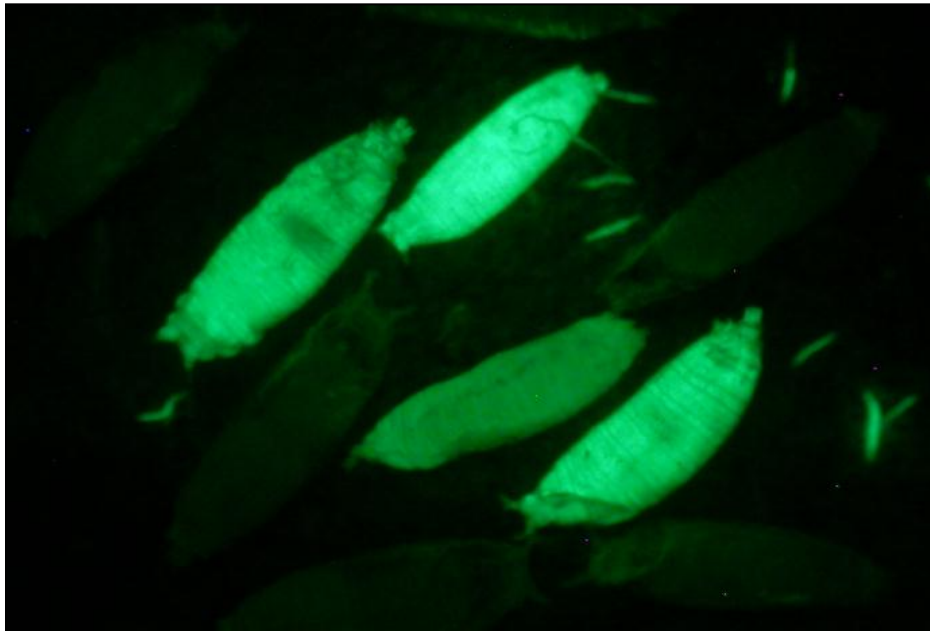


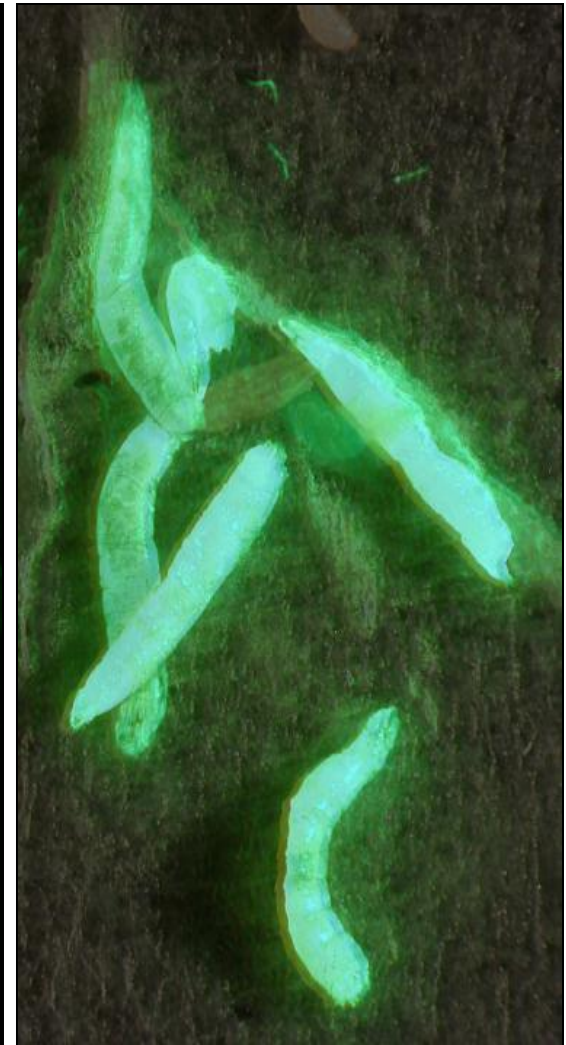
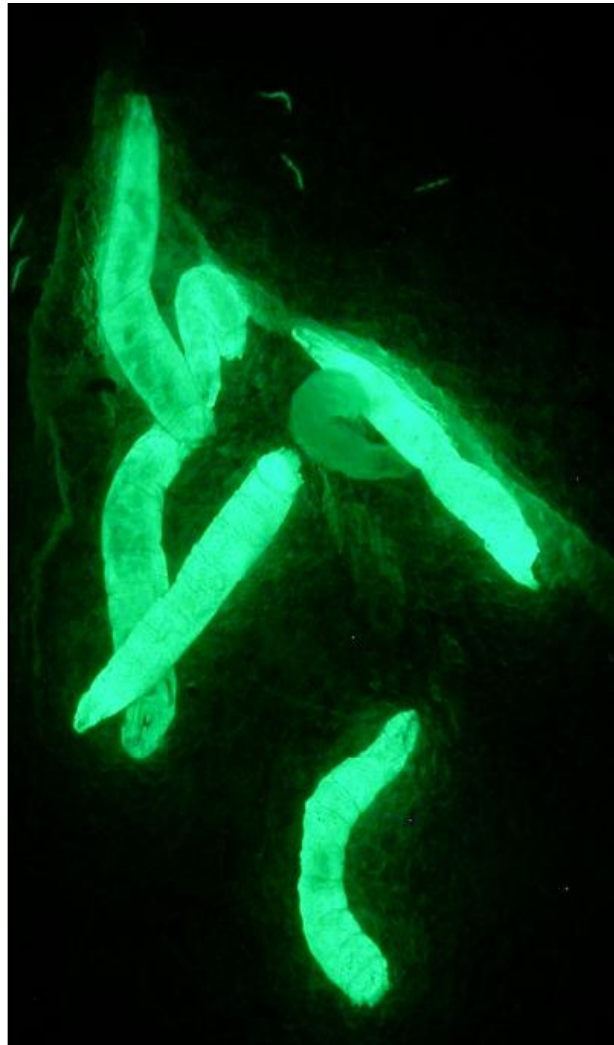
wet chambers for incubation in termostate



mortality counting under fluorescence microscope

- day light
- GFP plant
- merged





mortality counting under fluorescence microscope - day light, GFP plant, merged

Plastic bags settings:

- folded tissue paper
- *Heterorhabditis bacteriophora* TT01
- 25 IJs / 1 *Drosophila* larva / 29°C
(25 IJs in 10µl PBS/ 500 µl per bag)
- 50 *Drosophila* larvae / bag
- only 48 hrs mortality counting
under fluorescence microscope
- in triplicates

