

BED BUGS—EFFICIENCY OF NATTARO SAFE

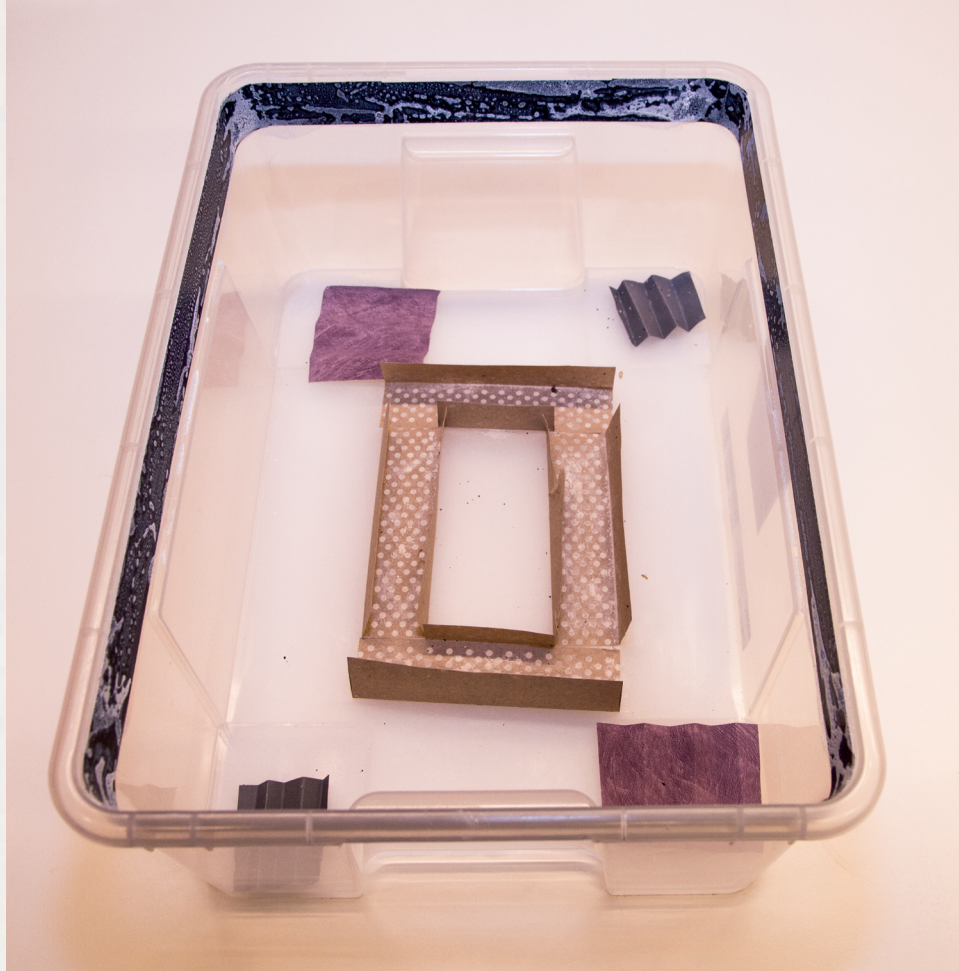


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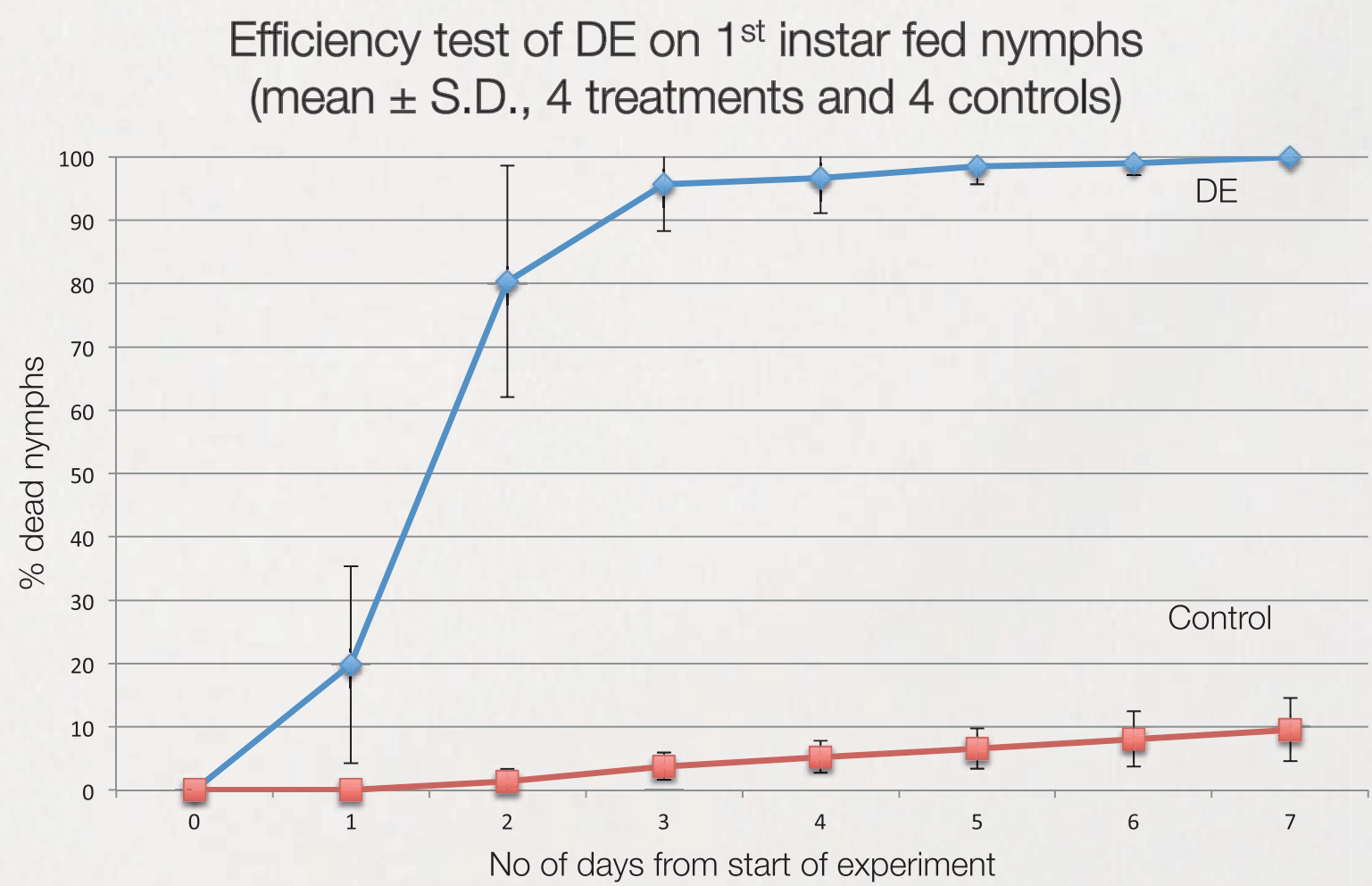
Following a very successful field trial with Nattaro Safe (NS), our Diatomaceous Earth (DE) filled tape, installed on beds in refugee accommodations, we wanted to verify the results in our lab. Our expectations were that the life cycle is cut short at one or more stages, hence preventing an increase in numbers and eventually eradicating the infestation as such. We tested the efficiency of NS to prevent 1) newly fed 1st instar nymphs from further development and 2) the founding and development of new bed bug colonies (life cycle test): Furthermore, we tested the efficiency of our DE to kill fed and hungry adult females and males enclosed in containers with DE. All 1st instar nymphs were killed within 7 days of exposure and our life cycle test showed that DE loaded Nattaro Safe efficiently prevented buildup of bed bug numbers in an artificial infestation initiated by 10 virgin females and 10 virgin males. Last we found that fed female bed bugs are the most persistent and hungry males the least persistent of adult bed bugs.

We conclude that Nattaro Safe efficiently prevents the buildup of new populations of bed bugs thanks to disruption of the bed bugs life cycle, fast for 1st instar nymphs and slower for adults.

Experimental mini-cosmos



Nymph and life cycle tests were performed in minicosmos in which four 12 x 3 cm pieces of Nattaro Safe (NS) formed a square at the bottom. Pieces of folded paper provided artificial harbourages. Bed bugs were introduced into the middle of the square. Our experiments simulate the bedroom situation in which adults and nymphs are contaminated with DE when they pass through NS on their way to feast on sleeping humans in the bed and again when they return to their harbourages below the bed.



Nymph tests: survival in mini-cosmos

Fifty to sixty 1st instar nymphs were released inside a square of Nattaro Safe loaded either with DE (treatment) or baking powder (control). One hour later the nymphs were recollected, fed on human blood and released anew inside the NS square. All dead nymphs in the boxes were counted and removed every day. When all DE treated nymphs had died the experiment was terminated and the numbers of living nymphs in controls were counted.

We found a significant difference in survival between DE treated and control treated nymphs ($p=0.00673$). All nymphs in the DE treated mini-cosmos died between day 2 and day 7. In the controls only 4 to 16 % of the nymphs had died on day 7.

Test statistics of DE survival curves compared to their respective controls. The trials were run on fed 1st instar nymphs. N=4 treatments and 4 controls each with 50-60 1st instar nymphs.

Treatment	Amount of DE (g/m ²)	N (Obs. deaths)	X ²	df	p-value (sign. level)
DE ^a	13	4 (4)	7.3	1	0.00673 (**)
Control ^b	0	4 (0)			

Field trial – Pilot study with Swedish Migration Board

59 apartments, of which all beds in 15 had Nattaro Safe (NS) mounted, were followed for 7-9 months. All apartments were treated for bed bugs before start of the study. Only one apartment in which NS was mounted was in need of a treatment while all without NS were treated 1-4 times during the same period



Percentage of treated apartments with and without Nattaro Safe mounted on the beds

Life cycle experiment in mini-cosmos

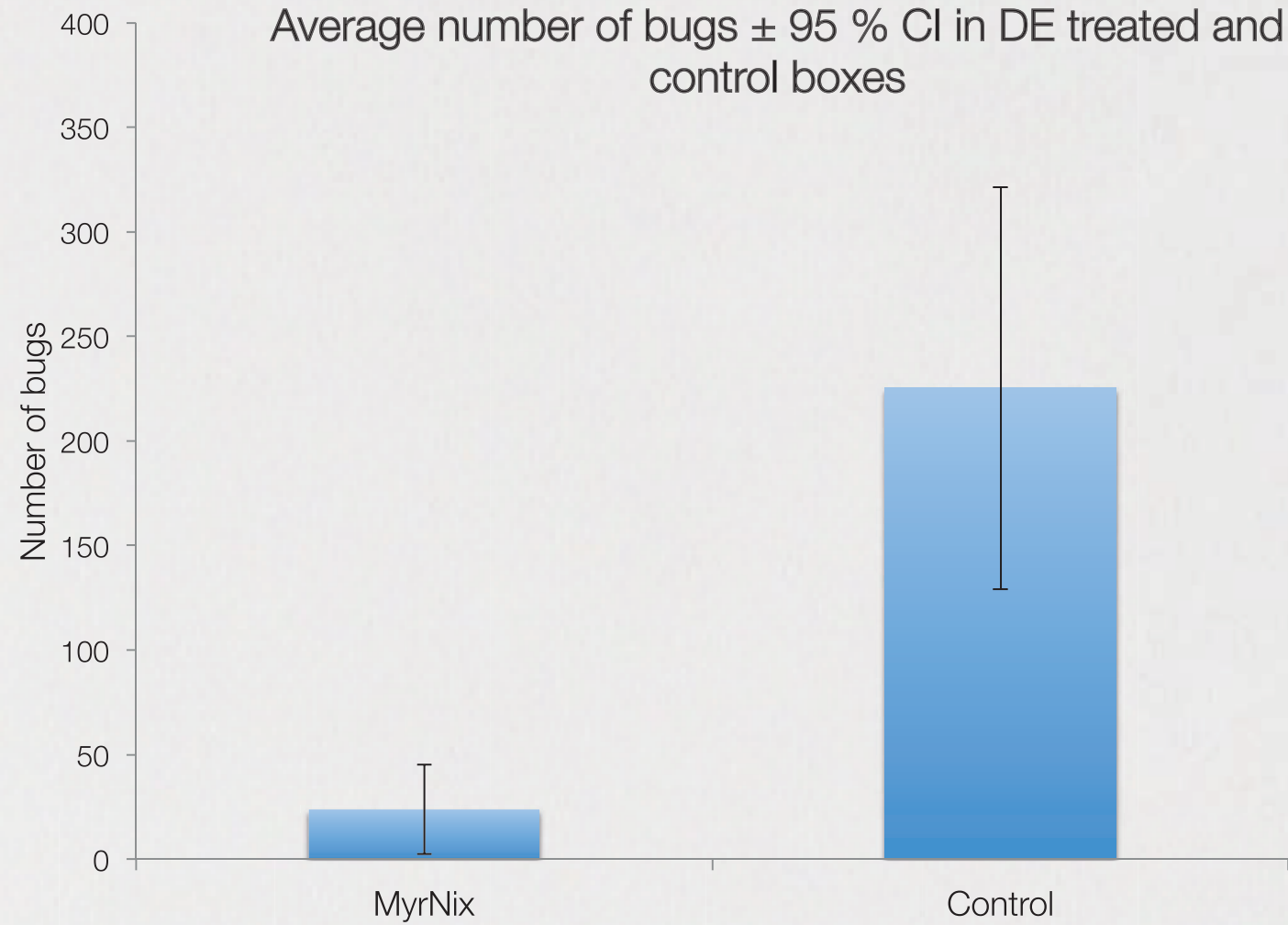
Ten virgin female and 10 virgin male bed bugs were released inside a Nattaro Safe (NS) square loaded either with DE (treatment) or baking powder (control). After one hour the bed bugs were recollected, fed and released anew inside the square of NS. At regular intervals (10.8 d \pm 2.9 d) until all adults and nymphs in the treatment boxes had died the bugs were recollected, counted and fed on human blood.



Nattaro Safe with DE and few signs of bed bugs.

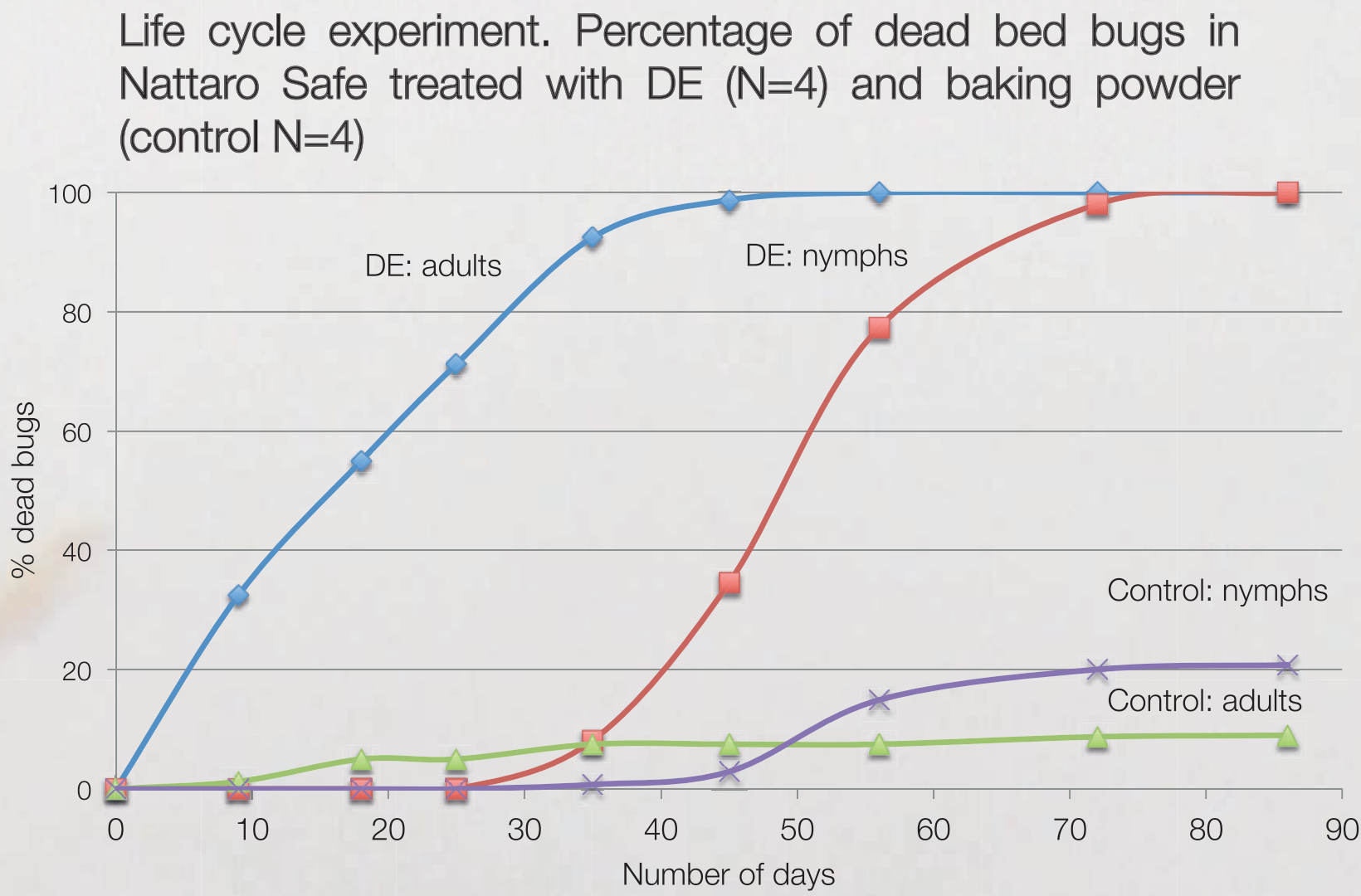


Nattaro Safe with baking powder (control) and a flourishing bed bug population.



There were significant differences in survival and population development of bed bugs in the DE treated NS and their controls (95 % confidence interval, CI). After between 35 and 56 days all adult bed bugs were eradicated in the DE treatment. Significantly (95% CI) fewer nymphs (range 20–38) hatched in boxes with DE treated NS compared to control boxes (range 112–250 nymphs). In the DE treated boxes all nymphs had died 37 to 51 days after the first nymph appeared.

Nattaro Safe loaded with DE was able to eradicate a population established by 20 virgin adults and the nymphs they bred within 76 days. Most nymphs probably were produced by a few females that survived and were able to continue egg-laying before they got contaminated with sufficient DE to kill them. However, only a few of the nymphs reached their second instar before they died. In contrast in the control boxes hundreds of nymphs developed and new adults appeared before the experiment was terminated after 86 days.



Influence of bed bug physiological condition and sex on efficiency of Diatomaceous Earth

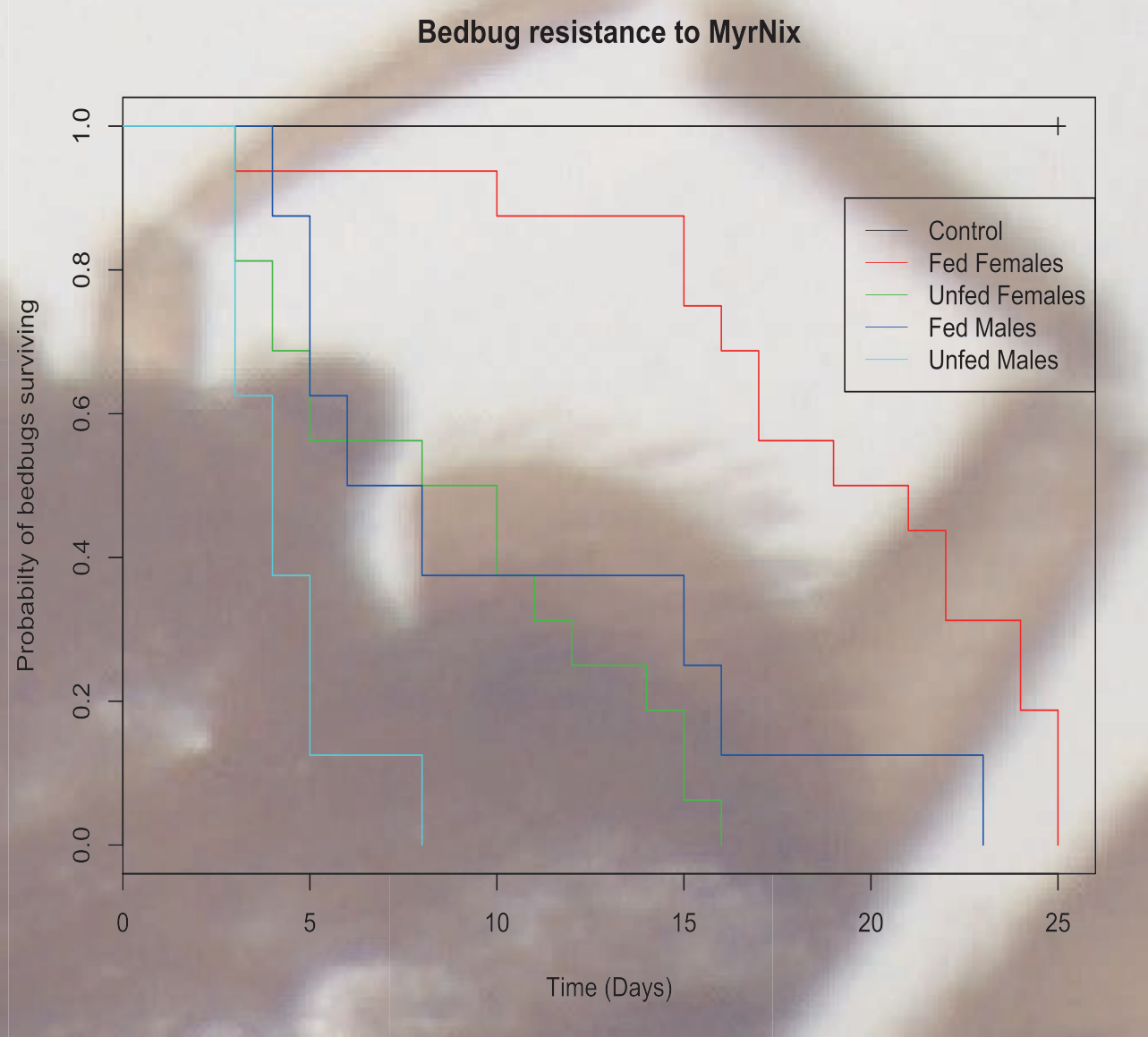
Diatomaceous earth is expected to function by either abrasion of and/or absorption of the outer wax layer that protects insects against water loss. We therefore expected that the physiological state (fed vs hungry) and sex (female vs male) of bed bugs influence their susceptibility to DE. To test that, fed and unfed female and male bed bugs were kept in containers with (treatment) and without (control) DE on a filter paper. Each replicate consisted of six bed bugs that were released and walked in the DE for 45 minutes after which they were transferred into clean containers. Controls without DE were set up similarly. Fed bed bugs were satiated less than 24 hours and unfed 6-7 days before start of the experiment. All were fed on sheep blood.

DE was significantly more efficient in killing the bed bugs than their respective controls ($p<0.001$). Fed females were the significantly most resistant to DE. There was no difference between unfed females and fed males while unfed males were most sensitive.



Left container: DE treated bed bugs.
Right container: control treatment without DE.

Survival curves for the fed and unfed female and male bed bug trials with DE and their controls.



Efficiency of DE on fed and unfed females and males

Treatment	N start	N dead	Sex	Fed	X ²	df	p-value (sign. level)
DE ^a	16	16	female	yes	19.0	1	1.34e-5 (***)
Control ^b	8	0					
DE ^{c,d}	16	16		no	20.2	1	7.1e-6 (***)
Control ^e	8	0					
DE ^{a,d}	8	8	male	yes	11.3	1	0.000785 (***)
Control ^b	5	0					
DE ^c	8	8		no	11.4	1	0.00074 (***)
Control ^e	5	0					

Log-rank test statistics of Kaplan-Meier survival curves comparing treatments to their respective controls. Significance levels between sexes and satiation setups follow the superscripted letters accordingly a-b: $p=3.33e^{-5}$, a-d: $p=0.00599$, a-e: $p=1.62e^{-5}$, b-e: $p=0.013$, d-e: $p=0.0154$ (superscripted letters within brackets are not significantly different with the equivalent letter but do not correspond to the mentioned sign. level).

Conclusion

Our experiments and our pilot study in the field strongly indicate that a mounting of Nattaro Safe on beds will control minor infestations because an infestation is eradicated before the bed bug population reach the exponential growth phase.

We conclude that Nattaro Safe efficiently prevents the buildup of new populations of bed bugs thanks to disruption of the bed bugs life cycle, fast for 1st instar nymphs and slower for adults.

Nattaro Safe is easy to mount on different bed frames and continues to function as long as there is DE left inside. Furthermore, there is little chance that bed bugs develop resistance to DE and it is environmental friendly.