RNA Interference of Potassium Ion Channel Genes in Crop Pests

Douglas Wightman*, Martin Edwards, Angharad Gatehouse | Newcastle University | Biology (Cellular and Molecular) | 130396350 | d.wightman2@ncl.ac.uk |

Aim

To identify an ion channel gene that can be 'knocked down' by RNA interference (RNAi) leading to *Tribolium castaneum* mortality.

Background

T. castaneum (Figure 1) is a crop pest and is responsible for a 10-40% loss of stored grains annually[1]. The current methods of control require insecticides which can be harmful to the environment[2] so alternatives such as RNAi are appealing. RNAi is a natural process of lowering gene expression (knockdown) through post-translational silencing and has already been shown to be effective in *T. castaneum*[3].

The gene (shaker) chosen for knockdown codes for a potassium voltage-gated channel protein which is a vital protein involved in producing action potentials across the nervous system. The hypothesis was that the absence of this protein would lead to malfunctioning neurons and death in the whole organism.

Figure 1. *Tribolium castaneum* captured by a scanning electron micrograph[4].

Figure 2. *Tribolium castaneum* larvae post injection[5]. Actual size=15mm.

Materials and Methods

RNA was extracted from ***T. castaneum*** **instar 6** larvae (Figure 2) which was then converted to cDNA and amplified in a PCR to amplify the shaker gene.

The shaker gene was added into a **pCR2.1 plasmid cloning vector** and combined with competent ***Escherichia coli*** to create a cloning system. **Shaker** was then extracted from the transformed *E.coli* and converted into **dsRNA**.

The **dsRNA concentration** for one **injection** dose was **37ng/larva** and one **feeding** dose was **475ng/larva. qPCR** determined mRNA abundance.

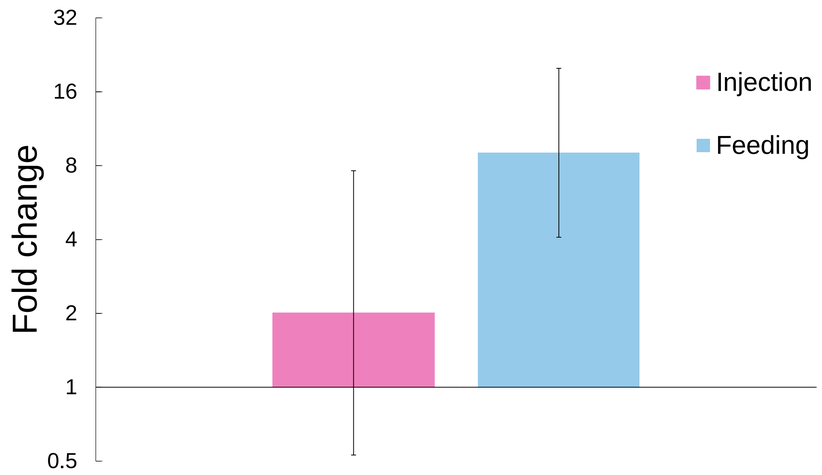

Figure 3. Fold change in shaker gene expression after the application of dsRNA or water as calculated by the ddCt method. n=40.

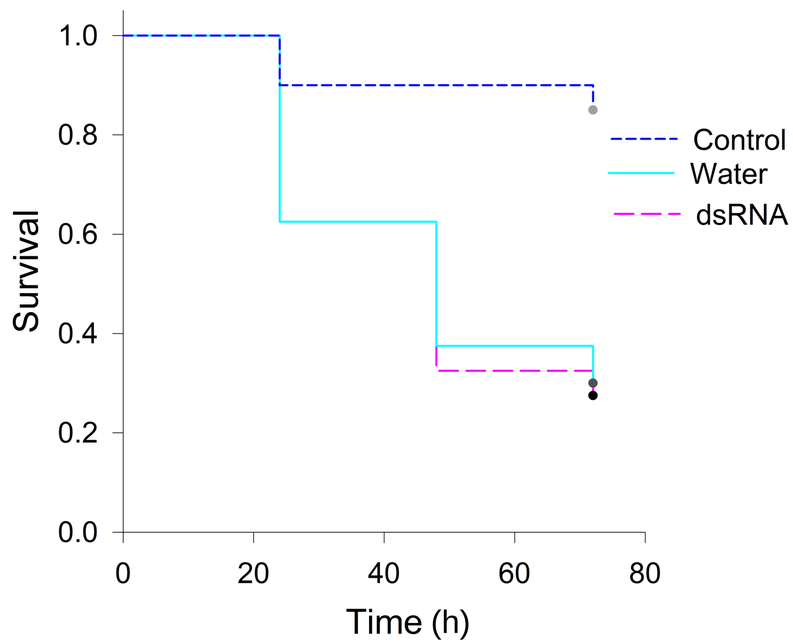

Figure 4. Survival rate after dsRNA, and water injections compared to an uninjected condition. Survival rate calculated through Kaplan-Meier mortality assay. n=40.

Results

Gene regulation assay

The difference between the change in dsRNA and water was not significant and neither was the difference between feeding dsRNA and flour diet (Figure 3).

P=0.104 between dsRNA & water injection.

P=0.133 between dsRNA & flour feeding.

Mortality assay

The difference in mortality between dsRNA and the control were not significant in both the injection and feeding assays (Figure 4).

P=0.821 between dsRNA & water injection.

P=0.576 between dsRNA & flour feeding.

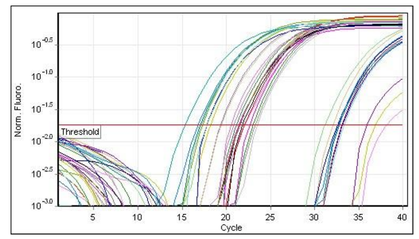

Figure 5. qPCR read out and Thermocycler[6].

Conclusions

Both the **mortality assay** and the **gene regulation assay** showed **no significant difference** between the dsRNA treatments and the controls.

Injecting or feeding **dsRNA did not increase mortality** which would indicate that shaker is not an effective gene to target to treat *T. castaneum* crop infestations. There are other potassium ion channel genes that can counteract the knockdown of shaker which could prevent an increase in mortality, however; there was not significant down-regulation of genes in either assay.

The **doses of dsRNA were low** so further study with **higher doses may cause an increase in mortality.** The number of samples used in the **qPCR** (Figure 5) was low (1 replicate) so **increasing sample size would increase the reliability** of the gene regulation data.

Acknowledgements

Many thanks to the Newcastle University Vacation Scholarship Scheme.

References

1. Mishra BB, Tripathi SP, Tripathi CPM (2012) Repellent effect of leaves essential oils from *Eucalyptus globulus* (Mirtaceae) and *Ocimum basilicum* (Lamiaceae) against two major stored grain insect pests of coleopterons. Nature and Science 10: 50-54.
2. doi:10.2478/v10102-009-0001-7
3. doi:10.1007/10_2013_208
4. http://i.telegraph.co.uk/multimedia/archive/01689/Red-Flour-beetle_1689999i.jpg.
5. http://ibeetle-base.uni-goettingen.de/images/91.
6. http://uncmain.sites.unc.edu/files/2013/07/Haines-lab-machines-440-300.jpg.