

References:
 Bensoussan N, Dixit S, Tabara M, Letwin D, Milojevic M, Antonacci M, Jin P, Arai Y, Bruinsma K, Suzuki T, Fukuhara T, Zhurov V, Geibel S, Nauen R, Grbic M, Grbic V (2020) Environmental RNA interference in two-spotted spider mite, *Tetranychus urticae*, reveals dsRNA processing requirements for efficient RNAi response. *Scientific Reports* 10(1):19126. doi: 10.1038/s41598-020-75682-6.

Bensoussan N, Zhurov V, Yamakawa S, O'Neil CH, Suzuki T, Grbic M and Grbic V (2018) The Digestive System of the Two-Spotted Spider Mite, *Tetranychus urticae* Koch, in the Context of the Mite-Plant Interaction. *Frontiers in Plant Science* 9:1206. doi: 10.3389/fpls.2018.01206

Grbić M, Van Leeuwen T, Clark RM, Rombauts S, Rouzé P, Grbić V, and 49 more. (2011) The genome of *Tetranychus urticae* reveals herbivorous pest adaptations. *Nature* 479:487-492.

Utilizing RNAi Technology to Develop Novel Agricultural Pesticides

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Background Information

Two-spotted spider mites (*Tetranychus urticae*) are a polyphagic agricultural pest that cause significant financial losses for agriculture

Polyphagic:
 able to eat from a diverse array of plants. Spider mites are unique, as most pests are only adapted to deal with defence compounds from one or a few plant species

Issues With Conventional Pesticides

Conventional treatments such as chemical pesticides are ineffective, as spider mites have an expanded gene family to digest, sequester, or excrete toxins

They are also capable of rapid reproduction to maintain high populations, resulting in significant plant damage

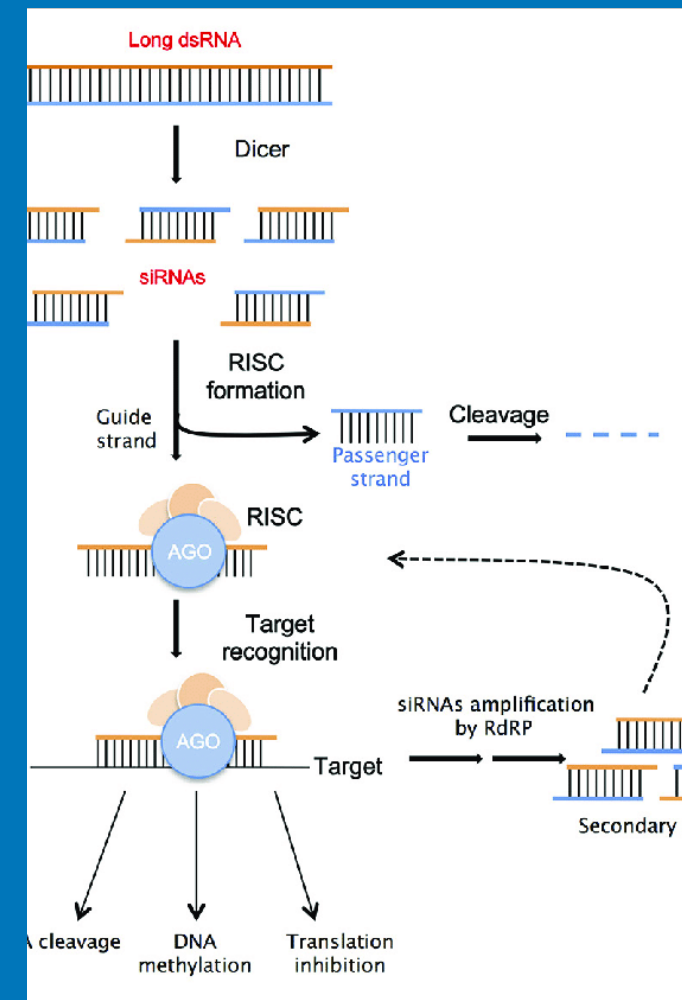
RNAi Significance

Unlike conventional chemical treatments, RNA interference (RNAi) treatments target specific mRNA products from necessary genes, killing spider mites without harming other biological life

Spider mites are capable of ingesting the miRNA via spraying them or leaves they inhabit, ensuring practical viability

Overview of RNAi Function

- Double stranded RNA (dsRNA) is manufactured in a lab. Depending on the gene we want to target, it contains code that corresponds to the target
- dsRNA can be orally introduced to spider mites via solution
- Once inside of spider mites, the dsRNA activates the endogenous Dicer in spider mites, which cleaves the dsRNA into 19-25bp fragments called short interfering RNAs (siRNAs)
- siRNAs are then integrated into an RNA induced silencing (RISC) complex which can bind to complementary (targeted) mRNA transcripts and prevent expression, such as by cleaving the sequence



Available from: https://www.researchgate.net/figure/Schematic-illustration-of-RNAi-mechanism-Double-stranded-RNA-dsRNA-molecule-binds-to_fig2_319124036 [accessed 8 Aug, 2022]

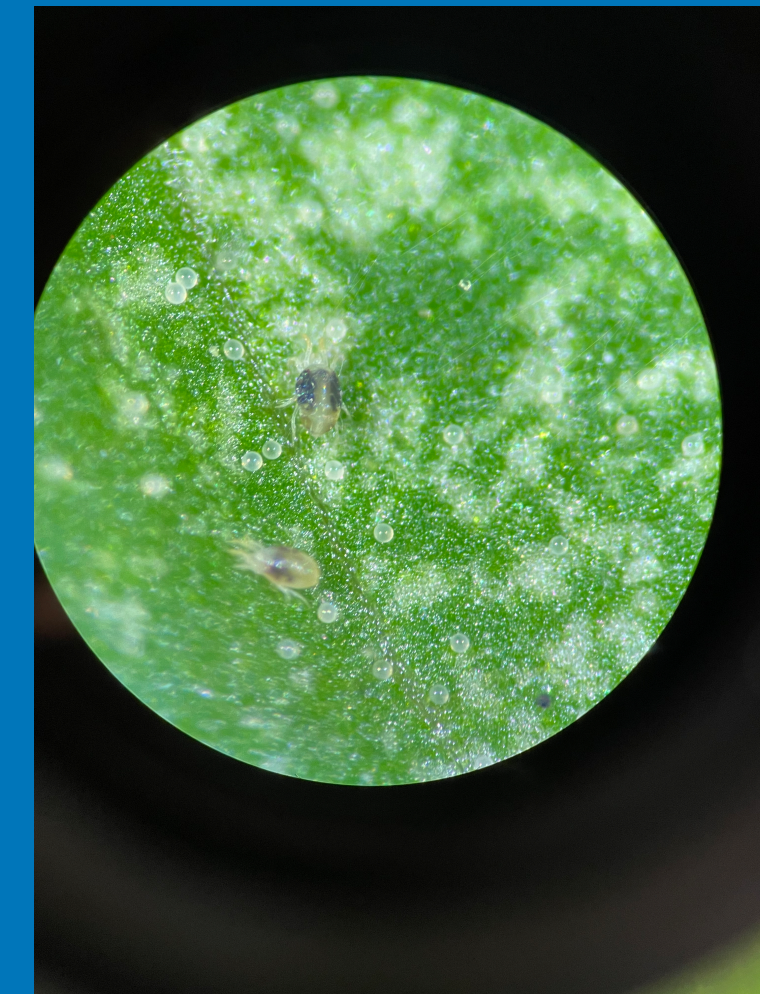
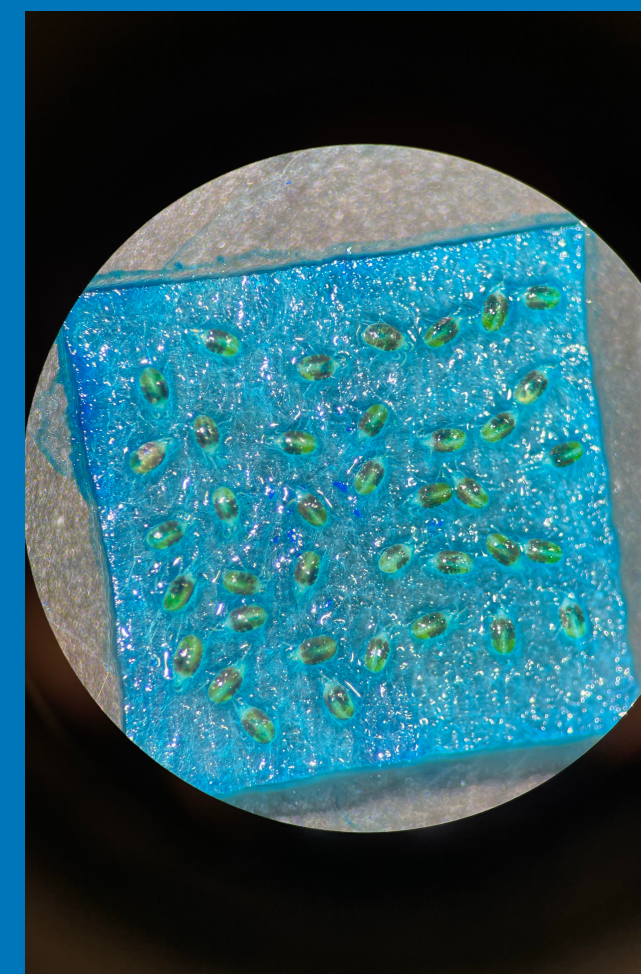


Results of effective dsRNA treatments

- Effective dsRNA treatments resulted in a dark spotting phenotype, and during observation lay much fewer eggs and having a lower survival rate

RNAi in the Lab

- To test the efficacy of RNAi, our lab subjects spider mites to solution with dsRNA that targets different gene targets



- Ineffective dsRNA treatments have no significant effect on spotting, showing a similar phenotype to control mites. Fecundity and survival rate are not different to control mites