Development of an AS-qPCR assay as a diagnostic tool to determine etoxazole resistance in *Tetranychus urticae* (two-spotted spider mite) populations

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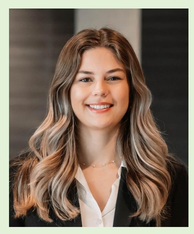


What is *Tetranychus urticae?*

The two-spotted spider mite (*Tetranychus urticae*, TSSM) is a **significant agricultural pest**, posing a threat to over 1,100 plant species worldwide[1].

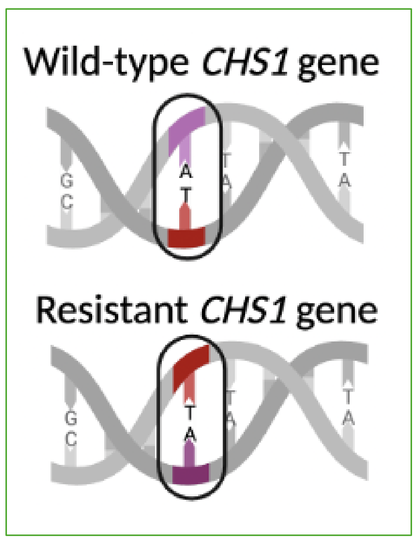
To manage TSSM populations, farmers often **depend on pesticides**; however, the mite's short life cycle and high reproductive capacity allow it to **quickly develop resistance**, challenging effective pest control[2].

What is Etoxazole?

Etoxazole is a widely used **pesticide** for controlling TSSM. As an ovicidal agent, it targets TSSM eggs by inhibiting chitin synthesis, essential for the mite's exoskeleton development[3].

Resistance to etoxazole is **linked** to a single nucleotide polymorphism (SNP) in the **chitin synthase 1 (*CHS1*) gene**[4].

Current detection methods for etoxazole resistance can take up to ten days, highlighting the need for rapid, cost-effective testing. Improved methods would allow growers to quickly identify and apply the most effective pesticide for TSSM infestations.

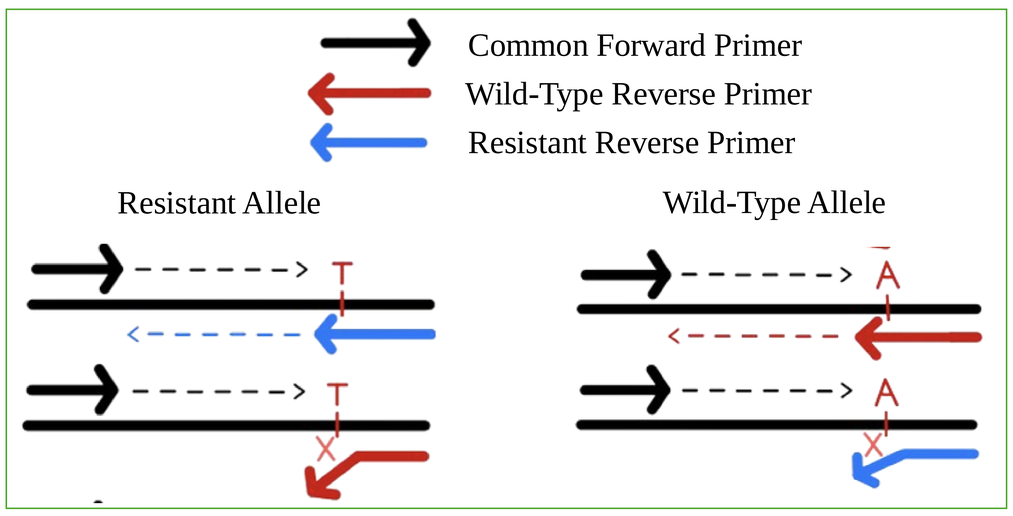


Objective:

Develop an efficient, reliable, and fast molecular assay for the detection of etoxazole resistance in TSSM populations.

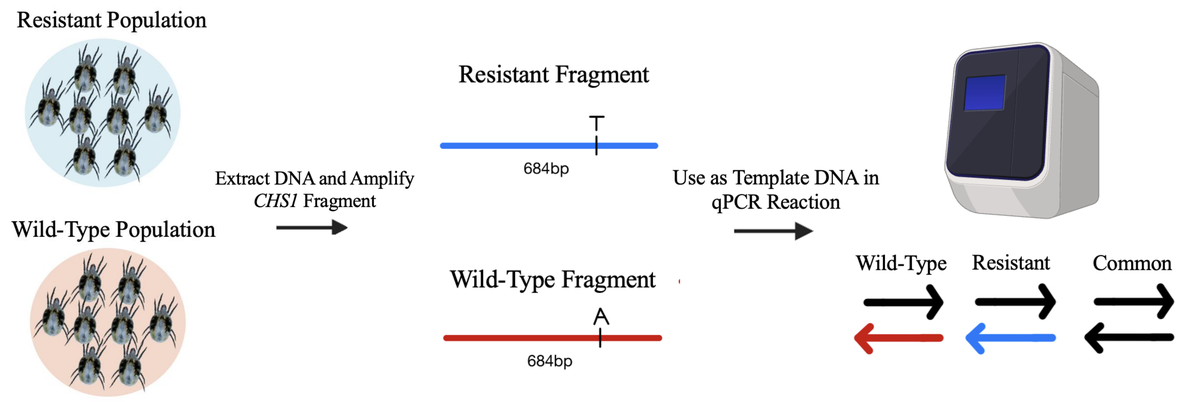
Assay Development

Previous studies demonstrated that **Allele-Specific qPCR (AS-qPCR)** is effective in **identifying SNPs**, providing a highly specific and rapid detection method essential for pest management[5]. However, AS-qPCR requires precise primer optimization to avoid non-specific amplification, which can be challenging to achieve.



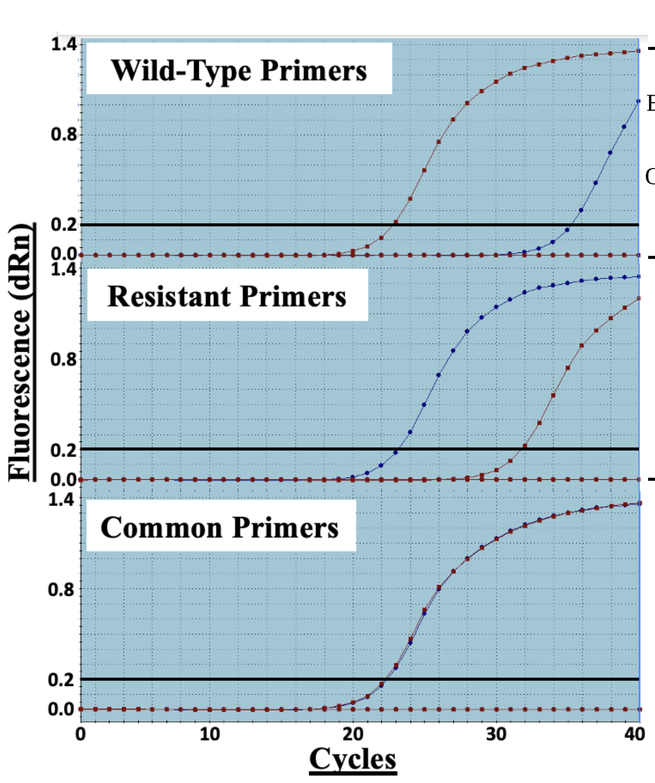
Two allele-specific primer pairs were designed: one for the resistant allele and one for the wild-type. Both pairs share a forward primer, with the **SNP** positioned at the **3' end** of their **specific reverse primers** to allow **allele discrimination**. A common primer pair was also designed to amplify both alleles as a positive control.

Assay Validation



Template DNA was purified and sequenced to confirm the presence or absence of the SNP. Both wild-type and resistant DNA templates were then used in **SYBR qPCR reactions**, testing each of the three primers in triplicates to **validate primer differentiation**.

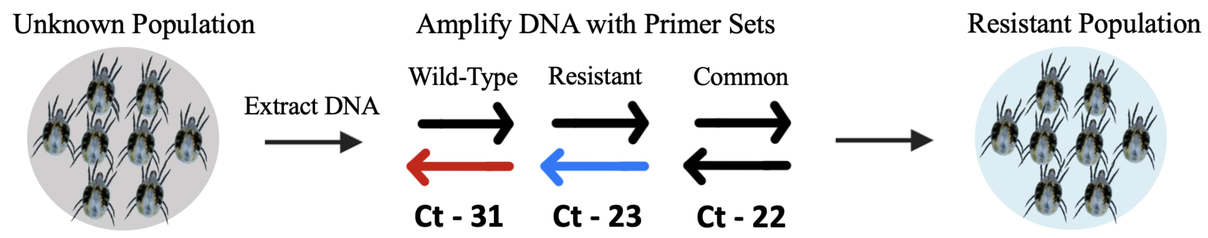
Results



	Wild-Type Template	Resistant Template
Expected →	↓ Ct Value	↑ Ct Value
Observed →	22.85	35.30
Wild-Type Primers	↑ Ct Value: 31.80	↓ Ct Value: 23.21
Resistant Primers	↓ Ct Value: 22.44	↓ Ct Value: 22.30
Common Primers		

The amplification plots confirm the effectiveness of each primer set in distinguishing between wild-type and resistant alleles. **The Cycle Threshold (Ct) values aligned with expected outcomes, validating the functionality of all three primer pairs in our assay.**

Significance



This method allows for the **detection of TSSM resistance to etoxazole** within 2 days, compared to the traditional 10 days, completing my objective and enabling growers to make informed pesticide decisions and therefore **minimize crop losses**.

References

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