Rapid field diagnostics for invasive fly detection

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1. Introduction

Insect molecular diagnostics is a new field that can be instrumental in monitoring the presence of invasive agricultural insect pests. A niche, yet significant application of molecular field diagnostics is in tephritid fruit fly detection and strain discrimination to support fruit fly exclusion and specialty crop protection efforts. To demonstrate the application of molecular field diagnostics to invasive species detection, we designed a single locus assay to differentiate between two strains of the same species, *Bactrocera dorsalis* (Fig. 1). Allele discrimination was performed using the MatMaCorp Solas 8 (Fig. 2), which uses iso-thermal amplification and fluorescence-based allele detection. The results of this study can be used to inform and improve various aspects of insect pest management practices to optimize accurate insect strain identification.

Figure 1: *Bactrocera dorsalis*

2. Workflow

Figure 2: MatMaCorp Solas 8

- A MatMaCorp custom C-SAND SNP assay was designed around a diagnostic locus discriminating two strains of *B. dorsalis*, a wild-type strain, and a genetic sexing strain (GSS) with a sex-linked pupal color polymorphism (Fig. 3) created for the purpose of releasing for the Sterile Insect Technique.

Figure 3: Sex-linked diagnostic phenotype with known causative mutation.

- The assay was tested on weathered flies with a heterozygous genotype at the locus of interest for up to 21 days to ascertain the number of weeks that sample quality is maintained and accurate discrimination can made.
- Both the MatMaCorp MagicTip (MT) DNA Isolation Kit and a traditional Kingfisher (KF) magnetic bead based nucleic acid extraction method were applied to test for differences sample quality and quantity over time.
- Solas 8 data was complemented with traditional qPCR to determine copy number of the target locus in the weathered samples to compare with the sensitivity of the Solas 8 in detecting the allele.

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3. Results

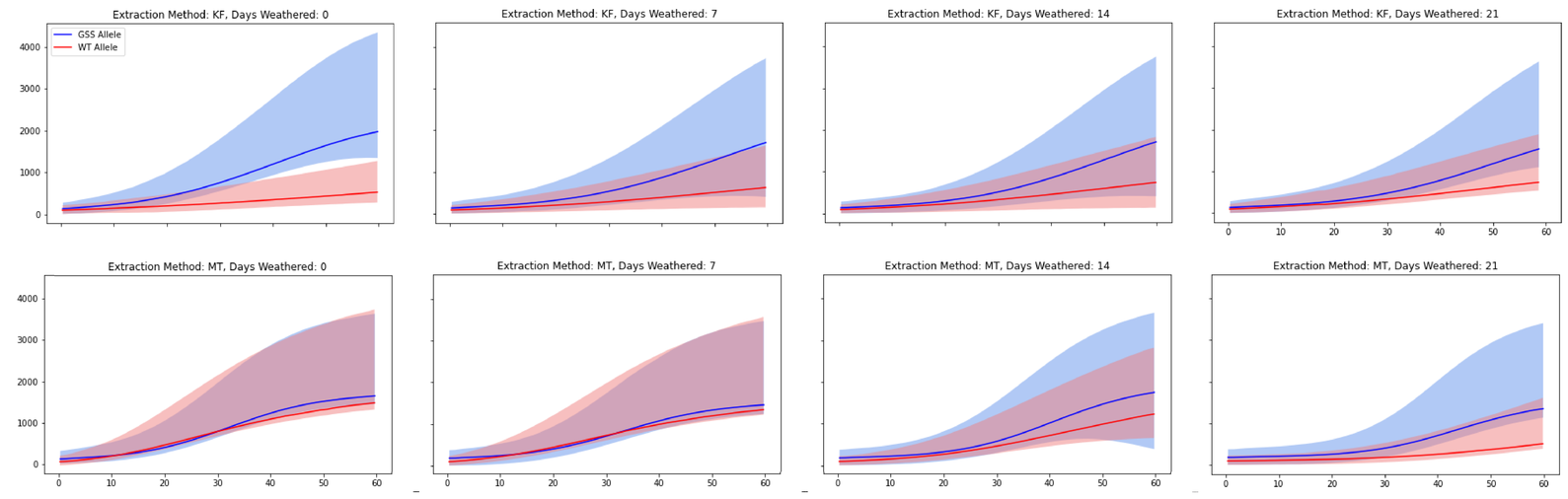

Figure 4: Allele amplification curves of heterozygous samples (N = 8 for each week) over three weeks of sample weathering.

- The samples extracted with the KF displayed higher DNA concentrations than the MT extractions, but they both performed similarly with the KF having fewer failed samples than the MT extractions.
- With the KF samples, the the shorter GSS allele was preferentially amplified, but this only affected the sample discrimination on non-weathered flies (Fig. 4).
- With the MT samples, both alleles were amplified equally until Day 21 at which point the wild-type was no longer detectable (Fig. 4).
- Validation of results and quantification of the target copy count for each sample using traditional qPCR revealed that regardless of copy count, the Solas 8 made the correct designation until the third week at which point both extraction methods failed to accurately determine strain identity (Fig. 5).
- Differences in target fragment size likely contributed to a decrease in accuracy when DNA concentration is high.

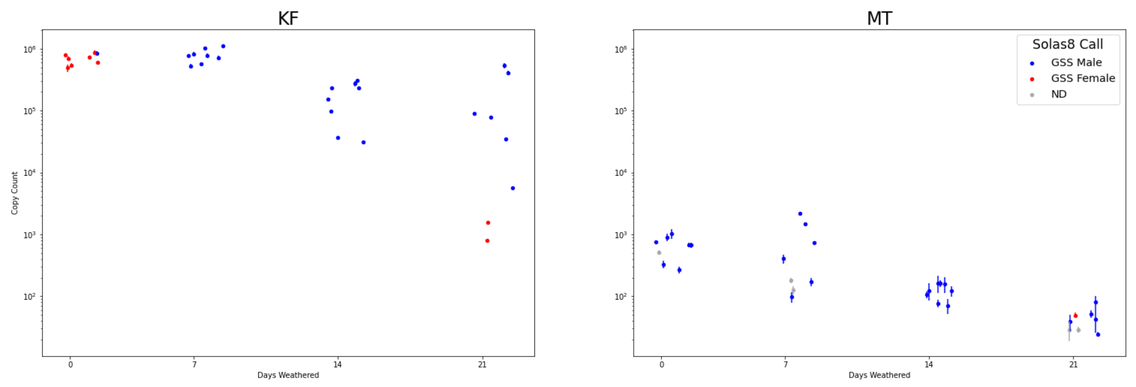

Figure 5: Degradation of target locus copy number over time.

4. Conclusions

1. The MatMaCorp Solas 8 and C-SAND assay were demonstrated to accurately differentiate between fruit fly strains of the same species after two weeks but no more than three weeks of sample degradation under Hawaii weather conditions.
2. The MagicTip field-based nucleic acid extraction method produced similar results to traditional magnetic bead based extraction methods.
3. Using a standard curve and the weathered samples amplified and detected using qPCR, we concluded that in this study, the Solas 8 can detect an amplified target with a starting amount of as few as 384 copies of the target locus.