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Analysis of competence in two potential insect vectors of Soybean vein necrosis virus

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Analysis of Competence in Two Potential Insect Vectors of Soybean Vein Necrosis Virus

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I. Background

Soybean vein necrosis virus-thrips interaction

- Soybean growers in the United States are facing a potential new threat to production due to an emerging viral disease, Soybean Vein Necrosis Virus (SVNV) (Fig. 1) [1].
- SVNV is transmitted from plant-to-plant via an insect species, soybean thrips (*Neohydatothrips variabilis)* (Fig. 2) [2].
- There is however, another potential thrips vector in soybean fields, tobacco thrips (*Frankliniella fusca*) (Fig. 3) [3].

Fig. 1. SVNV symptoms on soybean leaf.

Fig. 2. Soybean thrips adult and nymphs.

Fig. 3. An adult tobacco thrips.

Objectives

- Determine the time required for acquisition of SVNV molecules also called Acquisition Access Period (AAP) by soybean thrips and tobacco thrips.
- Determine the time required for successful transmission of SVNV to a healthy plant also termed Inoculation Access Period (IAP).

II. Vector Competence Assay

Acquisition Access Period

- First instar nymphs (n=10-15) of soybean thrips and tobacco thrips were exposed to SVNV-infected leaf tissue to acquire the virus for 24 hours i.e. Acquisition Access Period (AAP).

Latency

- The nymphs were then transferred to SVNV-uninfected leaf tissue until they reached adulthood, which allowed sufficient time for virus replication within the insect.

Inoculation Access Period

- Adult soybean thrips and tobacco thrips (n=10-15) were individually transferred to SVNV-uninfected leaf tissue to infect or inoculate the tissue with virus for 72 hours.

III. Experimental Workflow

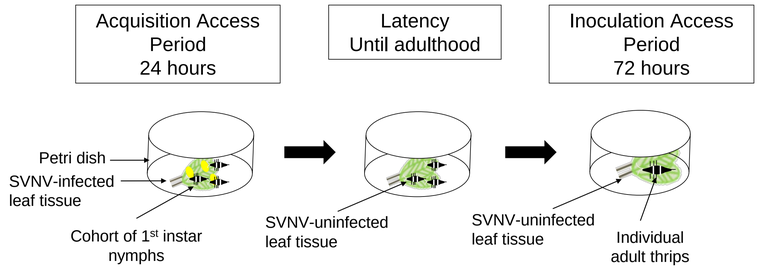

Fig. 4. Experimental workflow depicting test of vector competence in thrips species.

IV. Cloning of SVNV Gene

- RNA was extracted from SVNV-infected thrips and the SVNV gene was isolated using RT-PCR.
- SVNV nucleocapsid protein gene was cloned into E.coli using TOPO cloning kit (Fig. 5).
- E.coli was grown on LB agar plates and we obtained several positive colonies (Fig. 6A and B).

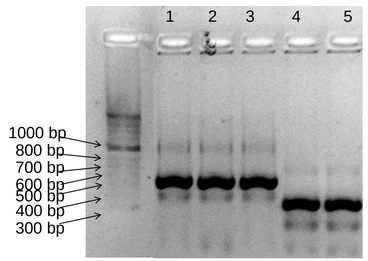

Fig. 5. Gel image showing plasmids with SVNV gene insert (Lanes 1, 2 and 3) and plasmid with no insert (Lane 4 and 5)

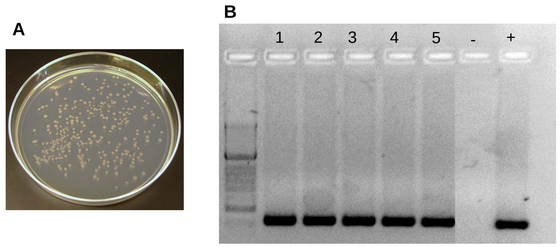

Fig. 6. A) E. coli colonies with plasmids + SVNV gene insert growing on agar plate. B) Gel image of five E. coli colonies tested for presence of SVNV gene using RT-PCR.

V. Detection of SVNV in Thrips

- To quantify the SVNV levels in individual thrips, a standard curve ranging from 10^8-10^1 copies/ul of RNA was prepared using a plasmid containing the SVNV gene (Fig. 7).
- Only one thrips (#8) successfully acquired SVNV after 24 hours of AAP and 72h of IAP (Fig. 8).

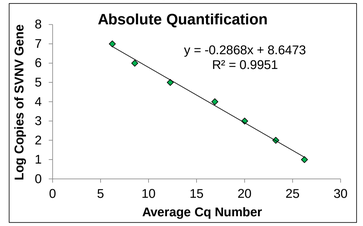

Fig. 7. Standard curve for absolute quantification of SVNV using quantitative RT-PCR (qRT-PCR).

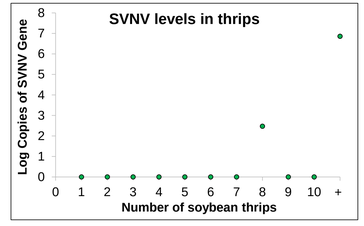

Fig. 8. SVNV levels in a subset of thrips using quantitative RT-PCR (qRT-PCR).

VI. Conclusions

- Our results suggest that soybean thrips likely require more than 24 hours of AAP to acquire SVNV from an infected leaf tissue.
- We did not detect any virus in tobacco thrips after AAP (data not shown).
- Future research will include extending the AAP and IAP durations to increase efficiency of virus infection.

VII. References

1. Khatabi B, Wen RH, Hershman D, Kennedy B, Newman M (2012) European Journal of Plant Pathology1-8.
2. Zhou and Tzanetakis (2013). Phytopathology 103: 966-971.
3. Irwin ME, Yeargan KV, Marston NL (1979) Environmental Entomology 8 (1): 131-140.

VIII. Acknowledgments

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