DOES *XIPHINEMA AMERICANUM* VECTOR GRAPEVINE FANLEAF VIRUS?

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Introduction

Fanleaf degeneration is one of the most severe viral diseases of grapevines due to its ability to disrupt fruit set and greatly reduce yields. It is caused by grapevine fanleaf virus (GFLV) and vectored from root-to-root by the dagger nematode, *Xiphinema index*. The objective of this research was to study the ability of a closely related dagger nematode, *X. americanum*, to vector GFLV. *Xiphinema americanum* is very common in California vineyard soils and vectors a closely related virus in grapevines, fruit trees and other crops. I*n vitro* and greenhouse experiments were performed to determine whether this closely related nematode is capable of vectoring GFLV among grapevines.

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In vitro experiment

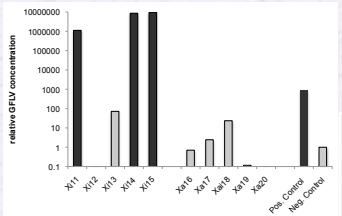
Roots of plantlets of GFLV infected Chardonnay were inoculated with 10 *X. index* or *X. americanum*. After **one week** nematodes were transferred to roots of healthy St George plantlets for another **week** and then removed. **Three weeks** later, roots were collected and frozen in liquid N. Each treatment was replicated 5 times. RNA extraction from roots was performed using a CTAB method and the RNA pellet was further purified using the RNeasy Plant Mini Kit (Qiagen). cDNA was synthesized from the prepared RNA, concentrated to 8 uL with a vacuum centrifuge, using Superscript III (Invitrogen). qRT-PCR was performed on a StepOnePlus PCR System using Fast SYBR Green Master Mix (Applied Biosystems). Primer sequences used for qRT-PCR were:
GFLV-F: GTTGTGTGTTAGGGGAGGTACTATTA; **GFLV-R;** TTCCACATACACCCCGGGATA;
18SrRNA-F: GTGACGGAGAATTAGGGTTCGA **18SrRNA-R:** CTGCCTTCCTTGGATGTGGT

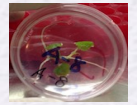
Greenhouse experiment

In vitro healthy St. George and GFVL infected Chardonnay were transplanted to 4-inch pots filled with autoclaved sand for acclimation to greenhouse conditions. **Four weeks** after transferring plants to greenhouse, GFLV infected Chardonnay plants were transplanted to 1 gallon pots, lined with fiberglass cloth and filled with autoclaved sand. Each pot was inoculated with 1 ml of inoculum containing 50 nematodes. Five pots were inoculated with *X. americanum*, 5 with *X. index* and 3 with water . **Three weeks** later, healthy St. George plants were transplanted to each of the 13 pots. At this point, every pot contained 2 plants: one infected Chardonnay and one healthy St. George. During the previous 21 days *X americanum* or *X. index* were feeding exclusively on infected Chardonnay. **Four months later,** qPCR for GFLV testing in leaves was run on all St George plants as described previously.

Results

In both experiments, only plants inoculated with *X. index* were clear positives for GFLV, although inoculation efficiency was low, with 3 positives out of 5 under *in vitro* conditions and only 1 positive in the greenhouse testing. The latter, possibly due to a low number of nematodes used as inoculum in proportion to the volume of soil.





GFLV concentration relative to housekeeping gene 18SrRNA and negative control (△△Ct). Negative control was healthy St George. Top*: in vitro* experiment Bottom: greenhouse experiment. Note log scale in y axis

Conclusions

These results suggest that *X. americanum* does not transmit GFLV or that at least is not as effective as *X. index*.