Project Title: Timing of field transmission of Grapevine red blotch associated virus

Investigators:

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Reporting Period

The work reported here are from July 1, 2016 through Feb. 9, 2018

Abstract

The goal of this project is to determine when Grapevine red blotch virus (GRBV) is spreading in the vineyard. Knowing when the virus is spreading will provide important information on effective management of GRBaV and help focus the efforts to identify additional vectors. This information will also help target control measures to times of the season when the virus is being transmitted in the field. Three vineyards where GRBV has been spreading are being used in this study. One vineyard has an adjacent riparian zone, with most virus spread occurring near that edge of the vineyard nearest the riparian zone. In this case the trap plants are placed in a grassy area between the riparian zone and the vineyard. The second vineyard has an adjacent alfalfa field and since the one vector reported to transmit the virus is the Three Cornered Alfalfa Hopper, the plants were placed perpendicular to the alfalfa field, and within vineyard rows. The third vineyard has most spread adjacent to a recently disturbed wooded area. In each vineyard, every plant has a unique number and the location of each plant is being mapped so that where virus spread occurs in each vineyard can be determined. Fifteen plants are placed in each vineyard each month starting April 15 through Sept 15, after one month in the field the plants are returned to Corvallis, treated with a systemic insecticide and maintained in a screenhouse. All 300 plants from the 2016 field trials were tested for GRBV in late October 2016, and in October 2017 and will be retested in fall of 2018. Given the lack of positive results in the 2016 trials, 25% of the 400 plants from the 2017 field trials were tested in early November of 2017. These plants will be tested again in the fall of 2018 and 2019.

Summary

The goal of this project is to determine when Grapevine red blotch virus (GRBV) is spreading in the vineyard. Knowing when the virus is spreading will provide important information on effective management of GRBaV and help focus the efforts to identify additional vectors. This information will also help target control measures to times of the season when the virus is being transmitted in the field. Three vineyards where GRBV has been spreading were used in 2016 and four vineyards are being used in 2017. One vineyard has an adjacent riparian zone, with most virus spread occurring near that edge of the vineyard nearest the riparian zone. In this case the trap plants are placed in a grassy area between the riparian zone and the vineyard. The second vineyard has an adjacent alfalfa field and since the one

vector reported to transmit the virus is the Three Cornered Alfalfa Hopper, the plants were placed perpendicular to the alfalfa field, and within vineyard rows. This vineyard was removed after the 2016 season, and another nearby vineyard with GRBV was substituted for the 2017 field trials. The third vineyard has most spread adjacent to a recently disturbed wooded area. In 2017 a fourth vineyard was added to the study, adjacent to a grassy/wooded area, where GRBV movement has been observed. In each vineyard, every plant has a unique number and the location of each plant is being mapped so that where virus spread occurs in each vineyard can be determined. Fifteen plants are placed in each vineyard each month starting April 15 through Sept 15 in 2016; and starting May 2 in 2017 and continuing until October. After one month in the field the plants are returned to Corvallis, treated with a systemic insecticide and maintained in a screenhouse. All 300 plants were tested for GRBV in November 2016 and were negative for GRBV in PCR testing. After overwintering, a set of 90 plants that represented trap plants for the 2016 growing season were tested by PCR in May of 2017. Again, all plants were negative for GRBV. The entire set of 300 plants was tested in October of 2017 and will be tested again in Sept. 2018. Twenty-five % of the plants from the 2017 trial were tested in November of 2017 and were negative for GRBV. All 400 of the test plants from the 2017 field trial will be tested in 2018 and 2019.

Introduction

In 2012, a new virus was identified in 'Cabernet franc' in the New York's Finger Lakes region and also in 'Cabernet sauvignon' plants in the Napa Valley. These plants exhibited leafroll-like symptoms but tested negative for leafroll viruses. At a meeting of the International Committee on the study of Viruses and Virus-like Diseases of Grapevine in October of 2012, the name Grapevine red blotch associated virus (GRBaV) was agreed upon for this new virus.

This research aims to determine when Grapevine red blotch associated virus (GRBaV) is spreading in the field. So far, the three cornered alfalfa hopper has been shown to transmit GRBV, but this vector is very minor in many vineyards where the virus is spreading. Movement of GRBV in vineyards after planting has been documented and can be quite rapid, which clearly indicates the presence an efficient vector, or a vector that is present in very high numbers. An increase in the incidence of GRBV over time in young, healthy vineyards that are adjacent to infected vineyards also suggests the existence of a vector. There has been much work done on trying to identify the vector(s) of GRBaV. Efforts looking at suspected vectors in California have resulted in the identification of the Three Cornered Alfalfa Hopper as a vector early in 2016. Regardless if this is the only vector or one of multiple vectors, the timing of transmission will be important information in developing a vector management plan.

If we know when the virus moves, efforts at vector control can be targeted to a specific time frame rather than throughout the growing season. Also, knowing when the virus is moving in the vineyards will help focus on transient insects, which may be present in vineyards for only a short period of time, or insects that feed on grapevines by have other preferred hosts. In either case these vectors could escape detection and identification in standard insect surveys. If transmission is more efficient in riparian areas adjacent to vineyards it will provide clues as where one should look to identify potential vectors.

This project was started in March using in-house (ARS) funds to ensure we could get the first year of field work done in 2016. Funding from CDFA Pierce's Disease Control Program became available July 1, 2016 and is being used for the remainder of the project. Three

hundred grapevines, Merlot on 3309 rootstock, were obtained (donated by) from Duarte nursery, repotted into three gallon pots and held in a screenhouse until being used in the field, or held in a canyard near Corvallis isolated from any vineyards. Plants were tested for Grapevine red blotch virus (GRBV) prior to use in the field experiment and all plants tested negative for GRBV in PCR assays using two sets of primers. Beginning in April 15 plants were placed in each of three vineyards, for a one month period (45 plants each month total). Then in mid-May these plants were returned to Corvallis, treated with a systemic insecticide and stored in a screenhouse. The second set of plants were taken to the vineyards in mid-May, the process repeated each month through September. The last set of plants was returned to the greenhouse in Corvallis in mid-October, there are a total of six sets of plants in each vineyard for a total of 270 trap plants with an additional 30 plants that have not been taken to a vineyard and remained in the screenhouse or canyard during the summer. In 2017, four vineyards are being used in the study, two in southern Oregon and two in the Willamette Valley, again 15 plants per vineyard per month. After the last set of plants was collected all 300 plants were tested for GRBV in November 2016. A subset of the plants were tested in May of 2017 and all were tested in October of 2017 and will be tested again in September of 2018. A subset (25%) of the trap plants for the 2017 study were tested in November of 2017 and all 400 will be tested in the fall of 2018 and 2019.

Objective:

Determine timing of field transmission of Grapevine red blotch virus

Results:

Three hundred plants were provided by Duarte Nursery for this work in 2016, 450 plants were provided in 2017. All plants were tested for GRBV prior to the start of the experiment in 2016 and a subset of the plants were tested for the trial prior to potting in 2017. Plants were potted in 3 gallon pots, and maintained in a canyard prior to taking them to the field. When plants were brought back to Corvallis from the fields, they were treated with a systemic insecticide and maintained in a screenhouse.

The three vineyards were selected because of documented spread of GRBV in these vineyards in previous years. Vineyard #1, was near Jacksonville in southern Oregon and has a small riparian area adjacent to the east edge of the vineyard. The trap plants were placed in a grassy area between the riparian zone and the vineyard. Vineyard #2 was near Medford in southern Oregon with the trap plants placed within the vineyard between every third plant in three rows near the west edge of the vineyard. There was an alfalfa field along the west edge of the vineyard. This vineyard was removed after the 2016 season, and the second vineyard used in southern Oregon in 2017 was also near Medford, Oregon, with documented spread of GRBV. The third vineyard is in the Willamette Valley near Yamhill, Oregon. In this vineyard the spread is occurring throughout the vineyard, with high rates of spread along the east edge of the vineyard where there has been recent removal of adjacent woodlands. In this case the trap plants were place between plants in a single row of the vineyard near the edge of where symptoms were observed. A fourth vineyard was added in 2017, another vineyard in the Willamette Valley, with spread of GRBV based on discussions with the grower.

Each plant was numbered, 1-300 (in 2016, and 1-400 in 2017) and the location of each plant and the month it was in the vineyard has been recorded. Thus, if GRBV spread is

happening from the alfalfa field, we will know which plants were nearest the source as well as which month the plants were in the field and exposed to potential GRBV transmission.

All plants were tested for GRBV in November of 2016 by PCR and all were negative for GRBV. A subset of 90 plants representing one vineyard in southern Oregon was tested in May of 2017 and all were negative for GRBV. All plants from 2016 were tested in October of 2017 and all were negative for GRBV. The last set of plants from the 2017 field experiments were brought back from the fields in mid-October. A subset of the 2017 plants (25% of the plants from the field) were tested the first week of Nov. 2017, and all were negative for GRBV. In all cases, the nucleic acid extracts were tested for the amplification of a plant gene to ensure the quality of the nucleic acid was such that it did not inhibit the enzymatic reactions of the PCR testing. All samples tested positive for the plant gene. Based on recent work from Dr. Fuchs lab at Cornell, showing the unreliable testing for GRBV until two years after infection, the plan is to keep these plants for two full years after coming back from the field. The plants from 2016 and 2017 will be tested in the fall of 2018 and 2019.

Discussion

The experimental setup went according to plan and plant rotation went smoothly. We had feeding damage similar to that observed with Three Cornered Alfalfa Hopper in one vine during the course of exposure in the vineyards. We placed sticky cards in the vineyard in the Willamette Valley and did not catch any Three cornered alfalfa hoppers. Recent work by entomologists Dr. Zalom (UC-Davis) and Dr. Walton (Oregon State University) suggests that sticky cards are not effective for monitoring the Membracid insects. The entomologists have been working on insect monitoring in vineyards in Oregon in 2016 and 2017. Based on recent information from Dr. Fuchs (May 2017 GRBV workshop in Davis, CA) it appears that detection of GRBV is very unreliable for the first two years after a plant is infected. Thus, the plan now is to maintain the trap plants for two full years after the end of the field part of the study and testing them after one and two years.

The entomologists working on membracids in Oregon (Walton and Hilton) did catch several species of membracids in Oregon vineyards in 2016 and 2017 and the feeding damage has been observed in the fields where we had our trap plants in 2017. Work on transmission by the membracid species identified from Oregon vineyards is ongoing by Vaughn Walton's group at OSU and as of meetings we had in January of 2018, they had not obtained any positive transmissions in the greenhouse using these two membracids.

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