Unified Grant Management for Viticulture and Enology

1. Summary

Grapevine red blotch-associated virus (GRBaV) from the genus *Grablovirus* in the family *Geminiviridae* (Varsani et al, 2017) is causing red blotch disease, a newly recognized threat to the grape and wine industry (Al Rwahnih et al., 2013; Cieniewicz et al., 2017a; Sudarshana et al., 2015). Analysis of the spatiotemporal incidence of GRBaV in the selected Cabernet franc vineyard in California over three consecutive years was consistent with the occurrence of virus spread (Cieniewicz et al., 2017b). Analysis of the potential to ingest GRBaV by insects visiting a diseased vineyard revealed the occurrence of four vector candidate species, including *Spissistilus festinus*. Follow-up transmission assays using colonies established in the laboratory confirmed *S. festinus* as a GRBaV vector of epidemiological significance (Cieniewicz et al., in preparation). In spite of remarkable progress, information on disease ecology remains scarce. Carrying out transmission experiments from infected grapes to alfalfa, a nonhost plant of GRBaV, in the laboratory indicated the presence of GRBaV in the majority of *S. festinus* even after 2-3 weeks of feeding on alfalfa, as shown by multiplex PCR (Krenz et al, 2014). This result revealed a persistent transmission mode of GRBaV. Experiments are under way to localize GRBaV in different organ tissue of *S. festinus*. Cultivated and wild *Vitis* sp. are the only hosts known for GRBaV (Bahder et al., 2016a; Perry et al., 2016). To determine alternative hosts of GRBaV in diseased vineyard ecosystems, cover crop stands within diseased vineyard middle rows were surveyed in mid-March. So far, all the samples collected, particularly legume species such as vetch, clover, bean, and pea, as well as alfalfa in unmanaged areas proximal to diseased vineyards, tested negative for GRBaV in multiplex PCR. Similarly, no *S. festinus* were found in cover crops, particularly legume species, in diseased vineyards through mid-March. Identical investigations will be further carried out in May. Finally, research results were communicated to extension educators, crop consultants, researchers, vineyard managers and regulators at winter school meetings in New York and Oregon.

2. Technical Report

3. Project Title: Biology and Spread of Grapevine Red Blotch-Associated Virus
UGMVE proposal number: 2015-1515

4. Principal Investigators: Marc Fuchs[1] and Keith Perry[2]

[1]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY 14456 (Phone: 315-787-2487; E-mail: mf13@cornell.edu)

[2]Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Ithaca, NY 14456 (Phone: 607-254-8243; E-mail: klp3@cornell.edu)

Principal Cooperators: Deborah Golino

University of California, Foundation Plant Services, One Shields Avenue, Davis, CA 95616 (Phone: 530 752-3590; E-mail: dagolino@ucdavis.edu)

5. Objectives and Experiments Conducted to Meet Stated Objectives

The overarching goal of this project was to advance our understanding of red blotch disease and its causal agent, GRBaV, with a major emphasis on horizontal spread in vineyards and optimized detection methodologies. Our specific objectives are to:

1. Characterize the spread of grapevine red blotch-associated virus (GRBaV)
    - Describe the transmission mode of GRBaV by *Spissistilus festinus*
    - Test sentinel vines established in a diseased vineyard where spread is documented for the presence of GRBaV

- Investigate the seasonal diversity and distribution of vector candidate populations in a diseased vineyard for which there is no evidence of spread

2. Determine if vineyard cover crops can host GRBaV and/or *S. festinus*
   - Survey cover crops in Napa Valley vineyards for *S. festinus*
   - Survey cover crops in Napa Valley vineyards for GRBaV
3. Determine the experimental host range of GRBaV and *S. festinus*
   - Agroinoculate commonly used vineyard cover crop species with infectious GRBaV clones and assess virus infection
   - Examine the reproductive potential of *S. festinus* on commonly used vineyard cover crop species
4. Determine the genetic variability of populations of *S. festinus*
5. Disseminate research results to farm advisors and to the grape and wine industry

To address objective #1 - Characterize the spread of grapevine red blotch-associated virus (GRBaV) -, specimens of *S. festinus* from alfalfa fields in Yolo and Kern counties were collected, transferred to Cornell University and established on alfalfa plants in a growth chamber with controlled temperature, humidity and photoperiod. Alfalfa is a host of *S. festinus* but not of GRBaV (Cieniewicz et al., unpublished). Conditions to rear *S. festinus* colonies were optimized so that a full development cycle, including oviposition, and the production of nymphs (Figure 1) and adults, could be completed within two months.

Fig. 1. Nymphs of *S. festinus* on alfalfa plants in a growth chamber at Cornell University.

To describe the transmission mode of GRBaV, *S. festinus* were allowed to feed on GRBaV-infected grapevines for 48-72 h. Then, groups of 2-4 individuals were transferred to alfalfa and allowed to feed for two weeks. These assays were duplicated. Subsets of *S. festinus* were tested for the presence of GRBaV after the acquisition and alfalfa feeding steps. After the acquisition period, 6 out of 8 *S. festinus* in experiment 1 and 3 of 5 *S. festinus* in experiment 2 were positive for GRBaV in PCR, confirming that *S. festinus* can ingest GRBaV. After feeding on alfalfa, most specimens tested (12 of 20 in experiment 1 and 6 of 11 in experiment 2) were positive for GRBaV, revealing that *S. festinus* is capable of keeping the virus even after the gut cleansing episode on a nonhost plant of GRBaV. These findings suggested a persistent transmission of GRBaV. To further our understanding of the transmission mode of GRBaV, additional work is under way to localize the virus in organ tissue of *S. festinus.*

To address objective #2 - Determine if vineyard cover crops can host grapevine red blotch-associated virus (GRBaV) and/or S. festinus -, eight different vineyards were selected. The eight vineyards are infected with GRBaV or proximal to vineyards infected with GRBaV. In addition, they carry legumes in their cover crop stands sown in November 2016 (Figure 2). Legumes, i.e. bell beans, peas, vetch, clover, alfalfa, medicagos, etc. are known hosts of *S. festinus*. Three additional vineyards were selected because they are not infected with GRBaV and carry legumes species in their cover crops stands. Our study aimed at surveying cover crops for *S. festinus* by sweep netting. From mid- to the end of March, two representative rows of each vineyard were swept 25-40 times depending on abundance of vegetation. Nets were inspected for treehoppers but none was found in any of the 11 vineyards surveyed. Additional sweep netting will be carried out later in the growing season.

The same two vineyard rows used to survey *S. festinus* were selected for a collection of cover crop samples (Figure 2). The vineyards were essentially divided into four quadrants. For each quadrant, bell beans, peas and vetch were collected. Occasionally other legume species were selected if there was a particularly high abundance, including various clover species. Alfalfa samples from unmanaged areas proximal to GRBaV-infected vineyards were also sampled. A total of over 200 legume samples from vineyard middle-row cover crops have been collected for GRBaV testing by multiplex PCR. So far, the 120 samples that have been tested were negative for GRBaV. The testing of additional cover crop samples from 11 vineyards is under way. Additional cover crop samples will be collected later in the growing season (early to mid May) and tested for GRBaV.

Fig. 1. Cover crops in a GRBaV-infected vineyard surveyed for GRBaV and *S. festinus.*

To address objective #3 - *Determine the experimental host range of GRBaV and S. festinus* -, snap beans were agroinfiltrated with an infectious clone of GRBaV isolate NY358 from phylogenetic clade 2. Experiments were duplicated with 10-20 biological replicates. Test plants were assayed for GRBaV in agroinfiltrated leaves at 5-7 days post-infiltration and in apical leaves at 14-21 days post-infiltration by multiplex PCR. The presence of GRBaV was detected in the majority of plants tested (12 of 20 in experiment 1 and 9 of 14 in experiment 2) in agroinfiltrated and apical leaves. Samples were also tested by RT-PCR to amplify a segment of a spliced GRBaV mRNAs. This assays was carried out to distinguish virus replication from virus expression-mediated by *A. tumefaciens* infection. Results revealed spliced GRBaV mRNA products in the majority of plants tested (12 of 20 in expeirment 1 and 8 of 14 in experiment 2). These findings were consistent with the capacity of snap bean to replicate GRBaV and serve as an alternative host for *S. festinus*-mediated transmission.

Fig. 3. Agroinfiltration of snap bean with an infectious clone of GRBaV. Necrotic rings correspond to the leaf area infiltrated with a needless syringe containing an *Agrobacterium tumefaciens* suspension carrying a binary plasmid with the infectious GRBaV clone.

To determine whether GRBaV-infected snap bean could serve as a virus reservoir for transmission by *S. festinus*, the vector of GRBaV (Bahder et al., 2016b), groups of 6-8 individuals were transferred to GRBaV-infected snap beans and allowed to feed for 48-72 hours. Then, groups of 2-4 *S. festinus* were transfrred to healthy snap beans and allowed to feed for 72 hours. Recipient plants were monitored over time for the presence of GRBaV by multiplex PCR. Results showed infection of recipient plants two weeks post-transmission. Thse findings showed that GRBaV-infected snap beans can serve as virus inoculum for transmission by *S. festinus*.

To address objective #4 - *Determine the genetic variability of populations of S. festinus* -, populations of insects were collected in vineyards and alfalfa fields in California, in peanut fields in Mississippi and in soybean fields in Alabama. All populations were transferred to Cornell for processing. DNA was

extracted from individuals and tested using primer sets specific to the mitochondrially-encoded *cytochrome c oxidase subunit I* (*COI*) by PCR. DNA amplicons were extracted from agarose gels after electrophoresis and subsequently sequenced bidirectionnally by Sanger sequencing. Sequences were analyzed and phylogenetic relationships among *S. festinus* from different hosts and geographic origin were inferred. Results indicated two distinct phylogenetic clades: one with 50 *COI* sequences of *S. festinus* from grape and alfalfa in California, and the other with 18 *COI* sequences of *S. festinus* from peanut and soybean in Mississippi and Alabama. A strong statistical support was found for the two clades. These findings seemed to suggest a genetic variability shaped by the environment. However, additional work is needed to ascertain this result. In the future, the internal transcribed spacer DNA between small- and large subunit ribosomal RNA will be targeted in addition to *COI* to determine genetic variability. Also, more populations of *S. festinus* populations will be tested.

To address objective #5 - Disseminate information to farm advisors and to the grape and wine industry, research results were communicated to extension educators, crop consultants, researchers, vineyard managers and regulators at winter school meetings in New York and Oregon. The targeted venues were (i) the Eastern Winey Exposition on March 23, 2017 in Syracuse, NY (40 participants), (ii) the Rogue Valley Grape Growers Conference on March 14, 2017 in Central Point, OR (100 participants), (iii) the Ontario Fruit and Vegetable Convention on February 23, 2017 in Niagara Falls, Canada (140 participants), and (iv) the 38th South African Society for Enology and Viticulture International Conference on August 23, 2016 in Somerset West, South Africa.

7. Outside Presentations of Research

Research results were communicated to stakeholders at the above-listed venues.

8. Research Success Statements

Using an elegant experimental approached, the transmission of GRaBV by *Spissistilus festinus* was shown to be persistent. Cover crops, particularly of legume species, in diseased vineyard middle rows, and of alfalfa in unmanaged areas proximal to diseased vineyards, do not harbor GRBaV, nor do they serve as hosts of *S. festinus* through mid-March. This work on the natural host range of the virus and its vector beyond *Vitis* sp. will be expanded throughout the growing season. Preliminary work on the experimental host range of GRBaV and *S. festinus* identified snap bean as an alternative host. This work will be expanded to other legume species. Analysis of the genetic variability of populations of *S. festinus* suggested two distinct phylogenetic clades with individuals collected from vineyards and alfalfa fields in California forming one clade, and individuals collected from peanut and soybean fields in Mississippi and Alabama forming a statistically-supported, second clade. This on-going research is filling important gaps of our knowledge on the ecology of red botch disease.

9. Funds Status

Funds were spent for salaries of key personnel (graduate student and technicians) involved in the research, supplies and greenhouse rent, travel from labs to and from vineyards for sample collection and surveys, and travel to grower's meetings to present research progress.

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