Status Report 11/30/2018

Initial Survey and Development of an Integrated Pest Management Approach to control grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in the Appalachian Mountains.

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Objectives:

(1) Develop an integrated pest management approach to control above- and belowground grape phylloxera infestations
(2) Investigate the spread of grape phylloxera in North Carolina, Georgia and Tennessee and outreach to the regional grower community

Justification and Description:

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch) is a root and leaf feeding aphid-like insect, which is parasitic to species of the grape genus (*Vitis* spec.). It is native to North America and can be found in natural environments of North Carolina, Tennessee and Georgia (Lund et al. 2017, Downie et al. 2001, Wapshere and Helm 1987).

Many American *Vitis* species are tolerant towards the insect, whereas on European *Vitis vinifera* cultivars and on French-American hybrids, grape phylloxera infestation can lead to severe damage, fruit quality and quantity reduction or complete die-off of the vine (see Powell et al. 2013). The most effective method to control damage caused by grape phylloxera is the grafting of *V. vinifera* cultivars to American rootstocks. This technique was developed in the early 20th century in the face of grape phylloxera induced losses to the European grape and wine industry (see Baida-Miro et al. 2010).

Today, it is common practice to graft hybrids or *V. vinifera* cultivars to a grape phylloxera resistant root stock, bred from American grape species. However, under field conditions, grape phylloxera can be frequently found even on resistant rootstocks (Hoffmann et al. 2011, 2015, 2016, Riaz et al. 2017). Field visits to the Appalachian region of the US during the summer and fall of 2017 revealed that the pest was present in 100% of the visited locations on different cultivars (hybrids and *V. vinifera*). Grape phylloxera was present on leafs and roots (Figure 1).

Although not fully understood in our region, we believe grape phylloxera is able to develop a 'full' life cycle (Figure 2) on some of the grape varieties commercially grown in the Appalachian region; that means the development of asexual and sexual life-cycle and below- and aboveground populations over late spring through the summer months (Forneck & Huber 2009, Hoffmann et al. 2016). Root infesting morphs of grape phylloxera can also lead to additional plant damage caused by fungal pathogens, which find access to the root system through grape phylloxera induced root swellings (Huber et al., 2009).

Question remain as to whether or not aboveground insecticide applications as recommended for the Appalachian region are sufficient enough to sustainably control a grape phylloxera infestation. Therefore we proposed to investigate a set of different methods to control above and belowground grape phylloxera infestations in the Appalachian region.

We requested funding for a one-year study, in which we proposed (1) to develop integrated grape phylloxera management approaches, based on cultivation methods, biocontrol agents and the recommended insecticide use and (2) to investigate the spread of grape phylloxera in vineyards in the Appalachian region. Our results will be part of grower, agent and specialist education and outreach programs.

However, we will ask for an extension the already allocated funding into a second year to continue our investigations.

Material and Methods:

Objective (1): Development of grape phylloxera IPM:

On Farm Trials: Field trials were conducted on two grape phylloxera infested commercial vineyards (see attached letters) in Clay County and Cherokee County, NC. Both vineyards were planted with 5-8 years old, mature, own rooted *Vitis aestivalis* hybrid cv. 'Norton'. The aim of this objective was to establish whether or not an integrated pest management strategy of insecticides, cultural practices and biocontrol agents can effectively control established grape phlloxera populations below and above ground. Therefore, field trials were established in a strip-split-plot design on two different field sites. In total, 3 plots were established with 3 replicates of following treatments (Figure 3). Each treatment contained 8-12 vines, depending on the field site.

(1) Movento (Spirotetramat) (6fl.oz/acre)
(2) Leaf removal
(3) Met52 Granular (*Metharizium anisopliae*) (200 lbs/a)
(4) Movento (6fl. Oz/acre) + Met 52 (200 lbs/a) + Leaf Removal
(5) Non Treated Control (NTC).

Field Site 1 (Clay County) is a vineyard with low-moderate foliar grape phylloxera infestation levels. No infestation of root feeding morphs could be detected. One split-strip plot was established. **Field Site 2** (Cherokee County) is a vineyard with heavy grape phylloxera infestation. Root and foliar infesting morphs were detected at Field Site 2. Two split-strip plots were established at field site 2. The timing of management applications was determined based on the assessment of leaf and root infestation levels of grape phylloxera, according to Porten & Huber (2003) and Hoffmann et al. (2011).

Insecticide and Leaf-removal

Insecticide: A foliar spray of Movento (6 fl/oz per ac) was applied June 5th, 2018 at Field Site 1 (backpack) and June 6th at Field Site 2 (air-blast). Before application, Movento was tank mixed with a spray adjuvant.

Leaf removal: 10 leafs which showed two or more leaf galls were removed per replicate on June 5th ,2018 (Field Site 1) and June 6th, 2018 (Field Site 2).

Belowground management

Met-52 is a commercially available granular which contains inoculum of the entomopathogenic fungus *Metarihizium anisopliae* strain Met-52. *M. anisopliae* is known to control soil-borne insect pests in greenhouses and water suspensions can be active against several large-scale insects. In our field trials, a rate of 200 lbs/acre Met-52 was manually incorporated into the soil under the vine foliage and 2 feet each site of a vine on June 5th 2018 (Field Site 1) and June 6th 2018 (Field Site 2).

Following parameters were assessed in June (pre-treatment), August and October 2018:

Leaf population and gall development:

To survey aboveground infestation, ten heavily infested leafs per treatment were collected and assessed for infestation. Leafs were stored on ice for transportation and then kept at 7 °C in the laboratory.

Root populations:

The belowground infestation levels of grape phylloxera were assessed, using a modified protocol based on Hoffmann et al. 2016. Three root samples per treatment were extracted in field, stored in water at 7 °C for transport and processed in the laboratory. Roots were inspected under the microscope for grape phylloxera infestation and root gall development. Root morphological parameters were measured using WinRhizo Pro 2018.

Re-Isolation of M. ansiopliae from soil:

Natural strains of *M. anisopliae* can occur in soil. To assess whether or not *M. ansiopliae* has developed due to granular treatments, it was recovered from root near soil samples pre-treatment (April 2018) and in October 2018. Fine roots were carefully extracted from the ground and attached soil was shaken into a sterile plastic bag. One mixed sample (3 sampling points) of root-near soil was taken per treatment. Soil was stored at 7 °C for transportation. In laboratory, the soil was air-dried, sieved through a 2 mm mesh. 100 ml of that suspension was plated on semi-selective CTAB medium in five replicates each (Posadas et al. 2011). Blue food coloring was added to the medium for better visibility of colonies. Inoculated petri-dishes were sealed and incubated at 25 °C for 4 days in the dark. Total number of colony forming units (cfu) was counted and the number of cfu/g soil was calculated.

Statistical analyses:

Data were analyzed using ANOVA and an adjunct Fisher LSD post-hoc test. Analyses were performed in RStudio v.1.1.442.

<u>Objective (2): Survey and outreach in the Appalachian Mountains</u>

We proposed to conduct an initial grape phylloxera survey across the Appalachian mountain regions in North Carolina, Georgia and Tennessee and (b) to reach out to the regional grower community through demonstration tools, meetings and extension service. However, this survey is still underway. We have collected and stored samples from leafs and roots of 5 infested field sites in NC. However, due to the priority of extending information of hurricane relief to growers after Hurricane Florence and Hurricane Michael in 2018, we decided to post-pone a more intense field survey into the following year.

Results:

Objective (1):

The application of Met 52 did lead in both field trials to a decrease of the amount of *Metarhizium anisopliae* found in soil (Table 1). In the **low pressure field trial (Field 1),** the application of Movento (8 fl. oz/ac) significantly reduced leaf infesting populations of grape phylloxera (Table 2) and did not lead to an increase of root infesting grape phylloxera populations (Table 3). The application of Met52 (200 lbs/ac) however led to a significant increase of belowground populations in the field, while the combined treatment controlled belowground grape phylloxera at the same level as the Movento application (Table 3).

However, a different picture unfolds in the **high pressure field trial (Field 2)**. While Movento (8 fl oz/ac) and the combined treatment significantly reduced leaf infestation with grape phylloxera (Table 2), <u>the Movento treatment significantly increased the root populations</u> of grape phylloxera (Table 3). However, leaf removal and the combined treatment of leaf removal, Met52 and Movento did lead to reduced belowground populations (Table 3). *In summary, only the combined treatment could control below and aboveground populations sufficiently.*

Objective (2):

Samples of foliar grape phylloxera were collected in five locations in four different counties in NC: Clay Co., Cherokee Co., Henderson Co., Surry Co. (Figure 4). Samples are currently stored in 95% Ethanol at NCSU, Kilgore Hall, Lab 1. The survey is still ongoing.

Impacts and Implications:

The application of Met52 had reduced the amount of *M. anisopliae* in soil. That can have several reasons. Most likely is Met52 not as competitive as natural *M. anisopliae* strains, which are adapted to the local soil-microbiological environment. Met 52 did lead under no circumstance to control of root infesting grape phylloxera populations. However, it did lead to a significant increase of root infestation in the low pressure field trial. This might *indicate the presence of a biocontrol agent* which effectively controls the local grape phylloxera population. However, further research is required.

The presented results show that in low-pressure Grape Phylloxera vineyards, a one-time application of Movento at 8 fl. Oz/ac at an early stage of population growth can sufficiently control grape phylloxera over the course of a year. However, in high pressure vineyards Insecticide treatments seem to increase below ground populations, indicating a the development of a vital connection between above and below ground life cycles (Figure 2). However, early leaf removal seems to lead to an increased control of root populations as well, but not aboveground populations.

Overall, our results indicate that with an integrated treatment of cultural methods, biocontrol and insecticide grape phylloxera can be controlled below and above ground. Grape phylloxera can be found in the main grape growing regions in North Carolina (Figure 4). However, further research is necessary to confirm our results. Therefore we will ask for an extension of the funds into the next growing season to continue our research.

Literature:

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Figure 1: Upper left and lower right: heavy grape phylloxera infestation of aboveground organs in Field Site 2, Cherokee Co. NC. Photographs from August 2017. **Upper right:** cut open leaf galls with asexual eggs of grape phylloxera (August 2017). **Lower left:** Root feeding wingless females (juvenile) (August 2017).

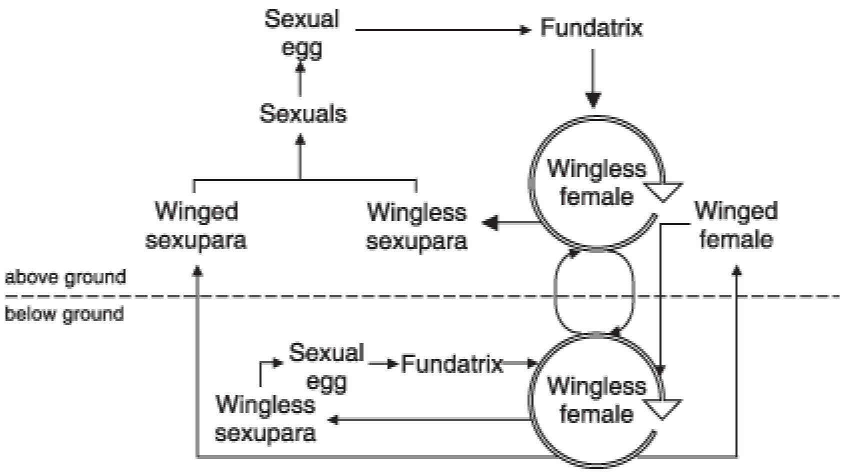

Figure 2: Assembled scheme of possible phylloxera life cycles according to Forneck & Huber (2009).

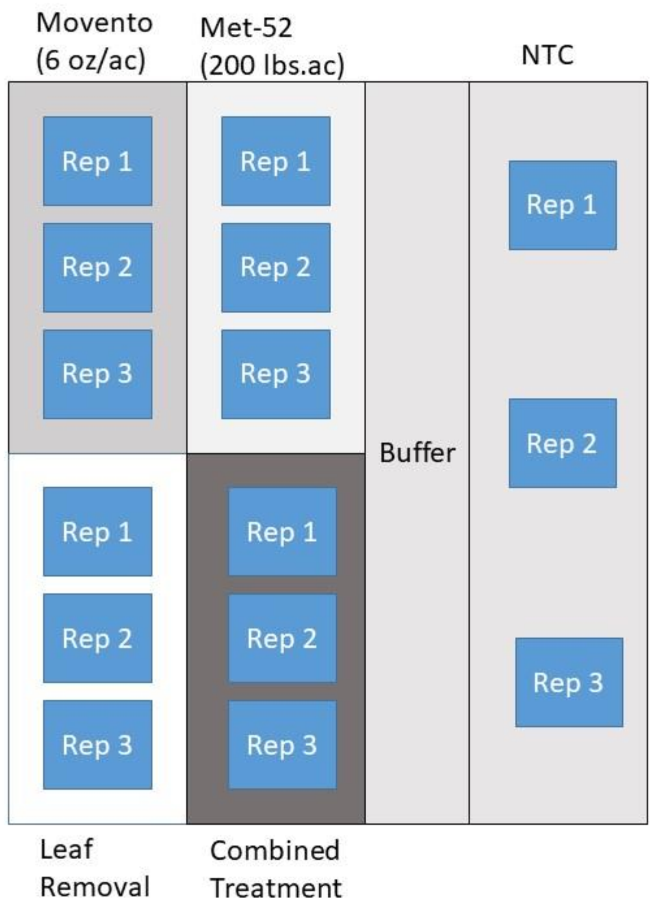

Figure 3: Split-Strip Plot Design. Each treatment and Buffer was established in one row. 8-12 vines per treatment, depending on the field site. 2 plots were established at the Field Site 2 Cherokee County), one plot at Field Site 1 (Clay County).

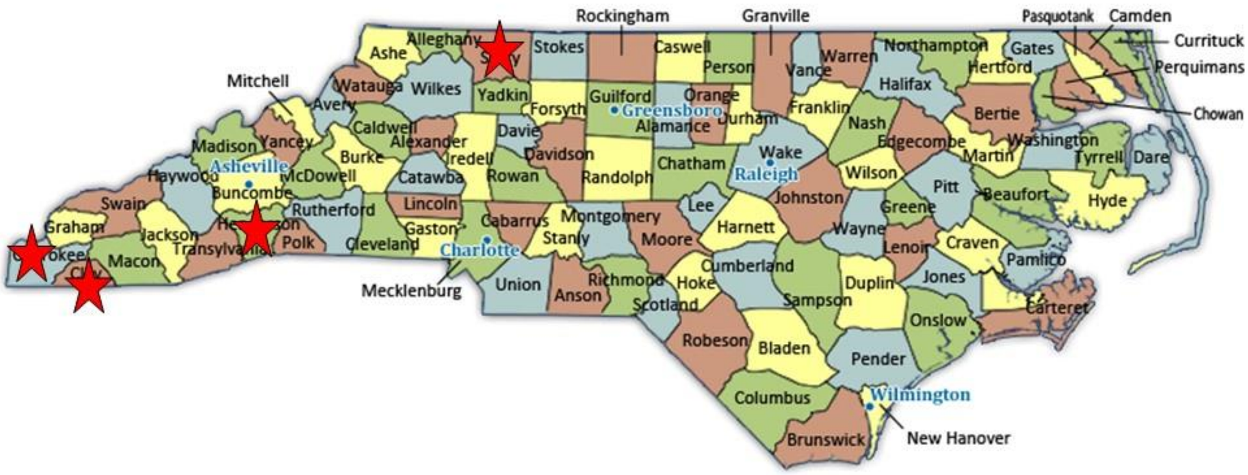

Figure 4: Stars indicate the counties in which leaf infesting ('aerial') grape phylloxera samples were collected from commercial vineyards (Cherokee Co., Clay Co., Henderson Co., Surry Co.)

Table 1: Densities of *Metharhizium anisopliae* in root-near soil samples. M. anisopliae levels were lower in Met-52 root-near soil samples in October, compared to all other treatments and the control (ANOVA, Fisher-LSD).

Field Site	*Treatment*	*April 2018 (cfu/g soil)*	*Oct. 2018 (g/g*
Field 1 *(Clay Co.)*	NTC	112	564 a
	Movento (8 fl.oz/ac)		630 a
	Met-52 (200 lbs/ac)		246 b
	Leaf Removal		690 a
	Combined Treatment		442 ab
Field 2 *(Cherokee Co.)*	NTC	112	340 ab
	Movento (8 fl.oz/ac)		584 a
	Met-52 (200 lbs/ac)		196 b
	Leaf Removal		260 b
	Combined Treatment		444 a

Table 2: Average densities of leaf galls as indicator for aboveground grape phylloxera pressure over the course of 2018 (ANOVA + Fisher LSD)

Field Site	*Treatment*	*June 2018 (pre-application)*	*August 2018*	*October 2018*
Field 1 *(Clay Co.)*	NTC	1.4	46 a	69.8 a
	Movento (8 fl.oz/ac)	7.8	**0.2 b**	34 b
	Met-52 (200 lbs/ac)	5.2	34.3 a	15 bc
	Leaf Removal	1.5	**0 b**	**0 c**
	Combined Treatment	0	0 b	**0 c**
Field 2 *(Cherokee Co.)*	NTC	15.5	230 a	146 a
	Movento (8 fl.oz/ac)	35.1	**15.6 c**	**67 b**
	Met-52 (200 lbs/ac)	37.35	159 bc	152 a
	Leaf Removal	5.4	223 a	150 a
	Combined Treatment	19.05	**3.6 c**	114 ab

Table 3: Average densities of root infesting individuals (instars/cm^2 root) as indicator for below ground grape phylloxera pressure over the course of 2018 (ANOVA + Fisher LSD)

Field Site	*Treatment*	*June 2018 (pre-application)*	*August 2018*	*October 2018*
Field 1 (Clay Co.)	NTC	0.009	0.011 c	*In progress*
	Movento (8 fl.oz/ac)	0.009	**0 c**	*In progress*
	Met-52 (200 lbs/ac)	0.007	0.21 a	*In progress*
	Leaf Removal	0.009	0.1 b	*In progress*
	Combined Treatment	0.012	**0.013 c**	*In progress*
Field 2 (Cherokee Co.)	NTC	0.016	0.16 b	*In progress*
	Movento (8 fl.oz/ac)	0.01	0.31a	*In progress*
	Met-52 (200 lbs/ac)	0.008	0.12 b	*In progress*
	Leaf Removal	0.014	**0.078 c**	*In progress*
	Combined Treatment	0.014	**0.06 c**	*In progress*

October 13, 2017

Dear Dr. Hoffmann

Eric and Judy Carlson of Calaboose Cellars support your efforts to develop grape phylloxera management strategies. This insect appears to be a major pest in our Appalachian winegrowing region. We are very happy to enable your research by providing the opportunity to develop on-farm trials for your proposed project "**Initial Survey and Development of an Integrated Pest Management Approach to control grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in the Appalachian Mountains".** We hope that your research will lead to improved regional grape phylloxera pest management strategies in the future.

Sincerely,

Eric Carlson

Stephen P. Weaver, Owner
Beaverdam Vineyard Inc.
527 Old Evans Rd.
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October 16, 2017

Dear Dr. Hoffmann

Beaverdam Vineyard Inc., a commercial vineyard, here in the Upper Hiwassee Highlands AVA of Southwestern NC supports your efforts to develop grape phylloxera management strategies. This insect appears to be a major pest in the Appalachian winegrowing regions. As you are aware we have need to develop those strategies to assure a vibrant vineyard specifically in protecting our Norton, Seyval Blanc, Vidal Blanc and Regent grapes. We are very happy to enable your research by providing the opportunity to develop on-farm trials for your proposed project **"Initial Survey and Development of an Integrated Pest Management Approach to control grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in the Appalachian Mountains".** We expect that your research will lead to improved regional grape phylloxera pest management strategies in the future.

Sincerely,

STEPHEN P. WEAVER