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Integrated Pest Management Approach to control grape phylloxera (*Daktulosphaira vitifoliae* Fitch) in the Appalachian Mountains.

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Objective:

Investigate the impact of Movento and Met 52 on population density of root- and foliar feeding grape phylloxera in two vineyards in the Appalachian Mountains.

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 American native grape varieties commercially grown in the Appalachian region ('Norton'); 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 two-year study, in which we investigated the impact of foliar insecticide applications as well as soil applied biocontrol agent on the population density of grape phylloxera above and below-ground. We chose two field sites, one with a low pressure of grape phylloxera, mostly in the foliar form and one with established root and foliar grape phylloxera populations.

Material and Methods:

Field trials were conducted on two grape phylloxera infested commercial vineyards 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 per treatments. Each treatment contained 8-12 vines.

(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 <u>low-moderate foliar grape phylloxera infestation</u> levels. Very low levels of root feeding morphs could be detected. One split-strip plot was established. **Field Site 2** (Cherokee County) is a vineyard with <u>heavy grape phylloxera infestation.</u> Root and foliar infesting morphs were detected in large and consistent numbers. 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

<u>Insecticide:</u> A foliar spray of Movento (6 fl/oz per ac) was applied June 5th, 2018 and August 17th 2019 at Field Site 1 (backpack); June 6th 2018 and August 16th 2019 at Field Site 2 (air-blast). Before application, Movento was tank mixed with a spray adjuvant.

<u>Leaf removal:</u> 10 leafs per vine which showed two or more leaf galls were removed on June 5th 2018, August 17th 2019 (Field Site 1) and June 6th 2018, August 16th 2019 (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/August 17th 2019 (Field Site 1) and June 6th 2018/August 16th 2019 (Field Site 2).

Following parameters were assessed in pre-treatment in both years as well as in August and October 2018, and in September and October 2019:

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. Average number of galls per treatment were counted.

Root populations:

The belowground infestation levels of grape phylloxera were assessed, using a modified protocol based on Hoffmann et al. 2016. Two 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:

To assess levels of *M. ansiopliae* in soil pre and post application, combined root-near soil samples per treatment were collected pre- and post 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.

Results:

In both field trials, the foliar application of Movento (6 fl. oz/ac) significantly reduced *leaf infesting populations* of grape phylloxera, but led to *an increase of individuals per sqcm root* in 2018 (Table 1). However, the application of Movento in the second year (2019) did not decrease

leaf populations in both field sites. The application of Met-52 (200 lbs/ac) did not lead to a decrease of root infesting grape phylloxera populations (Table 1), but did lead in both field trials to a decrease of the amount of *Metarhizium anisopliae* found in soil in 2018. Data from 2019 are still being analyzed at the time of the report. Leaf removal and the combined treatment of leaf removal, Met-52 and Movento did lead to reduced belowground populations in 2018 and to reduced aboveground populations in 2019 in the low pressure vineyard (Table 1).

Conclusions and Implications:

Our research shows that while the application of Movento as a foliar spray controls the aboveground populations of grape phylloxera in the first year, it also increases the population of root-feeding grape phylloxera in vineyards with high phylloxera pressure. Control of Grape Phylloxera with Movento was not successful in the second year of the study. Also Met 52 did not control grape phylloxera populations belowground. We hypothesize that the observed increase of root feeding grape phylloxera in Met 52 treatments could be caused by reduced densities of natural biocontrol agents, due to increased competition by an additional *M. anisopliae* strain (Met 52). Further investigations are planned in collaboration with the Agriculture Victoria Research Division in Australia. The data are currently being analyzed and will result in extension and sceitnific publications.

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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).

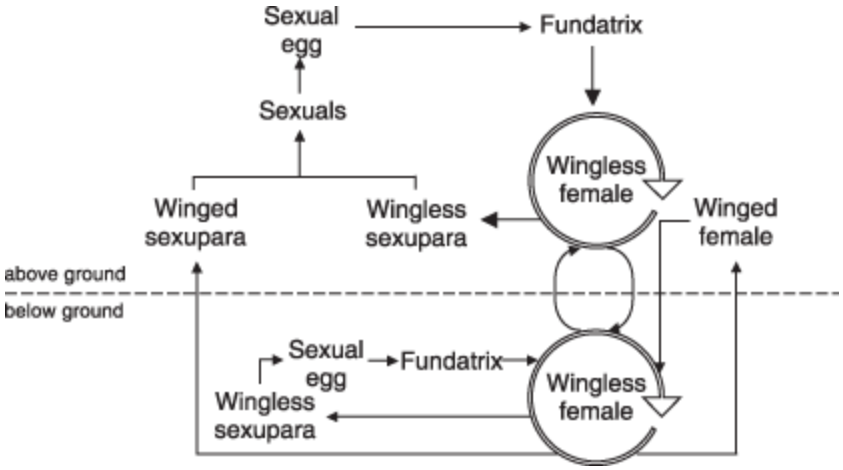

Figure 2: Assembled scheme of possible phylloxera life cycles according to Forneck & Huber (2009).

Table 1: Average densities of leaf galls and root feeding grape phylloxera (#/sqcm root) as indicator for aboveground grape phylloxera pressure in the years 2018/19 (ANOVA + Fisher LSD). Rest of the data are still being processed at the time of the report.

Field Site	*Treatment*	*Post-treatment 2018 (Aug 2018)*		*Post-treatment 2019 (Sept 2019)*	
		# of leaf-galls	#/sqcm of root	# of leaf-galls	#/sqcm of root
Field 1 Low pressure (Clay Co.)	NTC	46 a	0.011 c	31.6 b	*tbd*
	Movento (8 fl.oz/ac)	**0.2 b**	**0 c**	37.9 b	
	Met-52 (200 lbs/ac)	34.3 a	0.21 a	65.1a	
	Leaf Removal	**n.a.**	0.1 b	**27.6 b**	
	Combined Treatment	n.a.	**0.013 c**	**27.1 b**	
Field 2 High pressure (Cherokee Co.)	NTC	230 a	0.16 b	142.6 b	*tbd*
	Movento (8 fl.oz/ac)	**15.6 c**	0.31a	193.7 a	
	Met-52 (200 lbs/ac)	159 bc	0.12 b	135.4 b	
	Leaf Removal	223 a	**0.078 c**	136.9 b	
	Combined Treatment	**3.6 c**	**0.06 c**	137.7 b	