29th International Congress on Sound and Vibration
The annual congress of the International Institute of Acoustics and Vibration (IIAV)
9–13 July 2023
ICSV29

VIBRATIONAL MATING DISRUPTION AGAINST INSECT PESTS: FIVE YEARS OF EXPERIMENTATION IN THE VINEYARD

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Abstract

The use of vibrational signals in agriculture is becoming an important research topic as a new method of behavioural manipulation of insect pests. Semiophysicals include mechanical signals that have the potential to become environmentally friendly alternatives to pesticides. Like pheromones, vibrations endowed with specific spectral and temporal characteristics, can interfere with the mating behaviour of pests, thus preventing population outbreaks and crop damage. This approach is called "vibrational mating disruption" (VMD) and can be applied to control leafhoppers, insects that rely almost exclusively on vibrational signals for mating. Laboratory and semi-field tests have demonstrated that a species-specific mechanical stimulus transmitted to a plant (i.e., grapevine) by means of mini-shakers, can cause the total interruption of mating. In the present contribution, we report the results of a long-term research conducted on two target species, the leafhoppers *Scaphoideus titanus* and *Hebata vitis*. Since 2017 a field-scale experiment has been launched by setting up the first world 'vibrational vineyard' in the Trentino region (Italy) to evaluate the VMD efficacy. Every summer, the population density of the two insects has been measured by visual counting of the nymphs on leaves and yellow sticky traps for the adults. The efficiency of the actuator prototypes was monitored using highly sensitive equipment (laser Doppler vibrometer and accelerometers) and the transmission of vibrations in the trellis system was evaluated with a numerical model of the vineyard. Overall, the vibrational mating disruption technique proved to be effective in reducing the population density of both *S. titanus* and *H. vitis* as long as the disruptive signal was transmitted on the leaves above an active threshold of ca. 15 µm/s of amplitude. The use of vibrations

to control pests in vineyards seems to be a promising innovation. Next step will be the application of the method on large vine surfaces.

Keywords: pest management, insects, agriculture, sustainability, behaviour.

1. Introduction

In recent years, there has been a growing interest in the use of non-chemical methods to control insect pests in agricultural systems in order to reduce the negative impacts on ecosystems and human health [1]. One sustainable alternative to pesticides is the use of the mating disruption technique, which involves the release of species-specific signals to interfere with the mating behaviour of target insect pests to reduce their populations [2]. Historically, mating disruption has been based on the deployment of semiochemicals (i.e., pheromones) but recently the use of vibrations as disruptive signals, as semiophysicals, showed to be effective to control insect species whose intraspecific communication relies on substrate-borne vibrations [3–6]. Namely, a disruptive vibrational signal, the disturbance noise (DN), was specifically designed to interfere with the mating behaviour of the American grapevine leafhopper, *Scaphoideus titanus*, vector of the quarantine grapevine diseases flavescence dorée [3]. The method was successfully tested in semi-field conditions: vibrations were transmitted along a few vineyard rows using transducer prototypes (Tremos, CBC Europe) and adult leafhoppers were released on grapevine shoots enclosed in net sleeves for a period of 24 hours. The number of copulations in the treated samples was significantly lower than in the control [7].
Many variables can influence the outputs of the vibrational mating disruption technique (VMD) and its successful application in the field [8]. One crucial factor is the DN amplitude, as the system can only prevent mating efficiently if the signal amplitudes are above the safety threshold 2 x 10^{-2} mm/s previously calculated [7], which is the value of DN amplitude required to disrupt mating. Other elements that can potentially affect the DN propagation in plants, and thus impact the performance of VMD, including the trellis system, plant age, vegetation growth during the season, and the efficiency of the transducers. A dynamic approach, which describes how the vineyard responds under the application of the DN, can predict its working principles and propose possible optimizations.
In this study, we aimed to evaluate the efficacy of VMD through a six-year field trial. We conducted a regular biological monitoring to assess the population dynamics of the target insect pests, *S. titanus* and *Hebata* (*i.e., Empoasca*) *vitis*, another grapevine leafhopper pest susceptible to VMD [9]. To make the assessment, we conducted a technical monitoring and developed a dynamic numerical model to measure the intensity of the vibrations played back through the trellis system and predict their propagation. Numerical models can provide valuable information regarding the quality of the signal, its spread and possible improvements of the system [10].
This study presents the first long-term evaluation of using vibrations as a mating disruption technique in a commercial vineyard setting. The findings will contribute to the development of innovative sustainable pest management practices.

2. Material and methods

2.1 Experimental area and playback stimulus

The experimental vineyard (organic management, 1.5 Ha, Cabernet Franc) is located at Fondazione Edmund Mach in San Michele all'Adige, Trentino, Italy (46.18953 N, 11.13625 E, WGS84). In 2017, 110 electromagnetic transducers (Tremos, CBC Europe srl) were installed on the metal poles of the trellis system, with two transducers per row, at intervals of 50 m, emitting in loop the DN. In 2018, the DN was updated by adding a pure tone of 250 Hz, which previously had demonstrated to disrupt the mating of *H. vitis* [9]. A comparable (in terms of surface area, plant variety and management) neighbouring vineyard was monitored as a control. The two areas, treated and control, were monitored for six years (2017-2022).

2.2 Technical monitoring

Every year, before the growing season, the working status of shakers was checked and all not-functioning elements were replaced. To assess the DN amplitude variation on the trellis system, due to attenuation of vibrations over years (device wear) and distance from the transducer, measurements were taken using either a laser vibrometer (PDV-100, Polytec, Germany) or accelerometers (Model 352A24, PCB Piezotronics ICP®, United states) connected to a laptop through a LAN-XI data acquisition hardware (Brüel and Kjær Sound & Vibration Denmark) using the software BK Connect (Brüel and Kjær Sound & Vibration, Denmark).
Measurements were taken from at least two shakers per year, with the laser beam focused or the accelerometer attached to the second-to-last upper metal wire (about 1.6 m from the ground) of the trellis system, from two positions: 5 cm from the metal pole and in between two consecutive poles (3 m apart) (Fig. 1).

2.3 Numerical simulation

A numerical model of the vineyard was created using the Finite Element (FE) software *Abaqus Standard* (Dassault Systems, Simulia Corp., Providence, RI). The model was built according to the current vineyard structure (Fig.1) with one 60 m line modelled using 3D deformable beam parts for poles and 3D deformable truss parts for wires. A total of 11 poles and 4 wires were adopted for the entire vineyard line, resulting in 2909 elements and 2924 nodes. The material for both poles and wires is steel with elastic isotropic behaviour (Young's modulus 200 GPa, Poisson's Ratio 0.3, density 7800 kg/m^3 and structural damping equal to 0.01). A C-shaped profile was assigned to the pole part, to mimic the real section of poles in vineyard, while a circular section was defined for the wires, with cross-sectional area equal to 7.55E-06 m^2. To define the interactions between poles and wires, an assembled/complex connector was used, type *translator*, which allowed one relative displacement between the parts, while constraining the others.

The computational analysis was formed by three main steps: (i) a static step with imposed displacement to tensile the wires, (ii) a modal analysis of the entire system and (iii) a dynamic analysis with applied external perturbations (e.g., the DN generated by the shaker).
The DN generated by the shaker is given to the system as a combination of normal vertical dynamic force (F_y) and momentum (M_z). An additional case was included, representing a that is a different direction of excitation, with the purpose to provide improvements for the future. No extra (non-structural) masses were considered, thus modelling the vineyard structure without the presence of vegetation. The velocity v along the wires with increasing distance from the source of the excitation was analysed in both cases, with comparisons.

2.4 Biological monitoring

The abundance of *S. titanus* and *H. vitis* was measured with different techniques for immature and adult leafhoppers due to their distinct behaviour and distribution in the canopy. The number of immature individuals was visually inspected weekly by checking the same amount of leaves (20 per plant) in the treated and the control areas. Adult abundance was estimated by counting the number of individuals captured by yellow sticky traps (10x20 cm, Glutor, Biogard) deployed in both areas from

June to October and changed weekly. The number of monitored plants, traps, and weeks of the year is indicated in Table 1.

The biological performance of VMD was analysed with Generalized Linear Mixed Models (GLMMs) using the package glmmTMB with Rstudio (RstudioTeam, 2023). For immatures count, a GLMM was fitted for each species with the number of counted insects as the response variable, the treatment as explanatory factor, and the year and the week as random factors. For each year, only the weeks with at least one insect in the VMD or control area were considered. The number of counted individuals suffered from overdispersion and zero inflation, thus, a zero inflated negative binomial GLMM with a log-link function was used. For each species, the rate of adults in the treated and control area should be similar to the rate observed for immatures. To test this hypothesis, the total count of adults in the two areas was compared to the total count of immatures in the same areas with a chi-squared test (2x2 contingency table). The test was performed for each year independently.

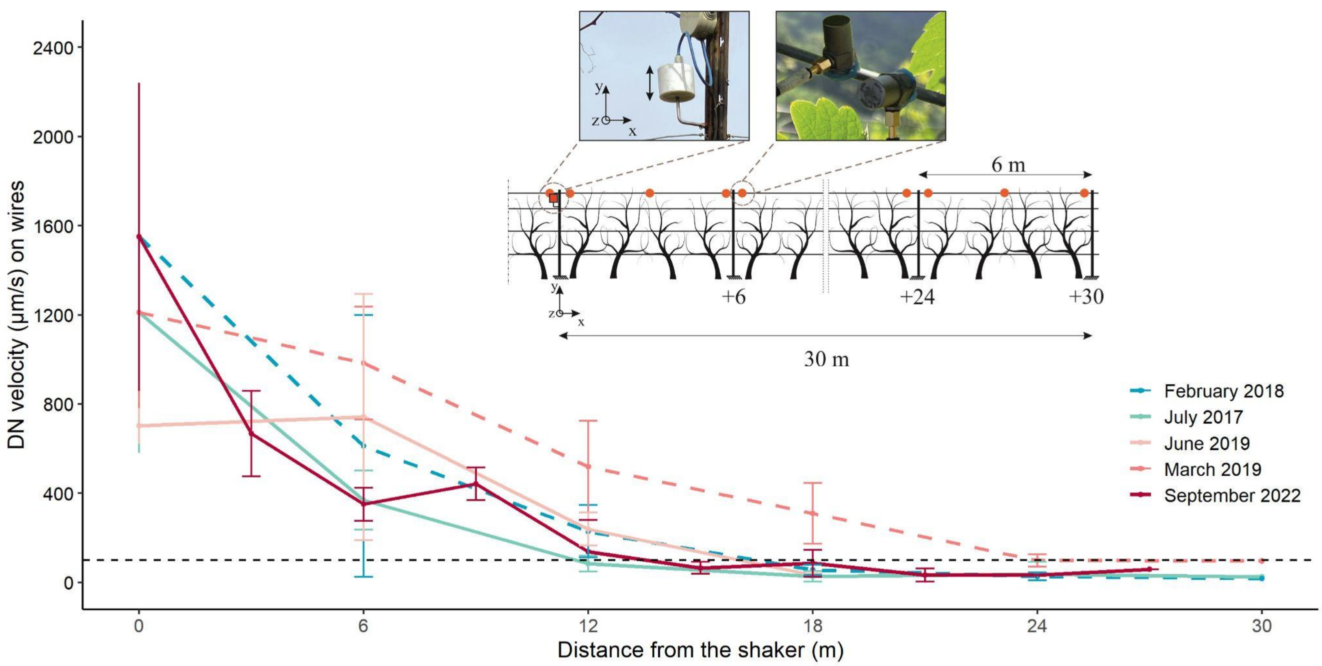

Figure 1: Diagram of the points used for measuring the DN intensity along the rows of the vineyard (red dots) and average and standard deviation of DN velocity at increasing distance from the shaker. The dashed line indicates 100 µm/s, the value of DN intensity needed on the wire to measure at least 15 µm/s on the plants.

3. Results

3.1 Technical monitoring

Since the installation in 2017, the efficiency of each shaker was highly variable (Fig. 1). Moreover, every year of functioning, some shakers stopped working properly: at the end of the growing season, the percentage of shakers emitting vibrations was 92%, 69%, and 89% in 2017, 2018 and 2021, respectively.

On the wire close to the source of the DN, at 5 cm from the pole where the shaker was installed, the velocity of displacement at 200 Hz was in the following ranges (min-max): 271.8 – 1581 µm/s in 2017, 1021.7 – 2368.2 µm/s in 2018, 642.0 -1724.3 µm/s in 2019, and 802.5-2248.8 µm/s in 2022. In all years, the DN amplitude decreased rapidly with increased distance from the source and it reached 100 µm/s about 15 m from the pole with the shaker (Fig. 1). Only in March 2019, the DN velocity value was above the 100 µm/s value until 24 m from the source. Additionally, the velocity of wires vibration was highest at all measuring locations in March 2019. In 2022, even if on average the intensity close to the source was the highest measured, it decreased very rapidly with increased distance from the source and the 100 µm/s was met at 12 m (Fig. 1).

3.2 Numerical simulations

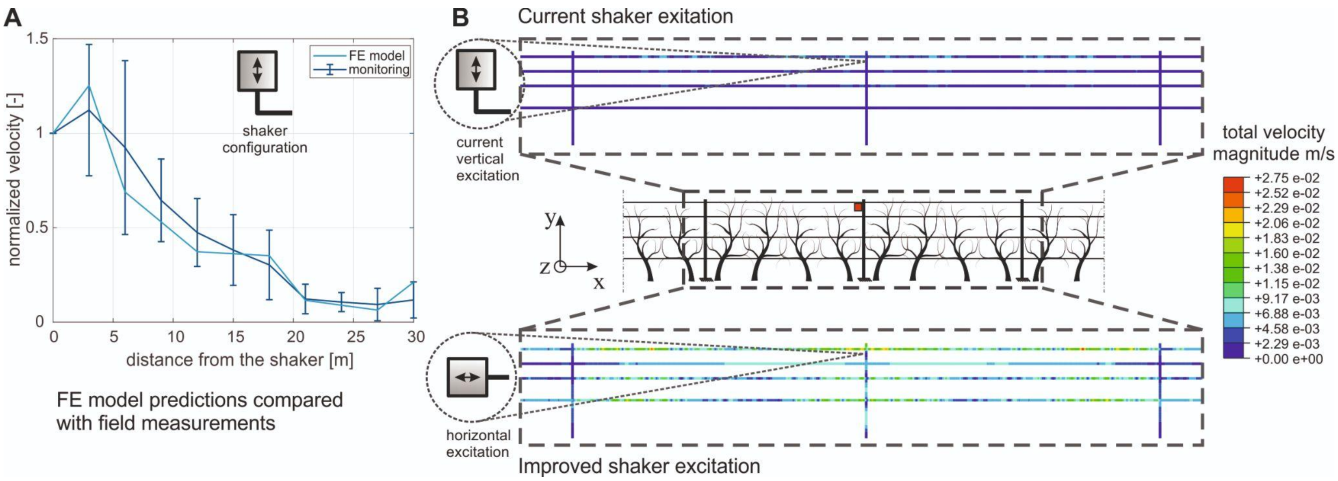

Figure 2: A) Comparison between monitored velocity along the wires (March 2019) and FE model prediction. B) FE simulation of the DN velocity along the wires with the current shaker excitation versus a possible improved solution. Shaker has been considered set within the red square for both the simulations.

Firstly, FE model predictions were compared with field monitoring signals (Fig. 2A). The dominant frequency (around 200 Hz according to the DN signal) was extracted from predicted velocities of the top wires of the model with the same Fast Fourier Transform algorithm that was used for field measurements (Hann window, 67% overlap, 8 Hz as frequency resolution). After model validation, another excitation direction was tested (moving from a vertical oscillation to a horizontal one by rotating the shaker of 90°). The velocity magnitude was compared between the two configurations (Fig. 2B), where the colour bar reports different intensity levels.

3.3 Biological monitoring

The model validation for all GLMMs indicated no problems. The GLMM results showed that VMD had a negative effect on the abundance of immatures for both species (Fig. 3, Table 1). For *S. titanus*, the number of immatures was lower in the VMD area for the first four years of application (2017 - 2020), with a reduction of 36%, 63%, 36%, 43 % every year; whereas, in 2021 and 2022, there was no difference between the two areas (z=-2.936, p=0.003). For *H. vitis*, the reduction was observed starting from 2018 (30% of reduction) for all years of monitoring, and it was particularly relevant for 2019 (52%) and the first generation of 2020 (44%), 2021 (44%), and 2022 (53%) (z=-7.734, p<0.001). In 2022, both species were not observed after week 28 of the year due to a pesticide treatment required by the Italian government as control strategy against flavescence dorée, the grapevine disease transmitted by *S. titanus*.

When comparing the number of adults and immatures, we observed that in general in the treated area the adults were more abundant than in the control (Table 1). The rate of captured adults was statistically different form the rate of immatures in all years for *H. vitis*, whereas for *S. titanus* this difference was significant only in 2018 and 2021, even though a trend was observed also in 2017 (Table 1).

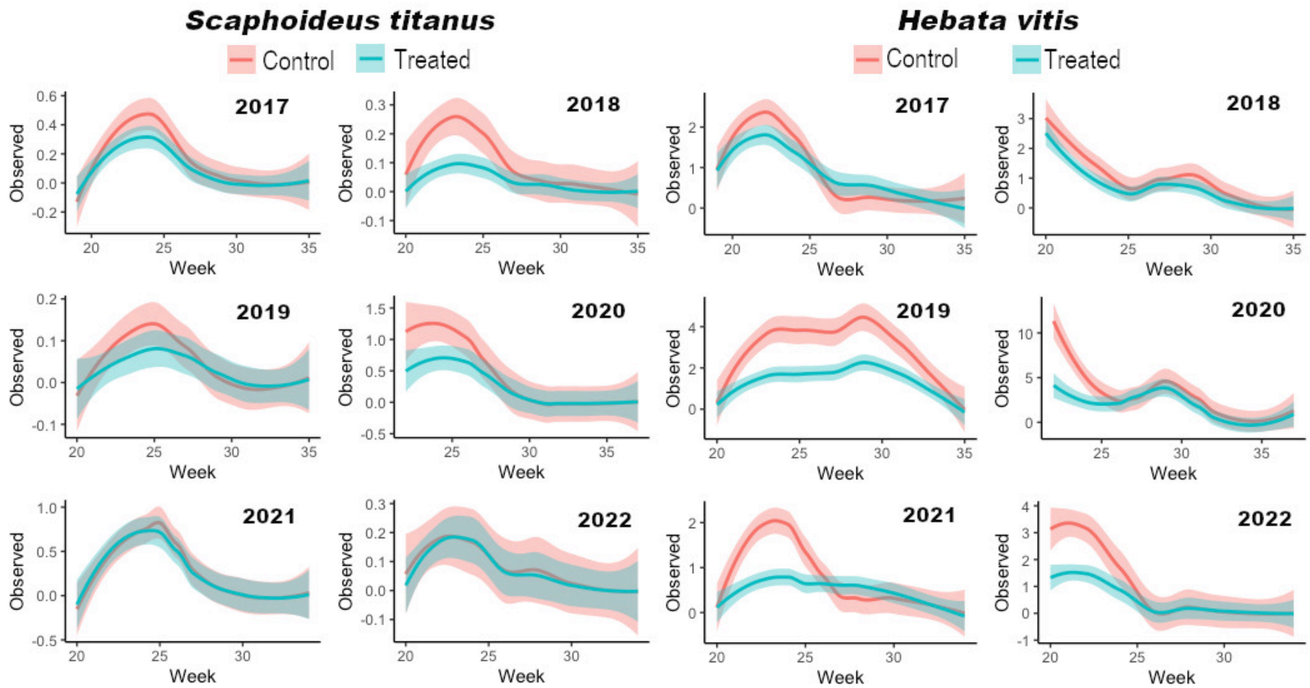

Figure 3: Number of immature stages of *S. titanus* and *H. vitis* in the treated and control areas along weeks of the six years monitoring (2017-2022). Lines show the LOESS smoother, with 95% standard error confidence interval.

Table 1: Total count of insects observed in the two vineyards, treated and control, divided by year, stage of development, and species. Weeks indicate the number of weeks of the year monitored. T= treated area and C = control area.

		Immatures				Adults					
		Plants	Weeks	*S. titanus*	*H. vitis*	Traps	Weeks	*S. titanus*	*H. vitis*	*S. titanus* χ^2 test	*H. vitis* χ^2 test
2017	T	24	19 - 35	34	309	5	29 - 42	82	4260	$\chi^2 = 3.02$ $p = 0.08$	$\chi^2 = 17.16$ $p < 0.001$
	C	24		53	339	6		80	3332		
2018	T	24	20 - 35	14	268	7	23 - 35	199	10419	$\chi^2 = 8.13$ $p = 0.004$	$\chi^2 = 75.8$ $p < 0.001$
	C	24		38	384	6		126	7465		
2019	T	16	20 - 35	7	345	8	20 - 39	643	19864	$\chi^2 = 2.2$ $p = 0.13$	$\chi^2 = 167.2$ $p < 0.001$
	C	16		11	718	8		496	17937		
2020	T	8	22 - 37	20	136	5	27 - 36	20	2455	$\chi^2 = 1.03$ $p = 0.31$	$\chi^2 = 82.5$ $p < 0.001$
	C	8		35	243	5		23	1639		
2021	T	21	20 - 34	71	116	5	21 - 36	92	6832	$\chi^2 = 27.5$ $p < 0.001$	$\chi^2 = 76.1$ $p < 0.001$
	C	21		72	206	5		21	4514		
2022	T	21	20 - 34	13	121	5	21 - 44	18	4429	$\chi^2 = 0.04$ $p = 0.83$	$\chi^2 = 127.5$ $p < 0.001$

4. Discussion

The installation of a VMD vineyard successfully reduced the number of immatures of two leafhopper species, *S. titanus* and *H. vitis,* of about 50% since the first year of activation, even though the transmission of the DN did not cover the entire treated surface with a minimum amplitude. Mating disruption techniques act on the adult stage of insects by interfering with mating, therefore the effect is expected starting from the second year from the disturbance activation. This is the reason why the number of immatures of both species was similar in the treated and in the control area in 2017 and gradually changed in the following years: *S. titanus* has only one generation per year, so the effect can be measured starting with the second year from installation; the DN was upgraded to disrupt *H. vitis* communication in 2018. The higher number of adults in the treated area compared to the control area are probably due to an increased flight activity of individuals exposed to DN, as it was recently demonstrated in laboratory conditions for *S. titanus* [11]. The results of this study support the hypothesis that the DN probably induces adult leafhoppers to fly more and thus they are captured more by sticky traps. This suggests a possible method of assessment for future field studies aimed at evaluating the efficacy of vibrational technologies. In fact, sticky traps are commonly used to assess the population abundance of leafhoppers in the field, but in presence of vibrational interference, captures can lead to a wrong estimate of the population; on the contrary, the count of immatures that are not able to fly and move far from the vibrated plants, seem to be more reliable. However, this result was evident throughout the study period only in the case of *H. vitis* while the effect was significant only in two years in the case of *S. titanus*. (2018 and 2021). Therefore, more research, also in different contexts is demanded for this species.

As for the immatures, the reason why the effect of VMD on *S. titanus* disappeared in the last two years is probably due to the reduced functioning of shakers over the course of each season and over the years. It is likely that the safety threshold (1.5×10^{-2} mm/s) was not guaranteed on most of the treated surface area, thus enabling insect pairs to mate [7,8]. The safety threshold for *H. vitis* is unknown, but it is likely that it is considerably lower than the one of *S. titanus.* In fact, usually, the amplitude of vibrational signals is proportional to the size of the emitter and *H. vitis* is smaller compared to *S. titanus* [12,13]. So, a DN with an intensity below 1.5×10^{-2} mm/s should be able to successfully mask the mating communication of *H. vitis.* This would explain why the VMD was effective on *H. vitis* in the last two years of monitoring and not on *S. titanus*. Even if the intensity of the DN close to the shaker was similar for all years of monitoring, we observed a very high variability between shakers. This was probably caused by lack of standardization in the manufacturing and installation process. In fact, the prototypes were hand-crafted and manually installed on the poles. Additionally, the propagation monitoring showed that after about 15 m, but even less for some shakers/rows, the DN amplitude was insufficient to reach the safety threshold. Considering that the shakers were 50 m a part from each other along each row, almost 20 m of each row were probably below the threshold. The shakers' variability and the high dissipation of energy along the row probably led to a non-homogenous coverage of the treated area with the DN, thus providing the insects with "unmasked" surface where mating could occur. These technical aspects must be implemented in future studies to increase the efficiency of VMD.

In this regard, some critical indications can be obtained by FE models, which are a powerful tool that can be used in a variety of fields to improve solutions, thus reducing the number of expensive and time-consuming experimental campaigns, as well as add information that cannot be obtained from experiments, such as the complete map of velocity field along the vineyard rows. Of course, firstly, a model needs to be validated through some experimental data; then, it can be adopted also to forecast alternative scenarios. The computational model showed a good agreement with the experimental results (Fig. 2A), with similar path from the signal source to the end of the row, highlighting a reduction of about 50% after 10 m from the signal source. This information is quite fundamental, since it was also reported that plants and their growth affected the signal intensity, which then dissipated more rapidly (Fig. 1). To provide possible improvements to the current system, a different excitation direction was tested. Results revealed how a rotation of 90° of the exciters could provide an increase in the signal intensity along the wires up to 10 times with respect to the currently adopted setup. Moreover, it could provide also the other wires with a more pronounced signal amplitude, able to cover and transmit to the plant the DN with multiple contact points.

The monitoring effort of the present study is pivotal in directing future application of applied biotremology. The large-scale nature of this study was critical to highlight the technical limitations of this new technology, such as the transmission of biologically active vibrations in a commercial vineyard. Notwithstanding the practical issues, VMD demonstrated to be effective in reducing the population of two leafhopper species simultaneously. The possibility to use vibrations to manipulate the behaviour of insect pests in the field will add on the toolkit of growers for implementing novel eco-friendly solutions that reduce the detrimental effects of agriculture on human health and ecosystem biodiversity.

5. Acknowledgments

We thank the technical help of Elisabetta Leonardalli, Ruggero Cetto, Elissa Daher and all the students of the University of Trento who helped with the field activities. We also thank Roberto Rosà and Jalal Fouani for their support with the statistical analysis of the biological data. Over the years, this work has been supported by the following grants: RN was supported by the European Union - FSE-REACT-EU, PON Research and Innovation 2014-2020 DM1062 / 2021; RN, AB, IA and NMP were supported by Fondazione CARITRO, Cassa di Risparmio di Trento e Rovereto, 502 No. 2019.0216.

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