European Knowledge Transfer

TECHNICAL DATA SHEET

Mating disruption by using vibrational signals

A new method for *Scaphoideus titanus* management

Network for the exchange and transfer of innovative knowledge between European wine growing regions

This project has received funding from the European Union's Horizon 2020 research and innovation programme under **grant agreement No 652601**

Mating disruption

Introductory note

During WINETWORK project, 219 interviews were conducted by the facilitator agents (FA's) of the 10 European winegrowing regions involved on the project. The FA's identified several practices to fight "Flavescense Dorée" phytoplasm and/or its vector, the cicadellidae *Scaphoideus titanus* Ball, from which one was the **Mating Disruption using Vibrational Signal**s to disturb the behaviour of *S. titanus*.

Unfortunately, this biotechnical method is **not yet available** commercially for winegrower's application. However, since this is a very innovative and promising tool, and there is a strong interest of the winegrowers and technicians on this technique, the consortium decided to create a technical datasheet to disseminate this technique.

Introduction

Scaphoideus titanus Ball (Hemiptera: Cicadellidae) is a leafhopper, the main vector of the phytoplasma that causes "Flavescence Dorée", the most threatening among European grapevine yellow diseases. Despite its importance, very little is known about this species besides its general biology and mechanism of disease transmission and communication.

In order to find alternatives to conventional pesticides, generally used against this pest, our attention needs to focus on some behavioral aspects, in particular, to the mating behavior of such species. Mate recognition and localization in Auchenorrhyncha (with the exception of most cicada) are mediated via acoustic signals, transmitted through the substrate. It has been estimated that 150 000 species use vibrations to achieve mating and among them there are several pests and important vectors of plant diseases.

In Europe, Flavescence dorée is a **quarantine disease** and there are compulsory measures to manage vector populations and prevent the spread of the disease which include large-scale insecticide treatments. To apply the mandatory control methods, growers may need to apply large amounts of pesticides, which have both environmental and economic impacts. Considering that there is an increasing market demand for pesticides-free products (organic market), and large food retailers are imposing more stringent limits than those in current legislation on residues, the EU Directive 128/2009, for the sustainable use of pesticides, is moving in the direction of finding alternatives to chemicals, the development of environmentally friendly alternatives is crucial.

Vibrational Mating Disruption (VMD) is an example of an innovative method, since it exploits vibrations used by this insect, both for mating and rivalry. Pair formation in *S. titanus* is characterized by a **continuous duet between a male and a female** that starts with partner reciprocal identification and proceeds with a location (search) stage before the final courtship that precedes mating. The mating duet is characterized by specific temporal pattern which change during the process of pair formation. In the identification duet, male pulses are delayed after female reply, while they are fully synchronized during location and courtship duets. One hypothesis is that **VMD is more successfully if applied during the identification stage** when external interferences could result in **loss of important information that is needed to correctly identify the mating partner.** However, given the complexity of the mating duet structure, interference caused by VMD could affect each stage of the mating process. The key to prevent *S. titanus* mating is the **interruption of the sound vibrational sexual communication by transmitting suitable disrupting signals**.

Figure 1: *S. titanus* Ball (Cristina Carlos, ADVID and Flavescence Dorée symptoms (Eng. José Freitas, DRAPN)

Methodology and application

Current knowledge about the sexual behavior of *S. titanus* and VMD experiments (Mazzoni et al. 2009a; Eriksson et al. 2012; Polajnar et al. 2014) suggested that playback of vibrational disturbance noise (VDN; based on vibrational signals used by males during rivalry) should suffice to **disrupt the courtship behavior and prevent mating**, thus representing a promising avenue for developing a non-chemical approach for controlling this invasive vector in Europe.

Principle of the mating disruption protection method for S. titanus.

An electromagnetic shaker was used to vibrate the wire with VDN that was transmitted to the plants. It was possible by testing transmission of male calling signals on different plant parts, establishing the active space of mating signals in order to adjust the power of the mating disruption signals into effective species-specific masking signals (VDN) (Mazzoni et al., 2009b; Polajnar et al., 2014).

Figure 2: Shaker designed to transfer disruptive noise to longer distances. (Source: Lucchi et. al)

Males call spontaneously, carrying out a specific "call and fly" behavior followed by a well-structured courtship song. Females emit signals only in response to males and rival males compete for mating, producing a disruptive noise aimed at interrupting a duet in place between pairs.

Scientific data and some results

After initial studies on the specie's mating behavior, attention was focused on the possibility of achieving mating disruption by playback of vibrational signals. Efficacy of playback with sufficient amplitude was first demonstrated in laboratory trials, and then in semifield conditions with insect pairs placed in cages in an experimental vineyard. The approach was to gather knowledge of basic reproductive biology first, which revealed a naturally occurring disturbance signal emitted by rival males (VDN) that masked the temporal structure of mating calls in antagonistic interactions between males. The use of VDN has a distinct advantage over synthetically generated pure tones waveforms, because its features have evolved for efficiency, so spectral features are expected to be optimal for this function. Although *S. titanus* is one of the few species known to use acoustic disruption, masking the vibrational signals, which are important for mate recognition, **should be effective in other species** as well.

Mazzoni et. al, in 2009 found that the playback of VDN reduced the level of male calling and interrupted established male-female duet that consequently resulted in a significantly reduced number of copulations. These results indicate that the vibrational communication channel is open to interference both from abiotic environmental noise and from signals produced by sexual competitors or even heterospecifics. The study also suggests that a **detailed understanding of a leafhopper behavior is essential** for trying new approaches in the development of more environmentally friendly control practices.

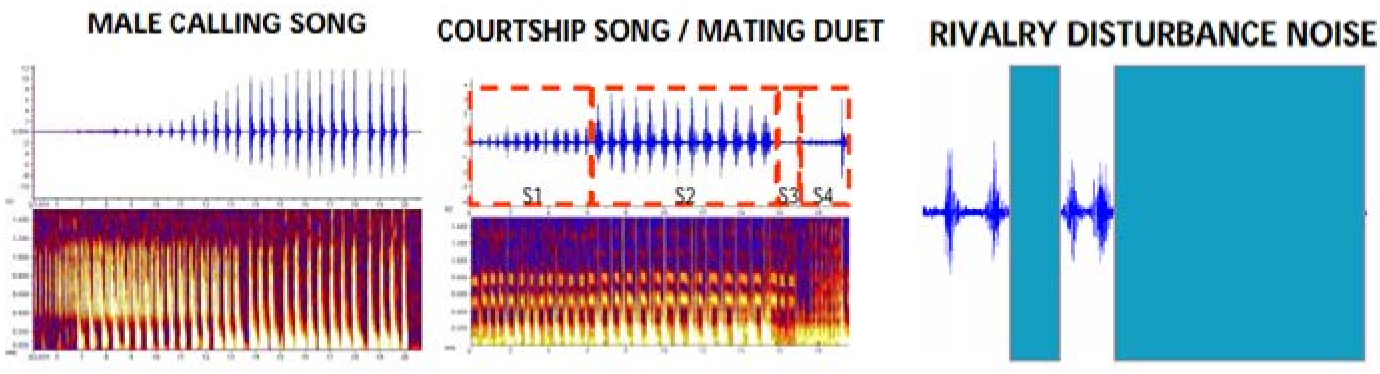

Figure 3: Male calling signal; Courtship song/Mating duet; Rivalry disturbance noise of *S. titanus* (Source: Mazzoni et. al, 2008)

Eriksson et. al (2012), showed, for the first time, that effective mating disruption based on substrateborne vibrational signals can be achieved in the field. When disruptive vibrational signals were applied to grapevine plants through a supporting wire, mating frequency of the leafhopper pest *S. titanus* dropped to 9% in semifield conditions and to 4% in a mature vineyard. The underlying mechanism of this environmentally friendly pestcontrol tactic is a **masking of the vibrational signals** used in mate recognition and location.
In 2012, Lucchi et. al, in open field conditions, found significant differences between vibrated and non-vibrated (C) grapevine plants treatments, concerning the number of virgin females, using an electromagnetic shaker to vibrate the wire with disruptive signal.

In 2013, the same investigators applied the methodology to longer distances. They found that is still possible to achieve 65% of mating disruption at 45 meters with 18 hours of shaker operation. They succeed in 80% of cases. The results indicate that the principles from which the mating disruption with vibrational signals was validated is **applicable even at field level, on mature plants**. The disruption has been effective on more than 90% of tested pairs when some conditions were respected. In particular, they found that mating is almost totally prevented, when the device is working for periods of more than 19 hours. Indeed, they detected important losses and dispersion of the signal, due to numerous points of contact between vibrating wires/plants/poles. This limit must be eliminated or strongly reduced, for instance by passing the signal or using dampers in correspondence of such critical points.

Polajnar et al. (2016), also published about using artificial vibrational noise to prevent mate recognition and localization mediated by vibrational signals in the grapevine pest S. titanus. Building on the proof of concept published previously, mating trials were set up in laboratory to determine the amplitude threshold for playback efficacy and reveal the mechanism of its function, while field trials were performed to validate this threshold and explore the possibility of reducing energy use by exploiting the diel pattern of this species' mating activity. The threshold obtained in laboratory trials—15 µm/s peak amplitude—was confirmed by measurements of attenuation and insect mating in field cages at successive

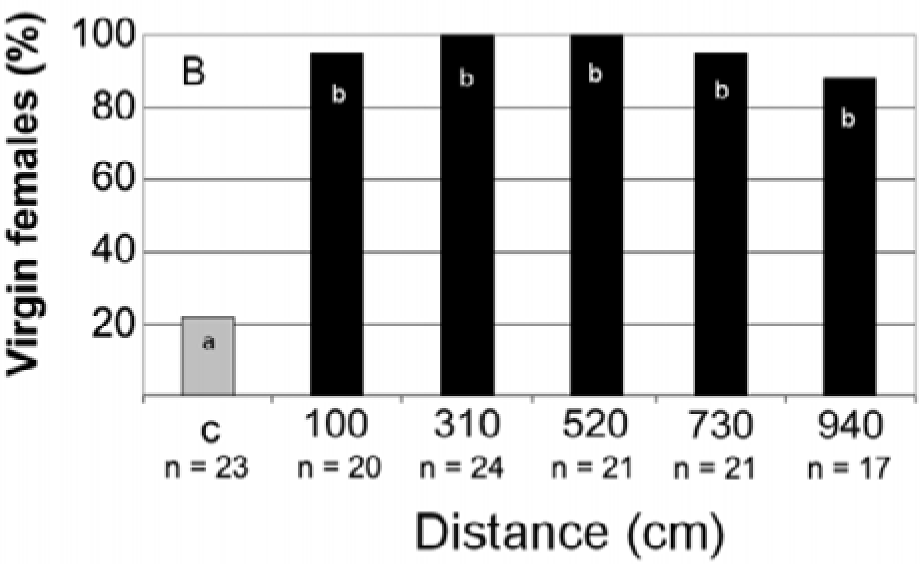

Figure 4: Number of virgin females found on vibrated and non-vibrated (C) grapevine plants in a field trial with mature grapevine plants in 2012 (Distance = distance to control (c) modality).

distances from the source. They also discovered that shutting off the disruptive noise between 1000 and 1800 h did not reduce efficacy of the method in the field, allowing energy saving in this period. The noise had an allor nothing effect on *S. titanus* mating behavior, and were unable to ascertain the exact mechanism of the communication breakdown, but the approach appears robust enough to merit largescale testing in the future.

Disruption amplitude (μm/s)	Males		Females		Fisher's exact test
	Total	Stopped calling	Total	Stopped replying	
<1.25	20	0	11	1	NS
1.251–2.5	14	0	14	1	NS
2.501–5	18	2	14	6	*
5.001–10	21	3	19	10	*
10.001–20	13	3	22	15	*
>20	11	4	11	10	***

Note that the classes are not paired, as male- and female-perceived amplitudes were measured separately

*$P < 0.05$; ***$P < 0.001$

Key Points for successful implementation of the MD method

The adoption of strategies based on acoustic tools would enable medium to long-term reduction in the use of chemical pesticides, which fits well within the IPM and organic concepts. However, in order to develop a technique that will be adopted by the public, it must become accessible and commercially viable. Such tools should therefore be (economically) competitive with other solutions already available on the market, namely considering the cost of the device (purchase + maintenance). Equipment costs, as deemed feasible by the industry, could be brought to within 300 €/ha, which is comparable to the cost of pesticide treatments if a 5-year lifetime of a unit is assumed. Another feature of such a system is the suspension of standard wires along the rows in vineyards, which can be used to deliver vibrational energy to individual plants, without the need for elaborate technical solutions.

However, it´s important to understand better the effect of different environmental conditions (rain, atmospheric pressure change), and also the collateral effects on beneficial insects or other pests.

Conclusions

The researchers believe that the use of acoustic devices in a sustainable way for growers is still to come, but the biological knowledge to make it work and the technology are already available. The lack of solutions would be overcome if more directed efforts were made to unify and optimize knowledge already available and to study and develop new solutions for practical application, according to the peculiarities of any crop-pest system where an acoustic based approach is feasible. However, it should be emphasized that many open questions remain to be answered, such as the struggles in the energy supply in the vineyards, before the method is ready for implementation, both technical and strategic.

Aside from the above-mentioned issues of attenuation and effect of vibrations on plants, there is plenty of room for improving design and usability of the devices, which are still in the prototype stage of development. Dealing with different trellising systems (including those without supporting wires) and abandoned vineyards should be carefully considered as well, although these are the issues largely shared with conventional mating disruption methods, so starting from scratch will not be necessary. That said, the researchers believe that this approach is worthy of being pursued further and has potential to replace or at least supplement chemical measures for control of *S. titanus* in Europe.

Source of information

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More information on

www.winetwork-data.eu

Technical datasheets :

- **Guide of good practices in FD management**
- **How to manage with more precision FD**
- **Hot Water Treatment**

Video seminar: State of the art of scientific research on Flavescence Dorée (François-Michel Bernard, IFV)

Experiences of collective management of FD in France (François-Michel Bernard, IFV)

Work realized in common by the facilitators agents of Winetwork project. Data came from practice, through the help of 219 interviews and from a review of scientific litterature.

Thanks to Valerio Mazzoni who participated to the development of this datasheet.