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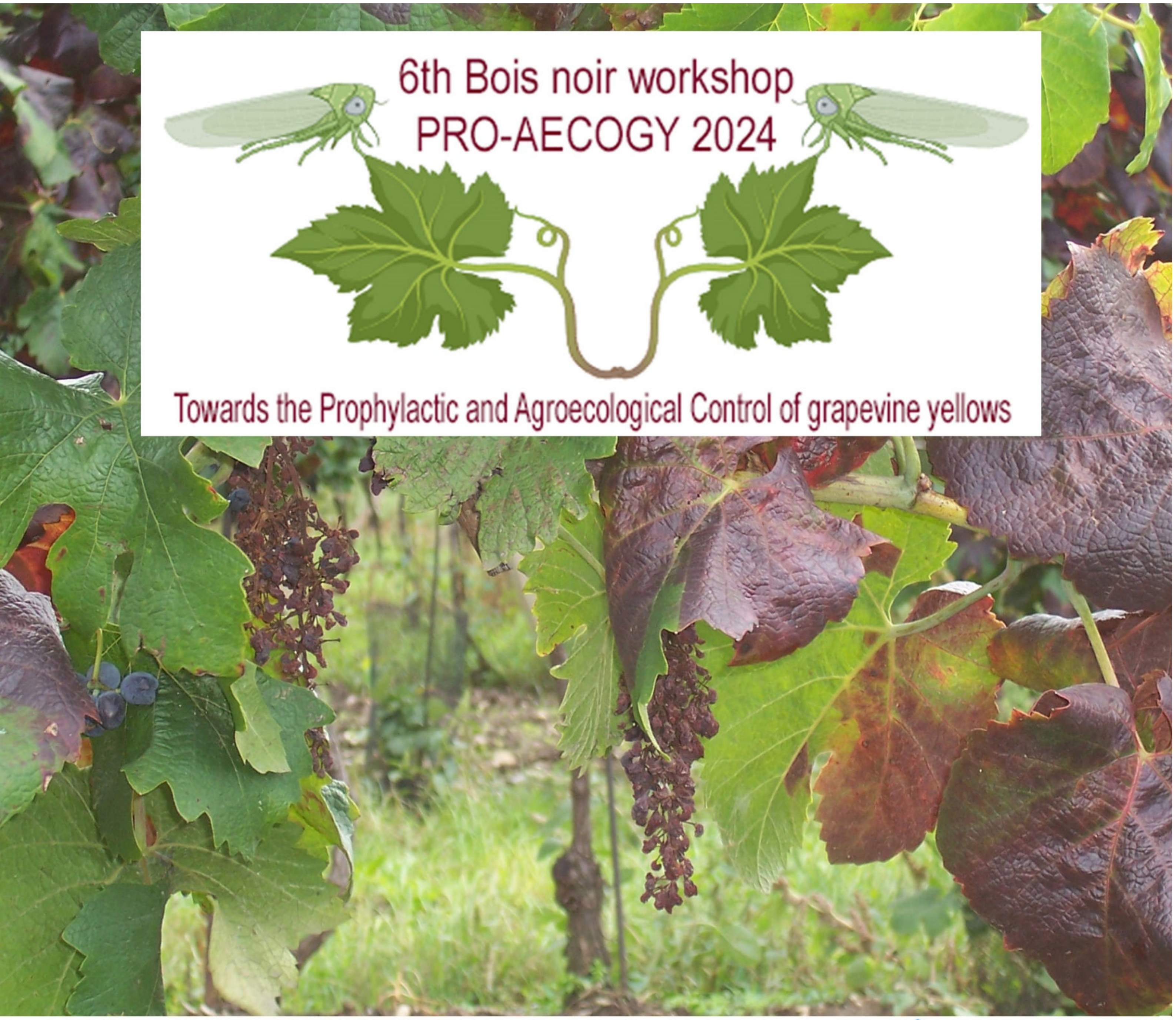

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Vibrational mating disruption and other techniques of behavioral manipulation against grapevine yellows'vectors

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BIOTREMOLOGY

Biotremology is the scientific discipline that studies behavioral and physiological interactions among organisms mediated by vibrational signals (Mazzoni et al., submitted). In insects, vibrational communication is widespread and involves most of the orders. The largest manifestation of it is found in Hemiptera, and in particular in Auchenorrhyncha, which include many pest species including grapevine Yellows' vectors (Virant-Doberlet et al., 2023). In fact, both *Scaphoideus titanus*, vector of Flavescence Dorée, and *Hyalesthes obsoletus*, vector of Bois Noir, primarily use vibrational signals for intraspecific communication, especially for mating (Mazzoni et al., 2014).

THE MATING BEHAVIOR OF *SCAPHOIDEUS TITANUS* AND *HYALESTHES OBSOLETUS*

The American grapevine leafhopper, *S. titanus*, relies on vibrational signals from the early stage of pair formation when males adopt the so-called 'call-fly' strategy to search for available females on the vine canopy. During the twilight hours of July and August, they emit Calling Songs, which consists of series of pulses emitted at regular intervals (ca. 0.3s) for at total duration of 15-20 s. After emitting one or two songs, they fly away (from leaf to leaf) and repeat the call. This routine is interrupted as soon as a female replies to the call, thus establishing vibrational duets with the male. After the first 'identification duet', devoted to reciprocal assessment (of partner identity and quality), males start searching for the females thanks to the 'location duet'. By emitting pulses from a stationary position, males invite females to respond, thus revealing their position on the plant. After a female's pulse, the male moves a few centimeters in the direction of the female. Finally, when the male reaches the female's leaf, they perform the 'courtship duet', during which the male emits the 'buzz', a harmonic sound at high frequency (900-1200 Hz), and that precedes the mating (Polajnar et al., 2014).
The cixiid planthopper, *H. obsoletus*, also communicate through vibrational signals, albeit with distinct mating behavior and different roles and usage of vibrations compared to *S. titanus*. In *H. obsoletus*, females initiate mating communication by emitting a long series of monotone pulses from a static position without exhibiting the call-fly behavior. Males can respond with single pulses (male syllable 1), establishing a first 'duet of identification'. Following this, there is a clear role reversal: males emit long series of pulses (male syllable 2) to which females reply with single pulses, prompting males to approach ('location duet'). When males are in close proximity of females (less than 1 cm) they emit a 'courtship song' preceding copulation (Mazzoni et al., 2010).

SIMILARITIES AND DIFFERENCES BETWEEN THE TWO SPECIES

Pair formation in both species involves an escalation of behaviors associated to phase-specific signals and songs from males and females, engaging in a duet where the integrity and functionality depend on the reciprocal adherence to temporal rules. The scheme follows a fixed sequence: 1) calling; 2) identification; 3) location; 4) courtship; 5) copula. In both species, males actively search for stationary females, who are stimulated to emit signals by male songs (Mazzoni et al., 2014). Despite numerous similarities, some important differences exist, relevant for the development of behavioral manipulation strategies. The most important difference lies in the role of the caller: males in *S. titanus* and females in *H. obsoletus*. In the leafhopper, females never call, necessitating male-driven duets; females need to be continuously stimulated or communication ceases, impeding copulation. In contrast, planthopper females signal their readiness to mate through calls, prompting males to emit series of pulses after identification, revealing their own position. However, a crucial point lies here, males could potentially locate females solely by following initial calls if they lasted long enough to determine their location.

IMPLICATIONS FOR BEHAVIORAL MANIPULATION

Different mating strategies lead to radically different approaches of behavioral manipulation, a pest control technique aimed at inducing target species to exhibit behaviors detrimental to their survival. By mimicking species-specific signals, it is possible to 'cheat' an insect into behaviors that, otherwise, would be advantageous in natural conditions. For example, sexual pheromones massively released in the field can compromise mating success in pest species like moths, where males cannot track natural pheromone trails released by females (Cardé, 1990; Foster and Harris, 1996). Similarly, in Auchenorrhyncha, vibrational signals can be used either to disrupt individuals from mating or attract them into traps. The former strategy has been developed over the last two decades to combat the infestations of *S. titanus* in vineyards (Mazzoni et al., 2019). In practice, by transmitting a disturbance noise (DN) mimicking the male's rivalry signal to vines, the *S. titanus* mating signals can be masked effectively reducing population density. This method, the 'Vibrational Mating Disruption (VMD)', after about 10 years of laboratory and semi-field trials, was tested in the field. Electromagnetic shakers were installed into the poles of a vineyard trellis at 50m intervals to transmit the DN to plants infested by wild *S. titanus* population. The field test was carried out for six consecutive years (2017-2022) in a commercial vineyard in the Province of Trento (Northern Italy) and promising results were observed. Monitoring of the pest population level was performed by visual inspections of immature stages and with yellow sticky traps for adults in two adjacent and similar areas: one vibrated by the shakers and one not-vibrated, the control. A significant reduction in *S. titanus* population density during the first three years of VMD application was observed. During this three-year period the shakers were capable to maintain the DN over the safety threshold (i.e., the least DN amplitude required to prevent male-female communication) in most of the plants. However, due to the senescence of the shakers, their efficacy diminished over time leading to *S. titanus* population rebounds (Nieri et al., 2023; Thiery et al., 2023).

Conversely, *H.obsoletus*, a species that occurs on vines only occasionally, renders VMD impractical. Transmitting disruptive signals to vines, which are not a host plants for the planthopper, would not cause any reduction in the species mating, while treating nettles and bindweed, the actual host plants, with vibrations does not appear feasible. On the other hand, laboratory tests confirmed males tracking the females' calls, suggesting the potential development of 'vibrotraps' to monitor their presence in the field and optimize protection strategies. A similar approach has been successfully developed for another pest, the brown marmorated stinkbug, *Halyomorpha halys*, with a bimodal trap releasing aggregation pheromone and sexual vibrational signals (Zapponi et al., 2022). In the case of *H. obsoletus*, an unimodal trap would suffice, considering the scarce or even null role of volatiles in pair formation (Mazzoni et al., 2010).

CONCLUSIONS AND PERSPECTIVES

In conclusion, the study of intraspecific communication and mating behaviors and their implications for behavioral manipulation in pest control strategies, particularly in the cases of Auchenorrhyncha, sheds light on innovative approaches to managing agricultural pests. These strategies underscore the importance of tailored approaches in pest management, which must account for the diverse mating behaviors of target species, ecological contexts and crop breeding peculiarities (i.e., trellis materials and structure, interplant distances etc.). By understanding this complex network of information and interactions, researchers and practitioners can develop more effective and sustainable pest management strategies that minimize environmental impact while maximizing agricultural productivity.

Looking ahead, future research should focus on refining and expanding behavioral manipulation techniques to address emerging pest threats and adapt to evolving ecological dynamics, taking into account the rapid technological progress that makes previously considered unpractical or costly solutions, practical and effective. An important mantra should be to anticipate future challenges in terms of energy supply, material costs, smart applications etc. and not view the present situation as a limitation. In conclusion, collaborative efforts between researchers, growers, and policymakers are essential for translating scientific insights into practical solutions that promote agricultural sustainability and resilience in the face of changing environmental conditions.

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