UNIVERSITY OF TRENTO

Doctoral School in Civil, Environmental and
Mechanical Engineering

Curriculum 1. Civil and Environmental Engineering

Sabina Avosani

Behaviour, ecology, and vibrational manipulation of insect vectors: the case of the meadow spittlebug *Philaenus spumarius*, with notes on a psyllid pest

2017 - Doctoral thesis

UNIVERSITY OF TRENTO - Italy

Department of Civil, Environmental and Mechanical Engineering

Doctoral School in Civil, Environmental and Mechanical Engineering Topic
2. Mechanics, Materials, Chemistry and Energy - XXXII cycle 2017/2020

Doctoral Thesis – June 2021

Sabina Avosani

Behaviour, ecology, and vibrational manipulation of insect vectors: the case of the meadow spittlebug *Philaenus spumarius*, with notes on a psyllid pest

Supervisors

Prof Marco Ciolli, University of Trento, C3A
Dr Valerio Mazzoni, Fondazione Edmund Mach
Dr Clara Tattoni, University of Florence

Funding

The project has been funded with support from Fondazione Edmund Mach (Department of Sustainable Agro-ecosystems and Bioresources, Research and Innovation Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige, Italy), CIHEAM-Bari (Via Ceglie 9, Valenzano, Italy), and The New Zealand Institute for Plant and Food Research Limited (Lincoln, Canterbury, New Zealand).

Funding information:

European Commission, Grant/Award Number: 727987 XF- ACTORS

Ministry of Business Innovation and Employment contract “Realising Potato Export Growth: C11 × 1308”

Abstract of the dissertation

Insects can cause economic and environmental damages by transmitting bacterial pathogens to plants, making the use of control and prevention measures imperative. On the other hand, methods aimed at preventing crop pests' outbreaks, such as tilling and insecticide applications, pose a threat to human and ecosystems health. Innovative and more sustainable control methods are therefore urgently needed to address farmers' needs and preserve ecosystem services provided by biodiversity. Biotremology is an emerging science that studies the substrate-borne vibrational signals emitted by insects to communicate and interact with their others in the environment. As many insect pests rely on these signals to communicate, their behaviour can be manipulated by transmitting vibrations to the substrate where they thrive. Vibrational signals can, for instance, interfere with target's behaviours such as mating, feeding, and residence time on the plant, and can therefore support integrated pest management strategies alternative to insecticides.

In this work, I investigated the mating behaviour and vibrational communication of two insect vectors having an important economic impact: *Philaenus spumarius* (Hemiptera: Aphrophoridae) and *Bactericera cockerelli* (Hemiptera: Triozidae). I also provided novel insights regarding the vibrational manipulation of their behaviour.

I characterised the sexual behaviour and associated vibrational signals of the meadow spittlebug *P. spumarius*, and I described other intraspecific interactions. I evaluated the correlation between the female signalling activity and development of the reproductive organs, suggesting that a vibrational mating disruption could be effective only if applied from the onset of egg maturation, which triggers the female proceptivity (from August onwards). Besides gathering behavioural data, I used occupancy models to estimate *P. spumarius* presence and detection probability in Trentino (Northern Italy), a rarely deployed approach to study insect distribution. Site covariates impacted *P. spumarius* detectability, suggesting that spittlebug presence can be overlooked if detection is not considered. As plant composition influences the presence and abundance of the spittlebug, I studied the association between *P. spumarius* and its host plants. Such information can support monitoring programs and control strategies aimed at reducing *P. spumarius* population in crops of interest by shaping the plant composition of the ground cover. After investigating the behaviours and the ecology of the spittlebug, I attempted to disrupt its feeding activity using vibrations, opening the gates for future research. In this regard, I combined for the first time biotremology with the Electrical Penetration Graph (EPG) technique.

The tomato potato psyllid *B. cockerelli* is an important threat to New Zealand crops. Besides the characterisation of the pair formation process and associated vibrational signals, I used vibrations to disrupt mate finding by transmitting vibrations to the host plant. Given that the stimulus attracted male psyllids at small scale, this strategy is a feasible approach that could be implemented for use in fields and greenhouses. In this way, an innovative trapping and mating disruption device could join the farmers' toolbox for monitoring and controlling *B. cockerelli.*

In conclusion, I provided new information regarding the ethology of two insect vectors and suggested that there is room for applied biotremology to support sustainable management strategies.

Le savoir humain sera rayé des archives du monde avant que nous ayons le dernier mot d'un moucheron.

Human knowledge will be erased from the archives of the world before we possess the last word that the gnat has to say to us.

Jean Henri Fabre
Souvenirs entomologiques (1870-1889)

Acknowledgements

There are indeed too many persons that I would like to thank.

Firstly, I thank any person that, for better or worse, led me to take the decisions and make the choices that brought me where I am. The PhD was a long journey, and I enjoyed it from the beginning to the end.

Secondly, I thank the persons that believed in me and gave me the opportunity to do what I love, research. Valerio, thank you for transmitting to me your knowledge and your passion, I could not ask for a better mentor and a more inspiring friend. You trusted me and let me do mistakes, explore, be creative and realise my ideas. By teaching me to be autonomous and be a good team member, you allowed me to grow as a researcher and as a person. Even when you sent back a manuscript overturned and asked more *pathos*, I felt lucky, as you were demanding me to improve and learn because you felt I could do it. Daniele, thank you for all the brainstorming discussions in front of a glass of wine, for the ideas, the projects, and your trust. You treated me as a valuable researcher and a good friend, you taught me many lessons, as I am delighted by the goals that we achieved. Vincenzo, thank you for helping me and for your trust, I will never forget it. Clara, Marco, thank you for your precious help and for pushing me to explore new frontiers. Max, *je te remercie* for bringing me on the other side of the world, you allowed me to build up a lab together and learn from you and from the Land of the Long White Clouds. Thank you for being a teacher and a friend, you and Claire became part of my family.

Prosperity is no just scale; adversity is the only balance to weigh friends (Plutarch). Valeria, Niccolò, Carola, Sara, Erika, Giacomo, Tom, Nicola, Lloyd, Silvia, Claire: you supported me during the glorious and darkest times. Plutarch could not have hoped for better friends, and I do not as well. I deeply thank my colleagues (and friends) of the biotremology team and of Plant and Food, for being the most helpful and delighting people I could encounter in a lab. Thank you for the good time spent together and the amazing BBQs as well. Indeed, I acknowledge all the members of other FEM/PFR/DICAM groups, as they always offered me their help and a smile. Marco, Franco, Betta, Dr Fraizingher, Dr Perini: *grazie*, I could not do research without you.

Mamma, Papo. There is nothing I could say for thanking you, as you believed in me, you gave me all I needed to follow my dreams and face the world. You are the pillars of a wonderful family, which is enriched of flaws and love. I am so proud of you, and I feel lucky as you are indeed the most amusing parents. I thank my brothers, which helped me to grow, and their families, which fill our lives with their love.

Merci Robin, *mon âme*, you saved me. I waited 27 years for you and now I am finally complete. Thank you for trotter the globe with me and for laughing with me every single day.

Finally, thank you for reading the results of my research, which meant learning, blood, smiles tears, laugh, pride, and delusions.

These are my *souvenirs entomologiques*.

Table of contents

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Chapter 5

Chapter 6

Chapter 7

Chapter 8

List of figures

List of tables

Chapter 1

General Introduction

1. Insect vectors of bacterial plant-pathogens

Hemipteran insects such as leafhoppers, psyllids and aphids pierce the plant tissues to reach the vascular system of plants with their stylets. These species usually probe the mesophyll but feed from the xylem or the phloem, where they find their primary nutrient source (Perilla-Henao and Casteel 2016). The phloem sap is rich in carbohydrates, proteins, and amino acids, while the xylem sap mainly transports water and contains less nutritive molecules then the phloem (Bové and Garnier 2002; Bae et al. 2015). Depending on the species, bacterial plant-pathogens can colonize either the phloem or the xylem. By feeding in vascular tissues, hemipteran insects interact with these microorganisms and become active players in their spread (Perilla-Henao and Casteel 2016). After acquisition of bacterial cells from infected tissues, they can transmit them to healthy plants (Purcell 1982). In this way, insect vectors are associated with outbreaks of devastating diseases in perennial and annual crops (Crosslin et al. 2010; Gottwald 2010; Munyaneza 2012; Chuche and Thiéry 2014; Godefroid et al. 2019). Nonetheless, by understanding the relationship between vectors and bacterial plant-pathogens, it is still possible to prevent further spread of diseases, considering that the specific interactions between pathogen and vector influence transmission efficiency (Purcell 1982). For instance, different microorganisms can replicate and propagate either outside or within cells of their vectors, which represent alternative hosts of the pathogen. Mechanisms mediating insect recognition, movement and replication are specific for each bacterium–vector interaction and need to be characterised to better understand disease epidemiology (Purcell 1982; Bové and Garnier 2002; Orlovskis et al. 2015; Perilla-Henao and Casteel 2016). While some microorganisms can be vertically transmitted to the offspring through the female ovary, others are only retained in the mouthparts (Purcell 1982; Backus and Morgan 2011).

Factors such as environmental conditions and especially biology of host plants, plant pathogens and vectors play a crucial role in the spread and spill over of plant diseases. By acquiring deep knowledge regarding these components, it is possible to identify main research needs, model diseases epidemiology, and develop valuable control methods (Perilla-Henao and Casteel 2016).

1.1. Gathering biological and ecological data on insect vectors

In the past decades, dramatic outbreaks of vector-borne plant diseases such as the Olive Quick Decline Syndrome, Pierce's disease of grapevine, citrus Huanglongbing and zebra chip resulted into a race to acquire information regarding the plant pathogen and its vector(s) (Gottwald 2010; Orlovskis et al. 2015; Almeida 2016b; Cornara et al. 2018a). Similar knowledge is a prerequisite for successful management of vector-borne diseases, since epidemics can be controlled by interrupting one or more variables involved in its pathosystem (Almeida et al. 2005). To achieve this goal, multiple techniques are combined within an integrated pest management framework.

Considering its crucial role in the pathosystem, data on the insect vector are fundamental to develop epidemiological models, risk assessment plans, and effective pest management techniques. For example, aspects such as life history, behaviour, ecology, population dynamics, reproductive biology and host plant associations are key factors driving transmission efficiency of the plant disease and may be manipulated for reducing its spread (Polajnar et al. 2015; Pertot et al. 2017; Cornara et al. 2018a).

1.2. Economic and environmental impacts of insect vectors

Vector-borne plant diseases cause important losses of food, income, or capital that impoverishes growers by reducing crop yields (Munyaneza 2010; Chuche and Thiéry 2014; Strona et al. 2017). Besides these economic losses, plant diseases foster increased management and regulatory costs, which result in a low cost-benefit ratio for the application of pesticides (Daugherty et al. 2015). Furthermore, methods addressed at managing plant pathogens and their vectors may represent a threat for the environment by affecting ecoservices and landscapes (Simberloff et al. 2013; Strona et al. 2017; Brühl and Zaller 2019; Desprez-Loustau et al. 2020; Ali et al. 2021). Plant removal, for instance, is mandatory for the control of some plant diseases (Chuche and Thiéry 2014; Cornara et al. 2018a; Bragard et al. 2019) but can reduce CO_2 sequestration and increase soil temperatures and evaporation rates (Ali et al. 2021). Management of a vector is often achieved by means of pesticides and soil tilling, the latter addressed to remove insects from the herbaceous cover within and surrounding a crop (Strona et al. 2017; Cornara et al. 2018a; Bragard et al. 2019). However, soil tilling is a double-edged weapon, considering that it can contribute to desertification and reduction of habitats that host beneficial arthropods, which provide important ecosystem services (Kairis et al. 2013; Karamaouna et al. 2019; Molinatto et al. 2020). The major threat to biodiversity, nonetheless, is represented by pesticides, given that they are causing the dramatic population decline of many terrestrial and aquatic organisms (Brühl and Zaller 2019; Sánchez-Bayo and Wyckhuys 2019). Intensive use of pesticides is currently driving to low abundance and restricted geographical distribution of species, leading many towards extinction (Diamond 1989; Brühl and Zaller 2019; Sánchez-Bayo and Wyckhuys 2019). Many introduced/native vectors are generalist species and can take advantage of the pesticide-driven biodiversity decline. In fact, ecological niches stroke by species extinction could be occupied by more adaptable species and, as consequence, shift to species-poor assemblages dominated by generalists (White and Kerr 2007; Sánchez-Bayo and Wyckhuys 2019). On the long-term, pesticide use can result in increased vector abundance, while its short-term effectiveness is often overestimated (Perring et al. 1999; Madden et al. 2000). An increased resistance to pesticides, for instance, can disrupt pest control and is considered one of the most challenging issues to overcome to ensure food security (Network 2016).

For all these reasons, innovative and environmentally safe strategies are needed to manage vector pests and control vector-borne plant diseases.

2. Applied biotremology: when vibrations meet pest control

2.1. A beginner’s guide to biotremology

Biotremology is an emerging discipline that studies the communication and behavioural processes relying on substrate-borne vibrations, and it is receiving growing interest from the scientific community (Hill and Wessel 2016; Hill et al. 2019; Cividini and Montesanto 2020). The term “biotremology” has been introduced only in 2016, and the number of related publications exponentially increased since then (Hill and Wessel 2016; Hill et al. 2019). In the beginning, these publications aimed at studying the vibrational communication of animals, whilst the scope of recent studies broadened, progressively including environmental noise, new technologies, and neurosciences (Hill et al. 2019).

Both vertebrates and invertebrates use substrate-borne vibrations to interact with other co- and heterospecifics and with their environment, and this form of communication is more widespread and ancient than previously thought (O'Connell-Rodwell et al. 2001; Virant-Doberlet and Cokl 2004; Hill 2009; Hill and Wessel 2016; Hill et al. 2019). "Well-studied" taxa (i.e., species whose vibrational communication has been studied for 15-30 years) include elephants, anurans, heelwalkers and termites. The number of studied species is dramatically increasing, and new behavioural data demonstrates that vibrations can be used by animals to accomplish crucial and diverse functions (Virant-Doberlet and Cokl 2004; Hill et al. 2019). Indeed, vibrational communication underlies an intricate network of intraspecific and interspecific interactions, in which senders, receivers, and eavesdroppers are concerned by a dynamic exchange of information (Figure 1.1) (Cocroft and Rodríguez 2005).

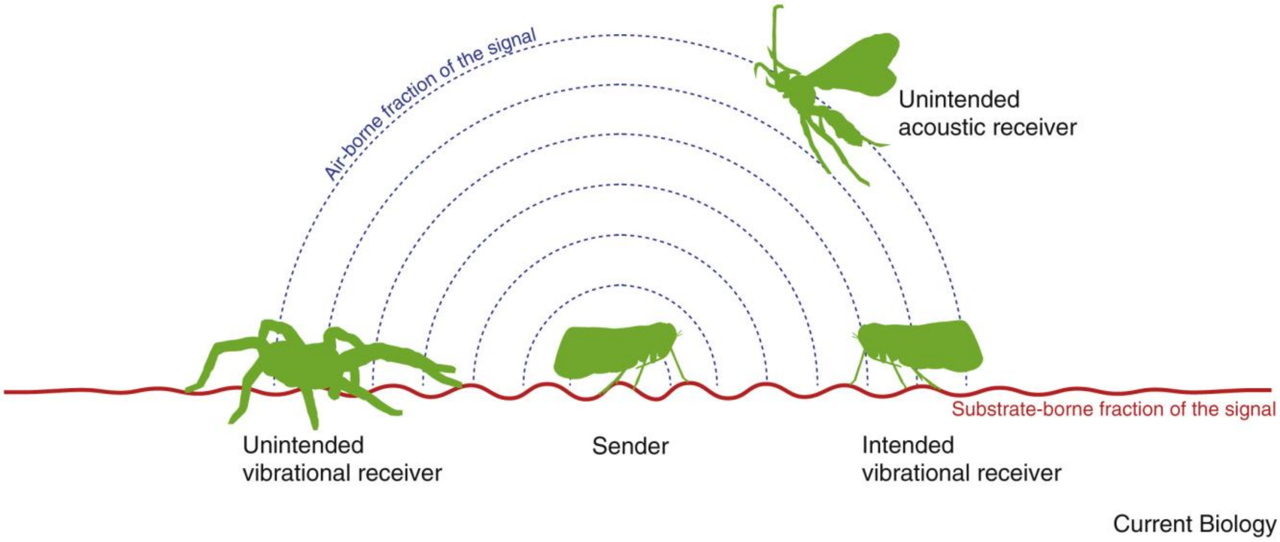

Figure 1.1. Complex vibrational communication network. An insect is emitting signals addressed to a conspecific receiver, but a predator able to perceive vibrations and an acoustical orienting parasitoid are eavesdropping (Hill 2009).

Within arthropods, sexual behaviours, defence, parental care, localisation of feeding sites, prey-predators' interactions and social behaviours rely on substrate-borne vibrations (Virant-Doberlet and Cokl 2004; Hill 2009; Hill and Wessel 2016; Cividini and Montesanto 2020). Substrate-borne communication is used by more than 200 000 insect species, encompassing bees (Michelsen et al. 1986), termites (Hager and Kirchner 2013), ants (Pielström and Roces 2012), lacewings (Henry 1979, 1980), Auchenorrhyncha (Nieri et al. 2017), stoneflies (Orci and Murányi 2021), psyllids (Eben et al. 2015) and beetles (Kasper and Hirschberger 2005). Depending on the species, vibrations are produced owing to specialised organs or to specific movements of body parts (Virant-Doberlet and Cokl 2004; Hill 2014). Four primary categories of vibration-producing mechanisms have been proposed: drumming, tremulation, stridulation, and tymbal buckling (Hill 2014). The most complex organ enabling production of vibrations and sounds is the tymbal, which was firstly described in cicadas. Within Auchenorrhyncha (i.e., Fulgoromorpha and Cicadoidea), tymbal-like organs enables substrate-borne communication and likely share with the tymbal a common origin that dates to more than 300 Mya. Given that tymbal and tymbal-like organs produce vibrations by involving the same set of muscles that cause distortions of specialized parts of the integument, the name Tymbalia was suggested for the taxon comprising Cicadomorpha, Fulgoromorpha, and Heteropteroidea (i.e., Hemiptera exclusive of Sternorrhyncha) (Wessel et al. 2014). Depending on the vibration-producing mechanism and species-specific structures, insects are capable to emit a great variety of signals. For instance, vibrational signals emitted by plant-dwelling stinkbugs (Hemiptera: Pentatomidae) are produced by tremulation of body parts and percussion of the substrate. Stinkbugs can modulate the frequency of the emitted signals, but dominant frequency is generally around 100 Hz, while higher harmonics reach 1000 Hz (Virant-Doberlet and Čokl 2004; Čokl et al. 2014). The substrate influences the signal features, since plants are frequency filters privileging frequencies around 100 Hz and attenuating those higher than 600 Hz (Čokl et al. 2014). Species-specific vibrational signals are therefore tuned to optimally transmit through a certain substrate, in order to be perceived by the desired receiver, which, on the other hand, must have specifically tuned organs to perceive vibrations (Čokl and Virant-Doberlet 2003; Virant-Doberlet and Cokl 2004; Hill 2009; Lakes-Harlan and Strauß 2014; Oberst et al. 2019). Within arthropods, the most sensitive mechanoreceptors are subgenual organs (Figure 1.2), which are in the proximal tibia, even though there are some exceptions among dipterans and coleopterans species. The function of this complex sensory organ is enabled by aggregations of scolopidia, the unit mechanoreceptor in invertebrates. Although organisation of subgenual organs differs between species, they are generally not connected to leg joints and can detect external vibrational signals transferred to the leg. The lower acceleration stimulus that can trigger a neuronal response, namely threshold, is a crucial parameter that determines the sensitivity of subgenual organs in different species, even when receptors can be tuned to different frequencies (Lakes-Harlan and Strauß 2014).

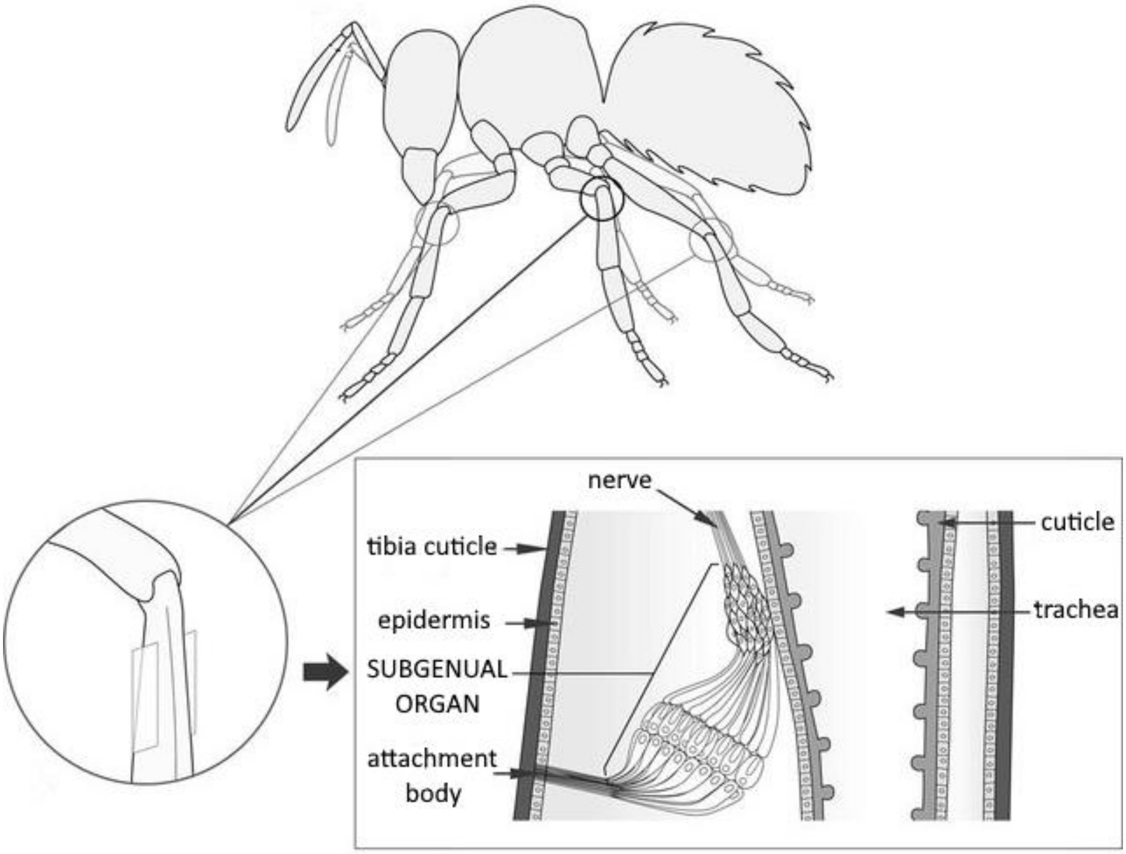

Figure 1.2. Position and internal structure of the subgenual organ of an insect. The internal structure of the organ is briefly illustrated in the square (adapted from “Hunt J & Richard FJ (2013). Intracolony

vibroacoustic communication in social insects. Insectes Sociaux, 60(4), 403-417. DOI: 10.1007/s00040-013-0311-9”).

Vibrational signals play a fundamental role in the sexual behaviour of numerous arthropod species, being used to identify and locate potential partners. In plant-dwelling insects, vibrations convey relevant information during pair formation phases such as identification, courtship, localisation, and mating. Spectral and temporal features of an emitted signal generally contain the information regarding the species (“who”), while intensity of the signal is likely the cue (“where”) used to reach a signalling partner (Virant-Doberlet and Cokl 2004; Polajnar et al. 2014; Cividini and Montesanto 2020). Calling signals are the first signals emitted during pair formation and allows recognition of a conspecific. A receiver interested in mating can, in turn, emit a response signal and establish a vibrational duet with the calling individual. This exchange of signals enables mate localisation, in that one partner searches whilst the other remains stationary on the plant (Virant-Doberlet and Cokl 2004; Mazzoni et al. 2010; Derlink et al. 2014; Polajnar et al. 2014; Nieri et al. 2017; Nieri and Mazzoni 2019). However, mate finding does not necessarily imply mating. Prior to copulation, females are usually reluctant to mate because their investment in offspring production is way higher than those of males (Gwynne 1991). After localisation of the female, males therefore produce courtship signals, which express the highest level of species-specificity, ensuring correct identification of a conspecific (Mazzoni et al. 2014, 2015). Courtship behaviours can be more or less complex, include tactile, visual, and chemical stimuli, and are aimed at triggering female acceptance to mating (Ringo 1996; Candolin 2003; Mazzoni et al. 2013; Lubanga et al. 2014). The male usually allocates most of his energies in this phase, since the quality of the courtship indicates male fitness and can determine whether the female will allow copulation (Mazzoni et al. 2014). The pair formation process is not an intimate interaction between male and female and can be disrupted or delayed by biotic and abiotic factors (Čokl and Virant-Doberlet 2003; Mazzoni et al. 2009a; Virant-Doberlet et al. 2019). For example, mate localisation and courtship are vulnerable to vibrational interference coming from intruders such as rival males or… biotremologists.

2.2. Applied biotremology: vibrational manipulation of insect behaviours

Applied biotremology is a growing discipline that attracts the interest of companies involved in pest control. Said interest is reinforced by the increasing awareness of insecticides-related risks. By studying vibrational signals associated with mating, researchers can provide useful information regarding insect behaviour and evolution. Besides conservation purposes, such knowledge is required to develop innovative pest control techniques. Thusly, in the last decade, promising technologies and vibrational strategies have been developed, opening the gates for further studies involving numerous species, notably hemipterans (Polajnar et al. 2015; Hill et al. 2019). For instance, a vibrational vineyard has been established in Northern Italy in 2017 for the control of *Scaphoideus titanus* (Hemiptera: Cicadellidae), a leafhopper capable of transmitting the phytoplasma causal agent of Flavescence dorée, a disease in grapevine (Chuche and Thiéry 2014; Mazzoni et al. 2019). That setup was made possible thanks to a fine understanding of *S. titanus’* sexual behaviour. In particular, it was observed that rival males produce a specific disturbance noise to interrupt an ongoing male-female duet and, eventually, mating (Mazzoni et al. 2009b; Polajnar et al. 2016). The recorded male disturbance signal was used to disrupt *S. titanus’* mating process, at first in the laboratory, then in the field. However, several technical aspects were considered to optimize the signal transmission through the trellis and from there to the plants. The novelty of this pest control strategy was not mating disruption per se, but the exploitation of vibrations instead of pheromones (Polajnar et al. 2015; Mazzoni et al. 2019). In California, disturbance vibrational waves were similarly transmitted through grapevine stems to interfere with the sexual communication of the glassy-winged sharpshooter *Homalodisca vitripennis* (Hemiptera: Cicadellidae), a vector of the noxious bacterium *Xylella fastidiosa* (Gordon and Krugner 2019). A mating disruption and trapping device for vibrational control of the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae) is also under development in the US (Lujo et al. 2016; Mankin 2019). Besides mating disruption, a trapping strategy that integrates chemical and vibrational signals has been proposed against the brown marmorated stink bug *Halyomorpha halys* (Hemiptera: Pentatomidae), while ongoing research is aimed at using vibrations to protect forests from invasive bark beetles (Mazzoni et al. 2017; Polajnar et al. 2019; Takanashi et al. 2019).

When considering the potential benefits of vibrational pest control strategies, some factors should be taken in account to ensure the efficiency of behavioural manipulation. For instance, some insect species rely on unimodal communication systems, in that they only use vibrations to identify, locate and court potential partners. In similar circumstances, disturbance vibrations alone could efficiently interfere with mating behaviours, while other stimuli should be integrated to interfere with species that use multiple cues for mate finding or aggregation, as seen in *H. halys* (Polajnar et al. 2016, 2019; Mazzoni et al. 2019). A case-by-case strategy is the best way to ensure an effective pest control method, given that identification of the most appropriate management of a species requires prior characterization of its life history. For instance, using signals that mimic a female attracting the *S. titanus* male into a trap does not seem feasible, since males and females establish a tightly synchronized mate finding duet. To operate, such a trap would require emitting the female signal in the proper time window by using a sophisticated device capable of replying to male signals (Mazzoni et al. 2019). Nevertheless, attractive signals can be applied when the male (or the female) is guided by the partner's signals without the need for a male-female duet, as observed in *D. citri* (Mankin 2019; Mazzoni et al. 2019; Polajnar et al. 2019). Besides mating, vibrational stimuli can interfere with residency on host plants and feeding, or trigger behaviours such as freezing and startle responses (Lee et al. 2012; Tsubaki et al. 2014; Takanashi et al. 2019).

Continuous transmission of vibrational signals involves high energy consumption and costs, but adoption of duty cycles could overcome this issue by considering behavioural factors such as sexual inactivity. In this regard, knowledge regarding insect phenology such as number of generations per year or reproductive seasonality suggests when and for how long stimuli must be applied to ensure pest control. Given that sexual behaviours can depend on environmental parameters (i.e., rain, temperature, and wind conditions), a real-time, computer-based analysis may support management approaches. In fact, they can determine the timing of signal application by coupling signal transducers with environmental sensors, enhancing vibrational stimuli effectiveness, and reducing costs related to signal transmission (Polajnar et al. 2016; Mazzoni et al. 2019).

These promising results demonstrate that vibrations share several similarities with semiochemicals, given that both can be used to manipulate relevant behaviours of insect pests (Figure 1.3). In this regard, researchers dealing with applied biotremology address vibrational stimuli as "semiophysicals" or "semiomechanicals". Chemical and vibrational stimuli differ in aspects such as signal active space, which represents the signal amplitude, sufficiently above the receiver detection threshold, eliciting behavioural responses. By modelling and enhancing propagation of vibrational signals through host plants, it is therefore possible to maximise the signal active space and consequently achieve high performances in pest control (Mazzoni et al. 2019). Besides improving signal propagation, specificity of the signal must be taken into consideration, given that vibrational behavioural manipulation techniques should be specifically addressed to the insect target, to reduce potential negative effects on beneficious arthropods (Polajnar et al. 2015; Hill et al. 2019; Mazzoni et al. 2019).

Figure 1.3. Vibrational mating disruption (a) of the leafhopper *Scaphoideus titanus* (b) by means of a vibration transducer mounted on a pole of the vineyard trellis system. This model resembles the mating disruption approach for (c) *Lobesia botrana*, achieved by means of (d) pheromone dispensers (Mazzoni et al. 2019).

Electronics and energetics are under steady development and will soon enable vibrational pest control devices to be applied in the field and integrated in farmers' and foresters' toolbox. Although more information regarding positive or negative effects of vibrations on non-target arthropods is needed, applied biotremology is opening the way for more sustainable pest control techniques alternative to pesticides (Hill et al. 2019).

3. *Philaenus spumarius:* an insect vector menacing the European agriculture

3.1. From old inhabitant to new threat

Modern agriculture favours invasion by alien species since it implies an increased transportation of goods over long distances, which promotes introduction of organisms (Hulme 2007). A biological invasion can alter the ecosystem's functioning if an organism can establish itself to the detriment of other species (Hulme 2007; Meyerson and Mooney 2007). These invasions of alien insect species have severe consequences, from damages to biodiversity and environment to socio-economic costs, which include direct damage or control-relative costs (Perrings et al. 2002; Hill et al. 2016). Furthermore, even autochthonous insects can become unwanted pests when a plant pathogen is introduced in an area. If the pathogen establishes itself and finds appropriate hosts, its introduction can be devastating. Insects vary in their ability to serve as vector, and transmission efficiency depends on temporal and spatial factors. For instance, disease epidemics can be governed by agricultural economics, geographic and environmental variables, which influence host distribution, insect populations, and plant-pathogen-vector associations (Perilla-Henao and Casteel 2016).

Recently, introduction of the Gram-negative bacterium *Xylella fastidiosa* in Europe initially resulted in an unprecedent social and economic turmoil in Southern Italy and then became a threat for European agriculture (Martelli et al. 2016; Saponari et al. 2017; Cornara et al. 2018a). *Xylella fastidiosa* originates from the Americas, where it causes diseases in crops such as grapevine, citrus, and almond, but also infects numerous wild and ornamental species (Purcell 1997; Sicard et al. 2018; EFSA et al. 2019; Bragard et al. 2019). A strain of the bacterium was recently introduced by contaminated material in Europe, where it infected olive plants, causing severe symptoms such as leaf scorch and dieback (Giampetruzzi et al. 2017; Saponari et al. 2019). First detection occurred in Southern Italy in 2013 and was followed by further reports in other European countries, where additional bacterial strains were identified (Saponari et al. 2013; Denancé et al. 2017; Olmo et al. 2017; EPPO 2018; Bragard et al. 2019; Frem et al. 2020; Schneider et al. 2020). Considering that *X. fastidiosa* is transmitted by xylem fluid-feeding insects in Auchenorrhyncha (i.e., Cercopoidea and Cicadoidea superfamily, Cicadellinae subfamily within Cicadellidae family) (Redak et al. 2004; Lopes et al. 2014; Almeida 2016b; Cornara et al. 2019a; Krugner et al. 2019), extensive surveys were performed to identify European vectors (Cornara et al. 2019a).

The native meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae) was recognized as the main vector of the bacterium and consequently became a menace for European agriculture. Before *X. fastidiosa* arrival, this common endemic insect was studied by European scientists for its unique colour polymorphism and the spittle produced by juvenile stages, whilst information regarding its biology, ecology, and ethology has been rather neglected (Yurtsever 2000; Silva et al. 2015; Cornara et al. 2018a). Dating back to the 1930's and 1950's, *P. spumarius* was considered a pest in the United States owing to damages caused by nymphs feeding on alfalfa and strawberry (Edwards 1935; Weaver and King 1954). Even so, economic losses caused by this insect were never so important as to encourage extensive research, especially in the Old World (Cornara et al. 2018a). Its role in *X. fastidiosa* transmission changed this trend and research is currently underway to collect information on *P. spumarius*, given that such data are fundamental prerequisites for developing future control approaches and modelling epidemiology of the plant disease (Birch et al. 2011; Pertot et al. 2017; Cornara et al. 2018a; Fierro et al. 2019).

3.2. Life history of *P. spumarius*

The meadow spittlebug *P. spumarius* L. (1758) is a hemipteran insect belonging to the superfamily Cercopoidea. Within this group, nymphal stages protect themselves from dehydration by producing a froth that covers their entire body. This "frothy dew" consists of a fluid secreted from the anus and integrated with secretions of abdominal glands and air bubbles (Wiegert 1964b; Yurtsever 2000; Rakitov 2002).

Genetics. As previously mentioned, *P. spumarius* attracted the interest of researchers owing to the adults' colour polymorphism, which also caused taxonomic confusion regarding this species

(Nast 1972; Cornara et al. 2018a). *Philaenus spumarius* provided a good model to test hypotheses about adaptation and maintenance of polymorphisms, because it is wide-spread, abundant, and genetically diverse (Rodrigues et al. 2014; Silva et al. 2015). At least 16 different dorsal colour pattern phenotypes (divided in non-melanic and melanic forms) occur across its geographic range (Halkka et al. 1990; Stewart 1996; Quartau and Borges 1997; Silva et al. 2015). Breeding experiments unveiled that a single autosomal locus (seven alleles with complex dominance and co-dominance relationships) determines the phenotype of adults (Halkka et al. 1990; Stewart and Lees 1996). Continuous maintenance of rarer phenotypes suggests that selective mechanisms are involved, and diverse evolutionary mechanisms act on colour polymorphism, even if all involved factors are still unknown (Halkka et al. 1974; Halkka et al. 1975; Silva et al. 2015). For instance, higher rate of attack by parasitoids of a pale phenotype may be compensated by higher longevity and fertility (Silva et al. 2015).

Biology, phenology, and behaviour. *Philaenus spumarius* is an univoltine specie that overwinters as an egg. After the egg stage, development occurs through five nymphal instars and takes five to six weeks, depending on weather and geographical location (Figure 1.4) (Weaver and King 1954; Wiegert 1964a; Cornara et al. 2018a). Males usually hatch before females, which, in turn, survive longer during the autumn(Edwards 1935; Halkka 1964; Drosopoulos and Asche 1991). First instar nymphs hatch in February-March, while adults can be found from April to December, even if environmental factors can affect adult emergence and survival (Weaver and King 1954; Wiegert 1964a; Cornara et al. 2018). An ovarian para-pause prevents development of eggs prior to August, whilst eggs maturation increases until November (Weaver and King 1954; Witsack 1973). Although ovaries are immature in spring, females can mate several times with different males and potentially store sperm inside their spermatheca (Robertson and Gibbs 1937; Yurtsever 2001). Polyandry is thought to provide genetic and evolutionary benefits to *P. spumarius* and other species, but more data are indeed needed to support this hypothesis (Yurtsever 2001). Oviposition starts in September and continues until death of females, which lay eggs in stubble, straw, and dead parts of plants, usually close to the ground (Barber and Ellis 1922; Weaver and King 1954; Yurtsever 2000). Egg masses consist of 18–51 elements that are held together by a frothy cement (Mundinger 1946; Weaver and King 1954; Ossiannilsson 1981). Environmental variables influence *P. spumarius'* life cycle, and among them, humidity and water availability represent the main driving forces. For instance, nymphs tend to avoid direct sun and drying winds by seeking sheltered places (i.e., plant rosettes and juxtaposing plant surfaces) and producing spittle (Weaver and King 1954; Grant et al. 1998; Bodino et al. 2020). Almost any plant providing sufficient moisture can support nymphal feeding and survival (Weaver and King 1954). A thick cornea protects adults from water loss, which is also avoided by migrating in cooler areas during summer (Keskinen and Meyer-Rochow 2004; Cornara et al. 2018a). Timing and site location of oviposition prevent dehydration of eggs, which likely hatch when humidity reaches certain values (Weaver and King 1954). Even if *P. spumarius* prefers moist environments its incredibly wide distribution suggests that this insect can live and thrive in different habitats, provided that host plants are not dried and support nutrition (Yurtsever 2000; Rodrigues et al. 2014; Cornara et al. 2018a). It is also possible that populations living in different geographical areas have undergone site-specific adaptations and hence present different humidity requirements. Humidity is not the unique pivotal factor governing *P. spumarius'* life, given that the ovarian development and oviposition are triggered by photoperiod changes (Witsack 1973; Cornara et al. 2018a; Morente et al. 2018a). Temperature influences *P. spumarius'* phenology, but data on thresholds and effects on spittlebug development is rather inconsistent (Weaver and King 1954; Medler 1955; Chmiel and Wilson 1979; Masters et al. 1998). In addition, population density may influence the fitness of *P. spumarius*, notably of nymphs. The latter tend to aggregate and share the same host and spittle, and it has been accordingly observed that nymph's mortality is inversely density dependent (Whittaker 1973). On some occasions, even different cercopid nymphs can be found with *P. spumarius* inside a spittle mass (Halkka et al. 1977). Aggregation can provide benefits if maintained below competition levels, because it ensures a bottom-up effect by overcoming negative tensions due to xylem feeding and improving thermoregulation (Wise et al. 2006). On the other hand, a density-dependent regulation may occur in adults (Whittaker 1973).

Although recent studies unveiled some interesting aspects of *P. spumarius'* biology and physiology such as feeding behaviour, structure of antennal sensilla, and physiological responses to plant volatile organic compounds, information regarding its sexual behaviour and intraspecific communication is lacking and urgently needed.

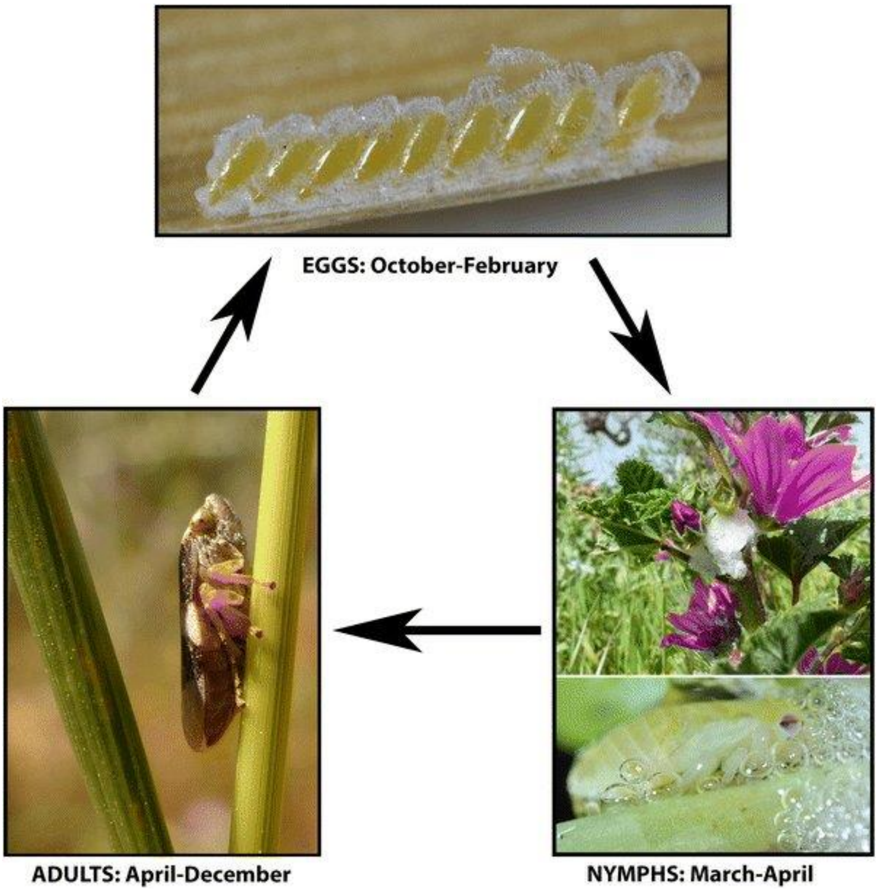

Figure 1.4. Life cycle of *Philaenus spumarius* in most habitats of the northern hemisphere (Cornara et al. 2018a)

*Ecology**.*** *Phileanus spumarius* is native of the Palearctic region but its geographic range expanded in Nearctic regions, as well as most of the temperate areas of earth and oceanic islands, likely promoted by anthropogenic introduction (Cornara et al. 2018a). The wide host plant range (both monocotyledonous and dicotyledonous) likely favoured its geographical expansion, considering that other monophagous Philaenus species are not widely distributed (Rodrigues et al. 2014; Cornara et al. 2018a). *Philaenus spumarius* nymphs and adults feed on xylem fluid, overcoming relatively high fluid tensions when ingesting from main transpiration stems (Wiegert 1964b; Horsfield 1977; Malone et al. 1999). Preferred hosts include nitrogen-fixing legumes and other plants with high amino acids concentration in the xylem sap, especially in the nymphal stages (Horsfield 1977; Thompson 1994, 2004). After *X. fastidiosa's* introduction in Europe, monitoring *P. spumarius* populations became mandatory. Researchers accordingly developed and shared with the scientific community reliable and cost-effective sampling techniques to provide useful and comparable data between locations. Once established an efficient sampling design, extensive surveys have been conducted to study *P. spumarius* distribution and its host exploitation in diverse European countries. These surveys provided fundamental knowledge about disease epidemiology and suggested how, in crops susceptible to *X. fastidiosa,* ground cover could be shaped to reduce vector settlement (Morente et al. 2018b; Dongiovanni et al. 2019; Bodino et al. 2020). In fact, plant composition in the herbaceous cover plays a major role in *P. spumarius* abundance, depending on site, period of the season, and insect developmental stage (Cornara et al. 2017b; Morente et al. 2018b; Bodino et al. 2020). In summer, for instance, adults colonise olive trees longer and in higher numbers in northern Italy than in southern Italy, suggesting that, in case of a *X. fastidiosa* outbreak, the risk of inoculation of the fastidious bacterium could be higher in northern Italian regions. Higher spittlebug population levels in northern Italy also support this hypothesis (Bodino et al. 2020). In chapter 4, we present the results of our surveys in northern Italy, which were based on the sampling methods used in other European surveys (Cornara et al. 2017b; Morente et al. 2018b; Bodino et al. 2019, 2020; Dongiovanni et al. 2019), but implemented with occupancy models. On that aspect, our approach proved innovative because it allowed the estimation of not only *P. spumarius* presence, but also its detection probability. In this way, we provided evidence that occupancy models could support pest management and risk assessment plans.

Migration and energetics. Philaenus spumarius geographical range depends on weather, since high temperatures and low humidity negatively affect eggs and young nymph survival. Although population density can be reduced by years of low precipitations and high spring temperatures, similar conditions are not sufficiently frequent to mitigate expansion of population, whilst other limiting factors such as predation likely operate (Weaver and King 1954; Wiegert 1964a). At any rate, adult population density within a crop can be bimodal, with peaks occurring in different periods of the season (i.e., in early July and mid-August in Central-Western America; Wiegert 1964a). Similar movements can be due to cultural practices (as mowing), habitat suitability or spring/summer drought (Wiegert 1964a; Drosopoulos and Asche 1991). Rapid habitat changes and periodic dispersion of the populations by summer mowing can lead spittlebugs to invade new areas, which can be reached by leaping and flying (Ossiannilsson 1981; Cornara et al. 2018a). Adults can perform impressive jumps with their hind legs, whose muscles and joint interlocking mechanisms enable generation of forces and accelerations (Burrows 2003). In this way, *P. spumarius* can reach heights from 15 to 70 cm from the ground, while a flight can span 30 m. Adults can move 100 m in one day (Weaver and King 1954). Migratory behaviours are well-known in this species, in which females move earlier in the season and reach further distances than males (Weaver 1951; Weaver and King 1954; Lavigne 1959; Halkka 1962; Wiegert 1964a; Drosopoulos and Asche 1991; Grant et al. 1998). As already mentioned, migration is feasible because *P. spumarius* can adapt to different habitats owing to its high polyphagy (Halkka et al. 1967). In autumn, adult migratory activity is limited, possibly because temperatures are cooler, males diminishing, and females spending time and energy to lay eggs (Weaver and King 1954; Halkka 1962).

3.3. *Philaenus spumarius* & *Xylella fastidiosa*

Even if *X. fastidiosa* subspecies *pauca* is the causal agent of the olive disease, it could not threaten an entire continent without the involvement of *P. spumarius*. Due to its adaptability, polyphagy, and abundance, it can thrive in almost all terrestrial habitats and mediate the spread of the fastidious bacterium from olive to olive and to alternative hosts (Saponari et al. 2013, 2014, 2017; Cornara et al. 2017a, b). With olive plants being the main reservoir of *X. fastidiosa*, *P. spumarius'* movements within the orchard influence disease epidemics (Cornara et al. 2017a, b). Plant's physiological and chemical characteristics usually guide movement of xylem-feeding insects, which shift from host to host following daily fluctuations of xylem sap nutrients (Andersen et al. 1992). In Southern Italy, colonisation of olive canopies by *P. spumarius* adults is likely due to the summer rise of temperatures, which causes desiccation of orchards ground cover. Presence of competent vectors within the olive canopy increase probability of infection and reduce the disease incubation period (Daugherty and Almeida 2009). In these circumstances, spittlebugs acquire the bacterium from infected olives and then disperse it when the weather cools, then move to other hosts (Cornara et al. 2018a). *Philaenus spumarius* is therefore a fearsome vector of *X. fastidiosa*, given that it promotes both secondary transmission between olive plants and primary transmission to wild plants surrounding the orchard (Cornara et al. 2017a, 2018a). Although *P. spumarius* carries low population of bacterial cells in the foregut, transmission probability also depends on the number of infective spittlebugs on the host plant, which can be very high in Italian olive orchards (Cornara et al. 2016, 2017a, 2018a; Bodino et al. 2020). Besides *P. spumarius*, the role of other auchenorrhynchans in *X. fastidiosa* spread was investigated, providing evidence that cicadas are not involved in disease outbreaks (Cornara et al. 2017b, 2019a, 2020b). On the other hand, the spittlebugs *Philaenus italosignus* and *Neophilaenus campestris* (Hemiptera: Aphrophoridae) can acquire and transmit *X. fastidiosa* subsp. *pauca* associated with the Southern Italy epidemics. Even so, *P. italosignus* and *N. campestris* are not relevant in mediating the transmission to olive, which is not a preferred host for these insects (Cavalieri et al. 2019). Epidemics accordingly depends on host–plant preference/selection, and multiple epidemiological cycles among wild and cultivated plant species are expected in Europe due to the wide host range of *P. spumarius*.

Overall, compelling evidence showed that *P. spumarius* is responsible for European *X. fastidiosa* outbreaks, and that it is not only a menace for olive and other cultivated crops, but potentially for forestry as well (Cornara et al. 2018a; Markheiser et al. 2019; Desprez-Loustau et al. 2020).

3.4. *Philaenus spumarius* control strategies and challenges

Monitoring surveys are essential tools of integrated pest management, since they allow insect detection, population estimation, and distribution mapping. For this reason, research has been addressed to identify the best sampling method for *P. spumarius,* as previously mentioned (Cornara et al. 2018a). Quadrat sampling consists in randomly positioning a sampling unit on the ground cover. It seems to be the most reliable sampling method for spittlebug nymphs, which are scarcely mobile (Whittaker 1973; Morente et al. 2018b; Dongiovanni et al. 2019; Bodino et al. 2020). Given the exceptional jumping ability of adults, sweep netting is more convenient than other methods for the estimation of *P. spumarius* populations on both ground vegetation and tree canopies (Morente et al. 2018b).

Management of *X. fastidiosa*-caused diseases would be possible only by targeting several aspects of the interaction between host plant, vector, and plant pathogen. Integration of multiple strategies would allow disruption of interactions within the pathosystem, resulting in effective and long-term disease management (Almeida et al. 2005; Almeida 2016b). Control approaches could simultaneously focus on all characters in play by removing infected plants, excluding contaminated ones, monitoring propagative material, and controlling vector populations (Lopes et al. 2016; Cornara et al. 2018a). In this regard, techniques aimed at reducing *P. spumarius* populations consists in the removal of herbaceous cover by both weeding, tilling, and mowing and application of insecticides (Almeida 2016a; Cornara et al. 2018a; Saponari et al. 2019). Although removal of ground cover may reduce nymph survival and emergence of adults, this approach affects soil quality and reduces habitats that foster populations of beneficial arthropods (Kairis et al. 2013; Karamaouna et al. 2019; Mesmin et al. 2020; Molinatto et al. 2020; Ali et al. 2021). Insecticides targeting adults conceal important side effects, as discussed above (Brühl and Zaller 2019; Sánchez-Bayo and Wyckhuys 2019; Baker et al. 2020; Chávez-Dulanto et al. 2020). By reducing the biodiversity in olive orchards, these control methods would accordingly promote the spread of *X. fastidiosa*, since generalist insects such as *P. spumarius* tend to occupy emptied niches (Civitello et al. 2015; Sánchez-Bayo and Wyckhuys 2019). Moreover, although pesticides offer a short-term control strategy, their efficacy in reducing the disease spread is highly variable (Purcell 1980; Madden et al. 2000; Daugherty et al. 2015). For these reasons, we need to rethink our approach of *X. fastidiosa* and *P. spumarius* control to find sustainable and efficient methods.

Although predation does not seem to affect *P. spumarius* populations (Whittaker 1973), research is underway to characterise potential biocontrol agents. Egg parasitoids of the genus Ooctonus (Hymenoptera: Mymaridae) and Centrodora (Hymenoptera: Aphelinidae) attack *P. spumarius*, but their parasitisation rates are not sufficient to reduce spittlebug populations (Weaver and King 1954; Whittaker 1973; Mesmin et al. 2020; Molinatto et al. 2020). Even if the endoparasitoid *Verrallia aucta* (Diptera: Pipunculidae) could have a direct effect on population dynamics owing to host sterilisation, it cannot prevent *X. fastidiosa* transmission given that adults die after 10–11 weeks of parasitism (Whittaker 1969, 1973). The predatory bug *Zelus renardii* (Hemiptera: Reduviidae) has recently been introduced in Europe and proposed as control agent for *P. spumarius*, but cannot be released in the environment given that this generalist predator constitutes itself a threat for other local species (Ables 1978; Cohen and Tang 1997; Davranoglou 2011; Mesmin et al. 2020). Other potential control agents include entomopathogenic fungi and nematodes, but their application and efficiency need to be tested (Weaver and King 1954; Harper and Whittaker 1976; Ben-Ze'ev and Kenneth 1981).

As mentioned before, *P. spumarius'* feeding behaviour was recently characterised together with *X. fastidiosa's* transmission mechanism by using electro penetration graph technique (Cornara et al. 2018b). This information can support development of strategies aimed at disrupting bacterium–spittlebug interactions, as demonstrated for other *X. fastidiosa* vectors (i.e., sharpshooters) (Labroussaa et al. 2016). Indeed, future feeding interference techniques are worthy to be tested (Cornara et al. 2016, 2019b, 2020a).

Innovative pest management approaches imply the manipulation of pest behaviours and include attraction or repellence to/from a signal and mating disruption (Foster and Harris 1997; Baker et al. 2020). Inter- and intraspecific communication are susceptible to interference, but knowledge regarding the nature of signals is crucial (Foster and Harris 1997; Polajnar et al. 2015; Pertot et al. 2017). For instance, sexual pheromones are released in the field for the control of several crop pests, especially lepidopteran species (Welter et al. 2005). Nonetheless, the sole reported spittlebug emitting pheromones is the rice spittlebug *Callitettix versicolor* (Hemiptera: Cercopidae) (Chen and Liang 2015), while other species use different cues to communicate. Although *P. spumarius* antennal sensilla carry chemoreceptors possibly responsible to plant attractants, these receptors are not involved in mating communication given that they number of antennal sensory structures is relatively low and that, within Auchenorrhyncha, pheromonal communication is rare (Ranieri et al. 2016). Moreover, monitoring and control methods based on chemical signals are very unlikely to be developed against Auchenorrhyncha, since chemical communication is limited among this taxon (Saxena and Kumar 1984; Claridge 1985). In fact, communication mostly relies on the exchange of vibrational substrate-borne signals, which have been exploited to develop pest control strategies (Čokl and Virant-Doberlet 2003; Polajnar et al. 2015; Gordon and Krugner 2019; Hill et al. 2019; Mazzoni et al. 2019; Cividini and Montesanto 2020).

Evidence suggests that *P. spumarius* males produce vibrations as well, but no information is available regarding signal role, intraspecific communication, and mating behaviours (Tishechkin 2003). A deep signal characterisation and description of pair formation is mandatory for the development of vibrational control strategies against *P. spumarius,* and first insights in this regard are presented here. Our investigations regarding *P. spumarius* pair formation process and associated intraspecific signals are presented in chapter 2, while the potential relationship between female signalling and reproductive biology is illustrated in chapter 3. In chapter 5, a vibrational stimulus based on a *P. spumarius* signal was used to impair its feeding activity, prompting for further research. Overall, outcomes of our investigations would facilitate development of diverse behavioural manipulation control strategies and provide new ethological data about *P. spumarius.*

4. An alien vector side story: *Bactericera cockerelli* in New Zealand

The accelerating trend of globalisation confronted New Zealand ecosystems and economics with a new challenge: the establishment of invasive species. In turn, this establishment triggered a declining effectiveness and acceptability of control methods, while intensifying land use and climate change. Considering the unique environment of this country, it is crucial to understand the bionomics and impact of invasive pest species and use this information to develop new control methods. Limitations of current pest management strategies call for new technologies and refinement of existing methods (Goldson et al. 2015).

The introduction of the tomato potato psyllid *Bactericera cockerelli* (Hemiptera: Triozidae) in New Zealand demonstrated that pest management is a policy as well as a technical challenge. After establishment of the psyllid in tomato and potato crops, farmers were forced to systematically apply pesticides, including toxic organophosphate products. Besides risks of resistance emergence, these compounds threat human health and the delicate ecosystems of New Zealand. Even the introduction of biocontrol agents poses a risk to native species. Hence, new management methods should be devised not only to ensure control of *B. cockerelli*, but environmental sustainability as well (Goldson et al. 2015). This challenge could be tackled thanks to manipulation of psyllid behaviours, but a deeper understanding of the ethology of this pest is required. Outstanding questions are to understand how *B. cockerelli* communicates, and how its pair formation occur.

4.1. A quick guide to the tomato potato psyllid

Bactericera cockerelli is a hemipteran insect belonging to the Sternorrhyncha suborder, superfamily Psylloidea (Ouvrard 2017). This psyllid is considered as a “temperature zone” species, since its life history is governed by climate, especially by temperature (Knowlton 1933; List 1939). *Bactericera cockerelli* adults emerge after five nymphal stages and are reproductively active soon after the last nymphal molt (Figure 1.5). Females lay hundreds of eggs, which are individually placed into plant tissue via a basal pedicel (Butler and Trumble 2012). Host plant, geographic origin of populations, sex and laboratory conditions are main factors influencing the psyllid development and life history (Liu and Trumble 2007; Yang and Liu 2009; Yang et al. 2010). *Bactericera cockerelli* feeds on phloem of different host species, with a preference for plants in the Solanaceae family (Wallis 1995). Liquid excreted by nymphs can foster fungal infestations and large psyllid populations often result in plant damage (Liu and Trumble 2007; Yang and Liu 2009).

Figure 1.5. Life stages of the psyllid *Bactericera cockerelli.* A) Eggs; B) nymphs; C) red and D) brown colour morphs of an adult (Butler and Trumble 2012).

Bactericera cockerelli is native to North America and found in Central America, Mexico, southern United States and New Zealand (Liu and Trumble 2007; Teulon et al. 2009). Besides damages due to direct feeding, it became a major pest of tomato and potato because it transmits the bacterium *Candidatus* Liberibacter solanacearum (Munyaneza et al. 2007a, b; Secor et al. 2009; Munyaneza 2010). This plant pathogen causes a disease known as “zebra chip” in potato, which resulted in millions of dollars in losses for the potato industry (Munyaneza 2012). *Candidatus* Liberibacter solanacearum can infect crops other than potato, although severity and type of symptoms vary depending on the bacterium haplotype (Munyaneza 2015). In New Zealand, introduction of *B. cockerelli* in 2006 represented a major challenge to integrated pest management in solanaceous greenhouse and field crops, due to psyllid’s high infestation rates (Teulon et al. 2009; Butler and Trumble 2012; Vereijssen et al. 2018). Current control strategies are expensive and rely on intensive use of pesticides, while there is an urgent need of integrated pest management programs incorporating natural biocontrol agents, resistant plant varieties, cultural practices aimed at preventing high infestations, and behavioural manipulation strategies (Butler and Trumble 2012; Butler et al. 2012).

4.2. Is there room for vibrations against *B. cockerelli*?

Vibrational communication is considered the main intra-specific communication channel in psyllids, being widespread among the majority of Psylloidea (Percy et al. 2006; Tishechkin 2007; Wenninger et al. 2009; Eben et al. 2015; Liao and Yang 2015; Liao et al. 2019). A proposed mechanism for signal production is stridulation, which involves the movement of the wings in a vertical plane, along the insect's body (Heslop-Harrison 1960; Tishechkin 2006; Eben et al. 2015; Wood et al. 2016; Oppedisano et al. 2019). Vibrations are emitted when sclerotised areas of anal veins of the wings rub against the meso- and metathorax axillary cords, which bear scale-like denticles. The anal vein acts as a 'plectrum' scraping against the surface of the axillary cord, which represents the 'pars stridens' (Taylor 1985; Tishechkin 2006). Some psyllid species are supposed to use two stridulatory mechanisms that work together, whilst frictional mechanisms such as rubbing between wing-wing, leg-abdomen or leg-wing do not play any role in signalling (Liao et al. 2019). The Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae), on the other hand, seems to use wingbeat signals for vibrational communication and have not been reported to stridulate (Wenninger et al. 2009). Rapid movements of wing is hypothesized to be the source of the faint sound that was first noted in Psylloidea (Heslop-Harrison 1960). Interestingly, stridulatory organs have been reported for species that did not emit vibrations, while these structures were not found in some Australian species, which possibly do not communicate by means of vibrations or use alternative stridulatory organs (Percy et al. 2006; Tishechkin 2006, 2007). Although stridulation appears to be a simple frictional mechanism, psyllids can produce signals with distinct amplitude and temporal patterns, enabling discrimination among different species (Percy et al. 2006; Liao and Yang 2015).

Given that psyllids can be a pest of important crops, interest in characterising their vibrational communication for the development of vibrational control strategies is increasing (Eben et al. 2015; Mankin 2019; Oppedisano et al. 2019). First encouraging attempts on Psylloidea have been reported for *D. citri*, which causes the devastating Huanglongbing disease in citrus trees by transmitting *Ca.* L. asiaticus (Hall et al. 2013). Researchers developed an innovative device consisting of a micro-controller platform operating a piezoelectric buzzer that mimicked a *D. citri* female by simulating her response after detection of a male call. In this way, vibrational signals of *D. citri* were used to monitor and lure male psyllids (Mankin et al. 2015; Hartman et al. 2017; Mankin 2019).

Chemical communication seems to have a role in the mating behaviour of *B. cockerelli,* since semiochemicals produced by females and males can attract males, whilst females are repelled by male odours (Guédot et al. 2010, 2013). Prior to copulation, *B. cockerelli* individuals tap the antennae over the body of a potential partner, suggesting that cuticular hydrocarbons can underlie mate assessment (Knowlton and Janes 1931). Even so, studies of the psyllid olfactory system have revealed a relatively simplified system, in that psyllid antennae bear few antennal sensilla and olfactory glomeruli (Arras et al. 2012). Chemical compounds potentially ensure short-range communication by enabling mate identification when a psyllid is close to a conspecific, while other signal modalities such as vibrations could underlie communication at greater distances. Within the family Triozidae, vibrational communication is rather common (Percy et al. 2006; Tishechkin 2006, 2007; Lubanga et al. 2014; Liao et al. 2016), validating the interest in behavioural studies aimed at characterizing mating related vibrational signals. If vibrations have indeed a major role in the pair formation process, similar information could allow the development of vibrational control strategies against *B. cockerelli*, in complement of other environmentally-friendly pest management strategies.

Outcomes of our studies regarding the vibrational communication and mating behaviour of *B. cockerelli* are presented in chapter 5, whilst chapter 6 illustrates first attempts to interfere with psyllid pair formation process.

5. Thesis outline

This doctoral thesis aims to shed light on the sexual behaviour and associated vibrational substrate-borne signals of two important vectors of plant pathogens. Even so, the final goal is to provide new information for future development of behavioural manipulation control strategies. The spittlebug *P. spumarius* is the pivot around which this thesis revolves, due to its role in the current *X. fastidiosa* European outbreak. Chapter 2 illustrates the outcomes of bioassays aimed at unveiling the pair formation process and associated vibrational signals of the spittlebug. In chapter 2, some aspects of intrasexual communication are unveiled, and first insights are provided on the use of playbacks to study *P. spumarius* behaviour. Chapter 3 is addressed at evaluating the potential relationship between female signalling activity and ovarian development, which was investigated by means of playback trials and morphological inspections. The outcomes of these chapters are important insights that contribute to a fully understanding of *P. spumarius* behaviour and suggest that behavioural manipulation approaches by means of vibration should be tested. Chapter 4 introduces a new approach for field monitoring *P. spumarius* and other Auchenorrhyncha that encompasses occupancy models, which enable estimation of presence and detection probability of the target species. Even if this was a preliminary work, the models suggested that *P. spumarius* detection can be overlooked due to the effect of site covariates (i.e., forest coverage, sunlight hours and plant height), and that occupancy models are useful tools to estimate true presence of pest species. Indeed, these models could support future disease epidemiology studies. In chapter 4, association between *P. spumarius* and its host plants in Trentino (Northern Italy) is illustrated as well. As reported in other field studies, similar knowledge is fundamental because the ground cover of susceptible crops can be shaped to reduce spittlebug residency. Chapter 5 provides a breakthrough application of vibrations, which were used to impair the feeding behaviour of *P. spumarius.* Even if this approach represents only a first step towards a future management of the olive disease, it deserves in our opinion further studies and eventual implementation.

Chapter 6 comprises the description of the vibrational communication of the psyllid *B. cockerelli* and provides first insights regarding vibrational behavioural interference against this pest. Chapter 7 illustrates how vibrational stimuli can disrupt psyllid pair formation by attracting males towards the source of the signal. These outcomes imply that vibrational stimuli may be used to monitor and control *B. cockerelli,* but further research should be addressed at improving signal transmission to ensure efficiency at greater scale.

Chapter 8 represents a general conclusion enclosing the main findings and further research needs. By discussing outcomes and challenges, we suggest that future research may enable materialisation of tailored vibrational control strategies against vectors of bacterial plant pathogens such as *P. spumarius* and *B. cockerelli*.

References

Ables JR (1978) Feeding behavior of an assassin bug, *Zelus renardii*. Ann Entomol Soc Am 71:476–478

Ali BM, van der Werf W, Oude Lansink A (2021) Assessment of the environmental impacts of *Xylella fastidiosa* subsp. *pauca* in Puglia. Crop Prot 142:105519. https://doi.org/10.1016/j.cropro.2020.105519

Almeida RPP (2016a) Can Apulia's olive trees be saved? Science (80-) 353:346–348. https://doi.org/10.1126/science.aaf9710

Almeida RPP (2016b) Chapter 12: *Xylella fastidiosa* Vector Transmission Biology. Vector-Mediated Transm Plant Pathog 165–173. https://doi.org/10.1094/9780890545355.012

Almeida RPP, Blua MJ, Lopes JRS, Purcell AH (2005) Vector transmission of *Xylella fastidiosa*: Applying fundamental knowledge to generate disease management strategies. Ann Entomol Soc Am 98:775–786. https://doi.org/10.1603/0013-8746(2005)098[0775:VTOXFA]2.0.CO;2

Andersen PC, Brodbeck B V, Mizell III RF (1992) Feeding by the leafhopper, *Homalodisca coagulata*, in relation to xylem fluid chemistry and tension. J Insect Physiol 38:611–622

Arras J, Hunter W, Bextine B (2012) Comparative analysis of antennae sensory arrays in asian citrus psyllid, *Diaphorina citri*, and potato psyllid, *Bactericera cockerelli* (Hemiptera). Southwest Entomol 37:1–12. https://doi.org/10.3958/059.037.0101

Backus EA, Morgan DJW (2011) Spatiotemporal colonization of *Xylella fastidiosa* in its vector supports the role of egestion in the inoculation mechanism of foregut-borne plant pathogens. Phytopathology 101:912–922. https://doi.org/10.1094/PHYTO-09-10-0231

Bae C, Han SW, Song Y-R, et al (2015) Infection processes of xylem-colonizing pathogenic bacteria: possible explanations for the scarcity of qualitative disease resistance genes against them in crops. Theor Appl Genet 128:1219–1229

Baker BP, Green TA, Loker AJ (2020) Biological control and integrated pest management in organic and conventional systems. Biol Control 140:104095. https://doi.org/10.1016/j.biocontrol.2019.104095

Barber G, Ellis WO (1922) Eggs of three Cercopidae. Psyche (Stuttg) 29:1–3

Ben-Ze'ev I, Kenneth RG (1981) *Zoophthora radicans and Zoophthora petchi* sp. nov. [Zygomycetes: Entomophthorales], two species of the "Sphaerosperma group" attacking leaf-hoppers and frog-hoppers [Hom.]. Entomophaga 26:131–142

Birch AN, Begg GS, Squire GR (2011) How agro-ecological research helps to address food security issues under new IPM and pesticide reduction policies for global crop production systems. J Exp Bot 62:3251–3261. https://doi.org/10.1093/jxb/err064

Bodino N, Cavalieri V, Dongiovanni C, et al (2020) Spittlebugs of mediterranean olive groves: Host-plant exploitation throughout the year. Insects 11:. https://doi.org/10.3390/insects11020130

Bodino N, Cavalieri V, Dongiovanni C, et al (2019) Phenology, seasonal abundance and stage-structure of spittlebug (Hemiptera: Aphrophoridae) populations in olive groves in Italy. Sci Rep 9:1–17. https://doi.org/10.1038/s41598-019-54279-8

Bové JM, Garnier M (2002) Phloem-and xylem-restricted plant pathogenic bacteria. Plant Sci 163:1083–1098

Bragard C, Dehnen-Schmutz K, Di Serio F, et al (2019) Update of the Scientific Opinion on the risks to plant health posed by *Xylella fastidiosa* in the EU territory. Efsa J 17:

Brühl CA, Zaller JG (2019) Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Front Environ Sci 7:2013–2016. https://doi.org/10.3389/fenvs.2019.00177

Burrows M (2003) Froghopper insects leap to new heights. Nature 424:509

Butler CD, Trumble JT (2012) The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. Terr Arthropod Rev 5:87–111. https://doi.org/10.1163/187498312x634266

Butler CD, Walker GP, Trumble JT (2012) Feeding disruption of potato psyllid, *Bactericera cockerelli*, By imidacloprid as measured by electrical penetration graphs. Entomol Exp Appl 142:247–257. https://doi.org/10.1111/j.1570-7458.2012.01219.x

Candolin U (2003) The use of multiple cues in mate choice. Biol Rev Camb Philos Soc 78:575–595. https://doi.org/10.1017/S1464793103006158

Cavalieri V, Altamura G, Fumarola G, et al (2019) Transmission of *Xylella fastidiosa* subspecies *pauca* sequence type 53 by different insect species. Insects 10:. https://doi.org/10.3390/insects10100324

Chávez-Dulanto PN, Thiry AAA, Glorio-Paulet P, et al (2020) Increasing the impact of science and technology to provide more people with healthier and safer food. Food Energy Secur 1–31. https://doi.org/10.1002/fes3.259

Chen X, Liang A-P (2015) Identification of a self-regulatory pheromone system that controls nymph aggregation behavior of rice spittlebug *Callitettix versicolor*. Front Zool 12:1–12

Chmiel SM, Wilson MC (1979) Estimation of the lower and upper developmental threshold temperatures and duration of the nymphal stages of the meadow spittlebug, *Philaenus spumarius*. Environ Entomol 8:682–685. https://doi.org/10.1093/ee/8.4.682

Chuche J, Thiéry D (2014) Biology and ecology of the Flavescence dorée vector *Scaphoideus titanus*: A review. Agron Sustain Dev 34:381–403. https://doi.org/10.1007/s13593-014-0208-7

Cividini S, Montesanto G (2020) Biotremology in arthropods. Learn Behav 48:281–300. https://doi.org/10.3758/s13420-020-00428-3

Civitello DJ, Cohen J, Fatima H, et al (2015) Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proc Natl Acad Sci U S A 112:8667–8671. https://doi.org/10.1073/pnas.1506279112

Claridge M (1985) Acoustic Signals in the Homoptera: Behavior, Taxonomy, and Evolution. Annu Rev Entomol 30:297–317. https://doi.org/10.1146/annurev.ento.30.1.297

Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323–334. https://doi.org/10.1641/0006-3568(2005)055[0323:TBEOIV]2.0.CO;2

Cohen AC, Tang R (1997) Relative prey weight influences handling time and biomass extraction in *Sinea confusa* and *Zelus renardii* (Heteroptera: Reduviidae). Environ Entomol 26:559–565

Čokl A, Virant-Doberlet M (2003) Communication with substrate-borne signals in small plant-dwelling insects. Annu Rev Entomol 48:29–50. https://doi.org/10.1146/annurev.ento.48.091801.112605

Čokl A, Zorović M, Kosi AŽ, et al (2014) Communication through plants in a narrow frequency window. In: Studying vibrational communication. Springer, pp 171–195

Cornara D, Bosco D, Fereres A (2018a) *Philaenus spumarius*: when an old acquaintance becomes a new threat to European agriculture. J Pest Sci (2004) 91:957–972. https://doi.org/10.1007/s10340-018-0966-0

Cornara D, Cavalieri V, Dongiovanni C, et al (2017a) Transmission of *Xylella fastidiosa* by naturally infected *Philaenus spumarius* (Hemiptera, Aphrophoridae) to different host plants. J Appl Entomol 141:80–87. https://doi.org/10.1111/jen.12365

Cornara D, Garzo E, Morente M, et al (2018b) EPG combined with micro-CT and video recording reveals new insights on the feeding behavior of *Philaenus spumarius*. PLoS One 13:1–20. https://doi.org/10.1371/journal.pone.0199154

Cornara D, Marra M, Morente M, et al (2020a) Feeding behavior in relation to spittlebug transmission of *Xylella fastidiosa*. J Pest Sci (2004) 93:1197–1213. https://doi.org/10.1007/s10340-020-01236-4

Cornara D, Marra M, Tedone B, et al (2020b) No evidence for cicadas' implication in *Xylella fastidiosa* epidemiology. Entomol Gen 40:125–132. https://doi.org/10.1127/entomologia/2020/0912

Cornara D, Morente M, Markheiser A, et al (2019a) An overview on the worldwide vectors of *Xylella fastidiosa*. Entomol Gen 39:157–181. https://doi.org/10.1127/entomologia/2019/0811

Cornara D, Ripamonti M, Morente M, et al (2019b) Artificial diet delivery system for *Philaenus spumarius*, the European vector of *Xylella fastidiosa*. J Appl Entomol 143:882–892. https://doi.org/10.1111/jen.12655

Cornara D, Saponari M, Zeilinger AR, et al (2017b) Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. J Pest Sci (2004) 90:521–530. https://doi.org/10.1007/s10340-016-0793-0

Cornara D, Sicard A, Zeilinger AR, et al (2016) Transmission of *Xylella fastidiosa* to grapevine by the meadow spittlebug. Phytopathology 106:1285–1290. https://doi.org/10.1094/PHYTO-05-16-0202-R

Crosslin JM, Munyaneza JE, Brown JK, Liefting LW (2010) Potato zebra chip disease: a phytopathological tale. Plant Heal Prog 11:33. https://doi.org/10.1094/PHP-2010-0317-01-RV

Daugherty MP, Almeida RPP (2009) Estimating *Xylella fastidiosa* transmission parameters: Decoupling sharpshooter number and feeding period. Entomol Exp Appl 132:84–92. https://doi.org/10.1111/j.1570-7458.2009.00868.x

Daugherty MP, O'Neill S, Byrne F, Zeilinger A (2015) Is vector control sufficient to limit pathogen spread in vineyards? Environ Entomol 44:789–797. https://doi.org/10.1093/ee/nvv046

Davranoglou L (2011) Zelus renardii (Kolenati, 1856) A New World reduviid discovered in Europe (Hemiptera: Reduviidae: Harpactorinae). Entomol Mon Mag Zelus 147:157–162

Denancé N, Legendre B, Briand M, et al (2017) Several subspecies and sequence types are associated with the emergence of *Xylella fastidiosa* in natural settings in France. Plant Pathol 66:1054–1064. https://doi.org/10.1111/ppa.12695

Derlink M, Pavlovčič P, Stewart AJA, Virant-Doberlet M (2014) Mate recognition in duetting species: The role of male and female vibrational signals. Anim Behav 90:181–193. https://doi.org/10.1016/j.anbehav.2014.01.023

Desprez-Loustau M-L, Balci Y, Cornara D, et al (2020) Is *Xylella fastidiosa* a serious threat to European forests? For An Int J For Res. https://doi.org/10.1093/forestry/cpaa029

Diamond JM (1989) The present, past and future of human-caused extinctions. Philos Trans - R Soc London, B 325:469–477. https://doi.org/10.1098/rstb.1989.0100

Dongiovanni C, Cavalieri V, Bodino N, et al (2019) Plant selection and population trend of spittlebug immatures (Hemiptera: Aphrophoridae) in olive groves of the Apulia region of Italy. J Econ

Entomol 112:67–74. https://doi.org/10.1093/jee/toy289

Drosopoulos S, Asche M (1991) Biosystematic studies on the spittlebug genus Philaenus with the description of a new species. Zool J Linn Soc 101:169–177. https://doi.org/10.1111/j.1096-3642.1991.tb00891.x

Eben A, Mühlethaler R, Gross J, Hoch H (2015) First evidence of acoustic communication in the pear psyllid *Cacopsylla pyri* L. (Hemiptera: Psyllidae). J Pest Sci (2004) 88:87–95. https://doi.org/10.1007/s10340-014-0588-0

Edwards WD (1935) Strawberry pests including the spittlebug. Ann Rep Oregon State Hortic Soc 27:58–65

EPPO GD (2018) EPPO global database. Eur Mediterr Plant Prot Organ

Fierro A, Liccardo A, Porcelli F (2019) A lattice model to manage the vector and the infection of the *Xylella fastidiosa* on olive trees. Sci Rep 9:1–14. https://doi.org/10.1038/s41598-019-44997-4

Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annu Rev Entomol Vol 42 42:123–146. https://doi.org/10.1146/annurev.ento.42.1.123

Frem M, Chapman D, Fucilli V, et al (2020) *Xylella fastidiosa* invasion of new countries in Europe, the Middle East and North Africa: Ranking the potential exposure scenarios. NeoBiota 59:77–97. https://doi.org/10.3897/neobiota.59.53208

Giampetruzzi A, Saponari M, Loconsole G, et al (2017) Genome-wide analysis provides evidence on the genetic relatedness of the emergent *Xylella fastidiosa* genotype in Italy to isolates from Central America. Phytopathology 107:816–827. https://doi.org/10.1094/PHYTO-12-16-0420-R

Godefroid M, Cruaud A, Streito JC, et al (2019) *Xylella fastidiosa*: climate suitability of European continent. Sci Rep 9:1–10. https://doi.org/10.1038/s41598-019-45365-y

Goldson SL, Bourdôt GW, Brockerhoff EG, et al (2015) New Zealand pest management: Current and future challenges. J R Soc New Zeal 45:31–58. https://doi.org/10.1080/03036758.2014.1000343

Gordon SD, Krugner R (2019) Mating Disruption by Vibrational Signals: Applications for Management of the Glassy-Winged Sharpshooter. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 355–373

Gottwald TR (2010) Current epidemiological understanding of citrus huanglongbing. Annu Rev Phytopathol 48:119–139. https://doi.org/10.1146/annurev-phyto-073009-114418

Grant JF, Lambdin PL, Folium RA (1998) Infestation levels and seasonal incidence of the meadow spittlebug (Homoptera: Cercopidae) on musk thistle in Tennessee. J Agric Urban Entomol 15:83–91

Guédot C, Horton DR, Landolt PJ (2010) Sex attraction in *Bactericera cockerelli* (Hemiptera: Triozidae). Environ Entomol 39:1302–1308. https://doi.org/10.1603/en10048

Guédot C, Horton DR, Landolt PJ, Munyaneza JE (2013) Effect of mating on sex attraction in *Bactericera cockerelli* with evidence of refractoriness. Entomol Exp Appl 149:27–35. https://doi.org/10.1111/eea.12104

Gwynne DT (1991) Sexual competition among females: What causes courtship-role reversal? Trends Ecol Evol 6:118–121. https://doi.org/10.1016/0169-5347(91)90089-G

Hager FA, Kirchner WH (2013) Vibrational long-distance communication in the termites *Macrotermes natalensis* and *Odontotermes* sp. J Exp Biol 216:3249–3256. https://doi.org/10.1242/jeb.086991

Halkka O (1964) Geographical, spatial and temporal variability in the balanced polymorphism of *Philaenus spumarius*. Heredity (Edinb) 19:383–401

Halkka O (1962) Equilibrium populations of *Philaenus spumarius* L. Nature 193:93–94. https://doi.org/10.1038/193093a0

Halkka O, Raatikainen M, Halkka L (1974) The founder principle, founder selection, and evolutionary divergence and convergence in natural populations of Philaenus. Hereditas 78:73–84. https://doi.org/10.1111/j.1601-5223.1974.tb01430.x

Halkka O, Raatikainen M, Halkka L (1990) Conditions requisite for stability of polymorphic balance in *Philaenus spumarius* (L.) (Homoptera). Genetica 46:67–76. https://doi.org/10.1007/BF00122518

Halkka O, Raatikainen M, Halkka L, Hovinen R (1975) The genetic composition of *Philaenus spumarius* populations in island habitats variably affected by voles. Evolution (N Y) 29:700. https://doi.org/10.2307/2407078

Halkka O, Raatikainen M, Halkka L, Raatikainen T (1977) Coexistence of four species of spittle-producing Homoptera. In: Annales Zoologici Fennici. JSTOR, pp 228–231

Halkka O, Raatikainen M, Vasarainen A, Heinonen L (1967) Ecology and ecological genetics of *Philaenus spumarius* (L.) (Homoptera). In: Ann. Zool. Fenn. Helsinki, pp 1–18

Hall DG, Richardson ML, Ammar ED, Halbert SE (2013) Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. Entomol Exp Appl 146:207–223. https://doi.org/10.1111/eea.12025

Harper G, Whittaker JB (1976) The role of natural enemies in the colour polymorphism of *Philaenus spumarius* (L.). J Anim Ecol 45:91. https://doi.org/10.2307/3769

Hartman E, Rohde B, Lujo S, et al (2017) Behavioral responses of male *Diaphorina citri* (Hemiptera: Liviidae) to mating communication signals from vibration traps in Citrus (Sapindales: Rutaceae) Trees. Florida Entomol 100:767–771. https://doi.org/10.1653/024.100.0425

Henry C (1980) Acoustical communication in *Chrysopa rufilabris* (Neuroptera: Chrysopidae), a green lacewing with two distinct calls. Proc Entomol Soc … 82:1–8

Henry CS (1979) Acoustical communication during courtship and mating in the green lacewing *Chrysopa carnea* (Neuroptera: Chrysopidae). Ann Entomol Soc Am 72:68–79. https://doi.org/10.1093/aesa/72.1.68

Heslop-Harrison G (1960) Sound production in the Homoptera with special reference to sound producing mechanisms in the Psyllidae. Ann Mag Nat Hist 3:633–640. https://doi.org/10.1080/00222936008651067

Hill MP, Clusella-Trullas S, Terblanche JS, Richardson DM (2016) Drivers, impacts, mechanisms and adaptation in insect invasions. Biol Invasions 18:883–891. https://doi.org/10.1007/s10530-016-1088-3

Hill PSM (2014) Stretching the paradigm or building a new? Development of a cohesive language for vibrational communication. In: Studying vibrational communication. Springer, pp 13–30

Hill PSM (2009) How do animals use substrate-borne vibrations as an information source? Naturwissenschaften 96:1355–1371. https://doi.org/10.1007/s00114-009-0588-8

Hill PSM, Mazzoni V, Narins P, et al (2019) Quo Vadis, Biotremology? In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International

Publishing, Cham, pp 3–14

Hill PSM, Wessel A (2016) Biotremology. Curr Biol 26:R187–R191. https://doi.org/10.1016/j.cub.2016.01.054

Horsfield D (1977) Relationships between feeding of *Philaenus spumarius* (L.) and the amino acid concentration in the xylem sap. Ecol Entomol 2:259–266. https://doi.org/10.1111/j.1365-2311.1977.tb00889.x

Hulme PE (2007) Biological invasions in Europe: Drivers, pressures, states, impacts and responses. Issues Environ Sci Technol 25:56–80. https://doi.org/10.1039/9781847557650-00056

Kairis O, Karavitis C, Kounalaki A, et al (2013) The effect of land management practices on soil erosion and land desertification in an olive grove. Soil Use Manag 29:597–606. https://doi.org/10.1111/sum.12074

Karamaouna F, Kati V, Volakakis N, et al (2019) Ground cover management with mixtures of flowering plants to enhance insect pollinators and natural enemies of pests in olive groves. Agric Ecosyst Environ 274:76–89. https://doi.org/10.1016/j.agee.2019.01.004

Kasper J, Hirschberger P (2005) Stridulation in Aphodius dung beetles: Songs and morphology of stridulatory organs in North American Aphodius species (Scarabaeidae). J Nat Hist 39:91–99. https://doi.org/10.1080/00222930310001018877

Keskinen E, Meyer-Rochow VB (2004) Post-embryonic photoreceptor development and dark/light adaptation in the spittle bug *Philaenus spumarius* (L.) (Homoptera, Cercopidae). Arthropod Struct Dev 33:405–417. https://doi.org/10.1016/j.asd.2004.05.010

Knowlton GF (1933) Length of adult life of *Paratrioza cockerelli* (Sulc). J Econ Entomol 26:730

Knowlton GF, Janes MJ (1931) Studies on the biology of *Paratrioza cockerelli* (Sulc). Ann Entomol Soc Am 24:283–291. https://doi.org/10.1093/aesa/24.2.283

Krugner R, Sisterson MS, Backus EA, et al (2019) Sharpshooters: a review of what moves *Xylella fastidiosa*. Austral Entomol 58:248–267. https://doi.org/10.1111/aen.12397

Labroussaa F, Zeilinger AR, Almeida RPP (2016) Blocking the transmission of a noncirculative vector-borne plant pathogenic bacterium. Mol Plant-Microbe Interact 29:535–544. https://doi.org/10.1094/MPMI-02-16-0032-R

Lakes-Harlan R, Strauß J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Studying vibrational communication. Springer, pp 277–302

Lavigne R (1959) Biology of *Philaenus leucophthalmus* (L.), in Massachusetts. J Econ Entomol 52:904–907. https://doi.org/10.1093/jee/52.5.904

Lee Y, Kim H, Kang TJ, Jang Y (2012) Stress response to acoustic stimuli in an aphid: A behavioral bioassay model. Entomol Res 42:320–329. https://doi.org/10.1111/1748-5967.12000

Liao Y-C, Wu Z-Z, Yang M-M (2019) Vibrational behavior of psyllids (Hemiptera: Psylloidea): Functional morphology and mechanisms. PLoS One 14:e0215196. https://doi.org/10.1371/journal.pone.0215196

Liao YC, Huang SS, Yang MM (2016) Substrate-borne signals, specific recognition, and plant effects on the acoustics of two allied species of Trioza, with the description of a new species (Psylloidea: Triozidae). Ann Entomol Soc Am 109:906–917. https://doi.org/10.1093/aesa/saw060

Liao YC, Yang MM (2015) Acoustic communication of three closely related psyllid species: A case study in clarifying allied species using substrate-borne signals (Hemiptera: Psyllidae:

Cacopsylla). Ann Entomol Soc Am 108:902–911. https://doi.org/10.1093/aesa/sav071

List GM (1939) The effect of temperature upon egg deposition, egg hatch and nymphal development of *Paratrioza cockerelli* (Sulc). J Econ Entomol 32:30–36. https://doi.org/10.1093/jee/32.1.30

Liu D, Trumble JT (2007) Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerelli*). Entomol Exp Appl 123:35–42. https://doi.org/10.1111/j.1570-7458.2007.00521.x

Lopes JRS, Krugner R, Brown J (2016) Chapter 14: Transmission Ecology and Epidemiology of the Citrus Variegated Chlorosis Strain of *Xylella fastidiosa*. Vector-Mediated Transm Plant Pathog 195–208. https://doi.org/10.1094/9780890545355.014

Lopes JRS, Landa BB, Fereres A (2014) A survey of potential insect vectors of the plant pathogenic bacterium *Xylella fastidiosa* in three regions of Spain. Spanish J Agric Res 12:795–800. https://doi.org/10.5424/sjar/2014123-5613

Lubanga UK, Guédot C, Percy DM, Steinbauer MJ (2014) Semiochemical and vibrational cues and signals mediating mate finding and courtship in psylloidea (Hemiptera): A synthesis. Insects 5:577–595. https://doi.org/10.3390/insects5030577

Lujo S, Hartman E, Norton K, et al (2016) Disrupting mating behavior of *Diaphorina citri* (Liviidae). J Econ Entomol 109:2373–2379. https://doi.org/10.1093/jee/tow202

Madden L V., Jeger MJ, Van Den Bosch F (2000) A theoretical assessment of the effects of vector-virus transmission mechanism on plant virus disease epidemics. Phytopathology 90:576–594. https://doi.org/10.1094/PHYTO.2000.90.6.576

Malone M, Watson R, Pritchard J (1999) The spittlebug *Philaenus spumarius* feeds from mature xylem at the full hydraulic tension of the transpiration stream. New Phytol 143:261–271. https://doi.org/10.1046/j.1469-8137.1999.00448.x

Mankin RW (2019) Vibrational Trapping and Interference with Mating of *Diaphorina citri*. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 399–413

Mankin RW, Rohde B, McNeill S (2015) Vibrational duetting mimics to trap and disrupt mating of the devastating Asian citrus psyllid insect pest. Proc Meet Acoust 25:. https://doi.org/10.1121/2.0000185

Markheiser A, Cornara D, Fereres A, Maixner M (2019) Analysis of vector behavior as a tool to predict *Xylella fastidiosa* patterns of spread. Entomol Gen XX: https://doi.org/10.1127/entomologia/2019/0841

Martelli GP, Boscia D, Porcelli F, Saponari M (2016) The olive quick decline syndrome in south-east Italy: a threatening phytosanitary emergency. Eur J Plant Pathol 144:235–243. https://doi.org/10.1007/s10658-015-0784-7

Masters GJ, Brown VK, Clarke IP, et al (1998) Direct and indirect effects of climate change on insect herbivores: Auchenorrhyncha (Homoptera). Ecol Entomol 23:45–52. https://doi.org/10.1046/j.1365-2311.1998.00109.x

Mazzoni V, Anfora G, Virant-Doberlet M (2013) Substrate vibrations during courtship in three Drosophila species. PLoS One 8:1–8. https://doi.org/10.1371/journal.pone.0080708

Mazzoni V, Eriksson A, Anfora G, et al (2014) Active space and the role of amplitude in plant-borne vibrational communication. In: Studying vibrational communication. Springer, pp 125–145

Mazzoni V, Lucchi A, Čokl A, et al (2009a) Disruption of the reproductive behaviour of *Scaphoideus titanus* by playback of vibrational signals. Entomol Exp Appl 133:174–185. https://doi.org/10.1111/j.1570-7458.2009.00911.x

Mazzoni V, Lucchi A, Ioriatti C, et al (2010) Mating behavior of *Hyalesthes obsoletus* (Hemiptera: Cixiidae). Ann Entomol Soc Am 103:813–822. https://doi.org/10.1603/an09145

Mazzoni V, Nieri R, Eriksson A, et al (2019) Mating Disruption by Vibrational Signals: State of the Field and Perspectives. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 331–354

Mazzoni V, Polajnar J, Baldini M, et al (2017) Use of substrate-borne vibrational signals to attract the Brown Marmorated Stink Bug, *Halyomorpha halys*. J Pest Sci (2004) 90:1219–1229. https://doi.org/10.1007/s10340-017-0862-z

Mazzoni V, Polajnar J, Virant-Doberlet M (2015) Secondary spectral components of substrate-borne vibrational signals affect male preference. Behav Processes 115:53–60. https://doi.org/10.1016/j.beproc.2015.02.019

Mazzoni V, Prěern J, Lucchi A, Virant-Doberlet M (2009b) Reproductive strategy of the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Bull Entomol Res 99:401–413. https://doi.org/10.1017/S0007485308006408

Medler JT (1955) Method of Predicting the Hatching Date of the Meadow Spittlebug1. J Econ Entomol 48:204–205. https://doi.org/10.1093/jee/48.2.204

Mesmin X, Chartois M, Genson G, et al (2020) *Ooctonus vulgatus* (Hymenoptera, Mymaridae), a potential biocontrol agent to reduce populations of *Philaenus spumarius* (Hemiptera, Aphrophoridae) the main vector of *Xylella fastidiosa* in Europe. PeerJ 2020:e8591. https://doi.org/10.7717/peerj.8591

Meyerson LA, Mooney HA (2007) Invasive alien species in an era of globalization. Front. Ecol. Environ. 5:199–208

Michelsen A, Kirchner WH, Lindauer M (1986) Sound and vibrational signals in the dance language of the honeybee, *Apis mellifera*. Behav Ecol Sociobiol 18:207–212. https://doi.org/10.1007/BF00290824

Molinatto G, Demichelis S, Bodino N, et al (2020) Biology and prevalence in northern Italy of *Verrallia aucta* (Diptera, Pipunculidae), a parasitoid of *Philaenus spumarius* (Hemiptera, Aphrophoridae), the main vector of *Xylella fastidiosa* in Europe. Insects 11:1–16. https://doi.org/10.3390/insects11090607

Morente M, Cornara D, Moreno A, Fereres A (2018a) Continuous indoor rearing of *Philaenus spumarius*, the main European vector of Xylella fastidiosa. J Appl Entomol 142:901–904. https://doi.org/10.1111/jen.12553

Morente M, Cornara D, Plaza M, et al (2018b) Distribution and relative abundance of insect vectors of *Xylella fastidiosa* in olive groves of the Iberian Peninsula. Insects 9:. https://doi.org/10.3390/insects9040175

Mundinger FG (1946) The control of spittle insects in strawberry plantings. J Econ Entomol 39:299–305. https://doi.org/10.1093/jee/39.3.299

Munyaneza JE (2012) Zebra chip disease of potato: Biology, epidemiology, and management. Am J Potato Res 89:329–350. https://doi.org/10.1007/s12230-012-9262-3

Munyaneza JE (2010) Psyllids as vectors of emerging bacterial diseases of annual crops. Southwest

Entomol 35:471–477. https://doi.org/10.3958/059.035.0335

Munyaneza JE (2015) Zebra chip disease, *Candidatus* Liberibacter, and potato psyllid: A global threat to the potato industry. Am J Potato Res 92:230–235. https://doi.org/10.1007/s12230-015-9448-6

Munyaneza JE, Crosslin JM, Upton JE (2007a) Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with "Zebra Chip," a new potato disease in southwestern United States and Mexico. J Econ Entomol 100:656–663. https://doi.org/10.1603/0022-0493(2007)100[656:aobchp]2.0.co;2

Munyaneza JE, Goolsby JM a., Crosslin JM, Upton JE (2007b) Further evidence that zebra chip potato disease in the lower Rio Grande Valley of Texas is associated with *Bactericera cockerelli*. Subtrop Plant Sci 59:30–37

Nast J (1972) Palaearctic Auchenorrhyncha (Homoptera). An annotated check list. Palaearct Auchen an Annot check List 550

Network RP (2016) Trends and challenges in pesticide resistance detection. Trends Plant Sci 21:834–853. https://doi.org/10.1016/j.tplants.2016.06.006

Nieri R, Mazzoni V (2019) Vibrational mating disruption of *Empoasca vitis* by natural or artificial disturbance noises. Pest Manag Sci 75:1065–1073. https://doi.org/10.1002/ps.5216

Nieri R, Mazzoni V, Gordon SD, Krugner R (2017) Mating behavior and vibrational mimicry in the glassy-winged sharpshooter, *Homalodisca vitripennis*. J Pest Sci (2004) 90:887–899. https://doi.org/10.1007/s10340-017-0840-5

O'Connell-Rodwell CE, Hart LA, Arnason BT (2001) Exploring the potential use of seismic waves as a communication channel by elephants and other large mammals. Am Zool 41:1157–1170. https://doi.org/10.1093/icb/41.5.1157

Oberst S, Lai JCS, Evans TA (2019) Physical Basis of Vibrational Behaviour: Channel Properties, Noise and Excitation Signal Extraction. In: Biotremology: Studying Vibrational Behavior. Springer, pp 53–78

Olmo D, Nieto A, Adrover F, et al (2017) First detection of *Xylella fastidiosa* infecting cherry (Prunus avium) and *Polygala myrtifolia* plants, in Mallorca Island, Spain. Plant Dis 101:1820

Oppedisano T, Polajnar J, Kostanjšek R, et al (2019) Substrate-borne vibrational communication in the vector of apple proliferation disease *Cacopsylla picta* (Hemiptera: Psyllidae). J Econ Entomol 1–8. https://doi.org/10.1093/jee/toz328

Orci KM, Murányi D (2021) Female answer specificity to male drumming calls in three closely related species of the stonefly genus Zwicknia (Plecoptera: Capniidae). Insect Sci 28:215–223. https://doi.org/10.1111/1744-7917.12759

Orlovskis Z, Canale MC, Thole V, et al (2015) Insect-borne plant pathogenic bacteria: Getting a ride goes beyond physical contact. Curr Opin Insect Sci 9:16–23. https://doi.org/10.1016/j.cois.2015.04.007

Ossiannilsson F (1981) The Auchenorrhyncha (Homoptera) of Fennoscandia and Denmark. Part 2: The families Cicadidae, Cercopidae, Membracidae, and Cicadellidae (excl. Deltocephalinae). Auchen Fennoscandia Denmark Part 2 Fam Cicadidae, Cercopidae, Membracidae, Cicadellidae (excl Deltocephalinae) 7:223–593

Ouvrard D (2017) Psyl'list–the world Psylloidea database. Nat Hist Museum https//www hemiptera-databases org/psyllist

Percy DM, Taylor GS, Kennedy M (2006) Psyllid communication: Acoustic diversity, mate recognition and phylogenetic signal. Invertebr Syst 20:431–445. https://doi.org/10.1071/IS05057

Perilla-Henao LM, Casteel CL (2016) Vector-borne bacterial plant pathogens: Interactions with hemipteran insects and plants. Front Plant Sci 7:1–15. https://doi.org/10.3389/fpls.2016.01163

Perring TM, Gruenhagen NM, Farrar CA (1999) Management of plant viral diseases through chemical control of insect vectors. Annu Rev Entomol 44:457–481. https://doi.org/10.1146/annurev.ento.44.1.457

Perrings C, Williamson M, Barbier EB, et al (2002) Biological invasion risks and the public good: An economic perspective. Ecol Soc 6:. https://doi.org/10.5751/es-00396-060101

Pertot I, Caffi T, Rossi V, et al (2017) A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Prot 97:70–84. https://doi.org/10.1016/j.cropro.2016.11.025

Pielström S, Roces F (2012) Vibrational communication in the spatial organization of collective digging in the leaf-cutting ant *Atta vollenweideri*. Anim Behav 84:743–752. https://doi.org/10.1016/j.anbehav.2012.07.008

Polajnar J, Eriksson A, Lucchi A, et al (2015) Manipulating behaviour with substrate-borne vibrations - Potential for insect pest control. Pest Manag Sci 71:15–23. https://doi.org/10.1002/ps.3848

Polajnar J, Eriksson A, Rossi Stacconi MV, et al (2014) The process of pair formation mediated by substrate-borne vibrations in a small insect. Behav Processes 107:68–78. https://doi.org/10.1016/j.beproc.2014.07.013

Polajnar J, Eriksson A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. J Pest Sci (2004) 89:909–921. https://doi.org/10.1007/s10340-015-0726-3

Polajnar J, Maistrello L, Ibrahim A, Mazzoni V (2019) Can Vibrational Playback Improve Control of an Invasive Stink Bug? In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 375–398

Purcell AH (1997) *Xylella fastidiosa*, a regional problem or global threat? J Plant Pathol 79:99–105

Purcell AH (1982) Insect Vector Relationships with Procaryotic Plant Pathogens. Annu Rev Phytopathol 20:397–417. https://doi.org/10.1146/annurev.py.20.090182.002145

Purcell AH (1980) Environmental therapy for Pierce’s disease of grapevines. Plant Dis 64:388–390. https://doi.org/10.1094/pd-64-388

Quartau JA, Borges PAV (1997) On the colour polymorphism of *Philaenus spumarius* (L.) (Homoptera, Cercopidae) in Portugal. Miscel.lania Zool 20:19–30

Rakitov RA (2002) Structure and function of the Malpighain tubules, and related behaviors in juvenile Cicadas: Evidence of homology with spittlebugs (Hemiptera: Cicadoidea and Cercopoidea). Zool Anz 241:117–130. https://doi.org/10.1078/0044-5231-00025

Ranieri E, Ruschioni S, Riolo P, et al (2016) Fine structure of antennal sensilla of the spittlebug *Philaenus spumarius* L. (Insecta: Hemiptera: Aphrophoridae). I. Chemoreceptors and thermo-/hygroreceptors. Arthropod Struct Dev 45:432–439. https://doi.org/10.1016/j.asd.2016.09.005

Redak RA, Purcell AH, Lopes JRS, et al (2004) The biology of xylem fluid-feeding insect vectors of *Xylella fastidiosa* and their relation to disease epidemiology. Annu Rev Entomol 49:243–270. https://doi.org/10.1146/annurev.ento.49.061802.123403

Ringo J (1996) Sexual receptivity in insects. Annu Rev Entomol 41:473–494. https://doi.org/10.1146/annurev.en.41.010196.002353

Robertson A, Gibbs AJ (1937) Spermatogenesis and fertilization in *Philaenus spumarius* Fallen. J Trop Med Hyg 40:257–262

Rodrigues ASB, Silva SE, Marabuto E, et al (2014) New mitochondrial and nuclear evidences support recent demographic expansion and an atypical phylogeographic pattern in the spittlebug *Philaenus spumarius* (Hemiptera, Aphrophoridae). PLoS One 9:1–12. https://doi.org/10.1371/journal.pone.0098375

Sánchez-Bayo F, Wyckhuys KAG (2019) Worldwide decline of the entomofauna: A review of its drivers. Biol Conserv 232:8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Saponari M, Boscia D, Altamura G, et al (2017) Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. Sci Rep 7:1–13. https://doi.org/10.1038/s41598-017-17957-z

Saponari M, Boscia D, Nigro F, Martelli GP (2013) Identification of DNA sequences related to *Xylella fastidiosa* in oleander, almond and olive trees exhibiting leaf scorch symptoms in Apulia (Southern Italy). J Plant Pathol 95:668. https://doi.org/10.4454/JPP.V95I3.035

Saponari M, Giampetruzzi A, Loconsole G, et al (2019) *Xylella fastidiosa* in olive in Apulia: Where we stand. Phytopathology 109:175–186. https://doi.org/10.1094/PHYTO-08-18-0319-FI

Saponari M, Loconsole G, Cornara D, et al (2014) Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy. J Econ Entomol 107:1316–1319. https://doi.org/10.1603/EC14142

Saxena KN, Kumar H (1984) Acoustic communication in the sexual behaviour of the leafhopper, *Amrasca devastans*. Physiol Entomol 9:77–86. https://doi.org/10.1111/j.1365-3032.1984.tb00683.x

Schneider K, van der Werf W, Cendoya M, et al (2020) Impact of *Xylella fastidiosa* subspecies *pauca* in European olives. Proc Natl Acad Sci U S A 117:9250–9259. https://doi.org/10.1073/pnas.1912206117

Secor GA, Rivera VV, Abad JA, et al (2009) Association of "*Candidatus* Liberibacter solanacearum" with zebra chip disease of potato established by graft and psyllid transmission, electron microscopy, and PCR. Plant Dis 93:574–583. https://doi.org/10.1094/PDIS-93-6-0574

Sicard A, Zeilinger AR, Vanhove M, et al (2018) *Xylella fastidiosa*: Insights into an emerging plant pathogen. Annu Rev Phytopathol 56:181–202. https://doi.org/10.1146/annurev-phyto-080417-045849

Silva SE, Rodrigues ASB, Marabuto E, et al (2015) Differential survival and reproduction in colour forms of *Philaenus spumarius* give new insights to the study of its balanced polymorphism. Ecol Entomol 40:759–766. https://doi.org/10.1111/een.12252

Simberloff D, Martin JL, Genovesi P, et al (2013) Impacts of biological invasions: What's what and the way forward. Trends Ecol Evol 28:58–66. https://doi.org/10.1016/j.tree.2012.07.013

Stewart AJA (1996) The colour/pattern polymorphism of *Philaenus spumarius* (L.) (Homoptera: Cercopidae) in England and Wales. Philos Trans R Soc B Biol Sci 351:69–89. https://doi.org/10.1098/rstb.1996.0005

Strona G, Carstens CJ, Beck PSA (2017) Network analysis reveals why *Xylella fastidiosa* will persist in Europe. Sci Rep 7:1–8. https://doi.org/10.1038/s41598-017-00077-z

Takanashi T, Uechi N, Tatsuta H (2019) Vibrations in hemipteran and coleopteran insects: behaviors and application in pest management. Appl Entomol Zool 54:21–29. https://doi.org/10.1007/s13355-018-00603-z

Taylor KL (1985) A possible stridulatory organ in some Psylloidea (Homoptera). Aust J Entomol 24:77–80. https://doi.org/10.1111/j.1440-6055.1985.tb00190.x

Teulon DAJ, Workman PJ, Thomas KL, Nielsen M-C (2009) *Bactericera cockerelli* incursion dispersal and current distribution on vegetable crops in New Zealand. New Zeal Plant Prot 62:136–144. https://doi.org/10.30843/nzpp.2009.62.4783

Thompson V (2004) Associative nitrogen fixation, C 4 photosynthesis, and the evolution of spittlebugs (Hemiptera: Cercopidae) as major pests of neotropical sugarcane and forage grasses. Bull Entomol Res 94:189–200. https://doi.org/10.1079/ber2004293

Thompson V (1994) Spittlebug indicators of nitrogen-fixing plants. Los salivazos como indicadores de plantas fijadoras de nitrógeno. Ecol Entomol 19:391–398

Tishechkin D (2006) On the structure of stridulatory organs in jumping plant lice (Homoptera: Psyllinea). Russ Entomol J 15:335–340

Tishechkin DY (2007) New data on vibratory communication in jumping plant lice of the families Aphalaridae and Triozidae (Homoptera, Psyllinea). Entomol Rev 87:394–400. https://doi.org/10.1134/S0013873807040021

Tishechkin DY (2003) Vibrational communication in Cercopoidea and Fulgoroidea (Homoptera: Cicadina) with notes on classification of higher taxa. Russ Entomol J 12:127–181

Tsubaki R, Hosoda N, Kitajima H, Takanashi T (2014) Substrate-borne vibrations induce behavioral responses in the leaf-dwelling cerambycid, *Paraglenea fortunei*. Zoolog Sci 31:789–794. https://doi.org/10.2108/zs140029

Vereijssen J, Smith GR, Weintraub PG (2018) *Bactericera cockerelli* (Hemiptera: Triozidae) and *Candidatus* liberibacter solanacearum in potatoes in New Zealand: Biology, transmission, and implications for management. J Integr Pest Manag 9:. https://doi.org/10.1093/jipm/pmy007

Virant-Doberlet M, Cokl A (2004) Vibrational communication in insects. Neotrop Entomol 33:121–134. https://doi.org/10.1590/s1519-566x2004000200001

Virant-Doberlet M, Kuhelj A, Polajnar J, Šturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol 7:1–15. https://doi.org/10.3389/fevo.2019.00203

Vos S, Camilleri M, Diakaki M, et al (2019) Pest survey card on *Xylella fastidiosa*. EFSA Support Publ 16:1667E. https://doi.org/10.2903/sp.efsa.2019.en-1667

Wallis RL (1995) Ecological studies on the potato psyllid as a pest of potatoes. The Department

Weaver C, King D (1954) Meadow spittlebug, *Philaenus leucophthalmus* (L.). Ohio Agric Exp Station Res Bull 741:1–99

Weaver CR (1951) The seasonal behavior of meadow spittlebug and its relation to a control method. J Econ Entomol 44:350–353. https://doi.org/10.1093/jee/44.3.350

Welter SC, Pickel C, Millar J, et al (2005) Pheromone mating disruption offers selective management options for key pests. Calif Agric 59:16–22. https://doi.org/10.3733/ca.v059n01p16

Wenninger EJ, Hall DG, Mankin RW (2009) Vibrational Communication Between the Sexes in *Diaphorina citri* (Hemiptera: Psyllidae). Ann Entomol Soc Am 102:547–555.

https://doi.org/10.1603/008.102.0327

Wessel A, Mühlethaler R, Hartung V, et al (2014) The tymbal: evolution of a complex vibration-producing organ in the Tymbalia (Hemiptera excl. Sternorrhyncha). In: Studying vibrational communication. Springer, pp 395–444

White PJT, Kerr JT (2007) Human impacts on environment-diversity relationships: Evidence for biotic homogenization from butterfly species richness patterns. Glob Ecol Biogeogr 16:290–299. https://doi.org/10.1111/j.1466-8238.2007.00298.x

Whittaker JB (1969) The biology of Pipunculidae (Diptera) parasitising some British Cercopidae (Homoptera). Proc R Entomol Soc London Ser A, Gen Entomol 44:17–24. https://doi.org/10.1111/j.1365-3032.1969.tb00814.x

Whittaker JB (1973) Density regulation in a population of *Philaenus spumarius* (L.) (Homoptera: Cercopidae). J Anim Ecol 163–172

Wiegert RG (1964a) Population energetics of meadow spittlebugs (*Philaenus spumarius* L.) as affected by migration and habitat. Ecol Monogr 34:217–241. https://doi.org/10.2307/1948501

Wiegert RG (1964b) The ingestion of xylem sap by meadow spittlebugs, *Philaenus spumarius* (L.). Am Midl Nat 71:422–428. https://doi.org/10.2307/2423300

Wise MJ, Kieffer DL, Abrahamson WG (2006) Costs and benefits of gregarious feeding in the meadow spittlebug, *Philaenus spumarius*. Ecol Entomol 31:548–555. https://doi.org/10.1111/j.1365-2311.2006.00814.x

Witsack W (1973) Experimentell-okologische Untersuchungen uber Dormanz-Formen von Zikaden (Homoptera, Auchenorrhyncha). 2. zur Ovarial-Parapause und obligatorischen Embryonal-Diapause von *Philaenus spumarius* (L.)(Aphrophoridae). Zool Jahrbücher Abteilung für Syst Okol und Geogr der Tiere 100:517–562

Wood RM, Peters RA, Taylor GS, Steinbauer MJ (2016) Characteristics of the signals of male *Anoeconeossa bundoorensis* Taylor and Burckhardt (Hemiptera: Aphalaridae) associated with female responsiveness. J Insect Behav 29:1–14. https://doi.org/10.1007/s10905-015-9539-8

Yang XB, Liu TX (2009) Life history and life tables of *Bactericera cockerelli* (Homoptera: Psyllidae) on eggplant and bell pepper. Environ Entomol 38:1661–1667. https://doi.org/10.1603/022.038.0619

Yang XB, Zhang YM, Hua L, Liu TX (2010) Life history and life tables of *Bactericera cockerelli* (Hemiptera: Psyllidae) on potato under laboratory and field conditions in the Lower Rio Grande Valley of Texas. J Econ Entomol 103:1729–1734. https://doi.org/10.1603/EC10083

Yurtsever S (2000) On the polymorphic meadow spittlebug, *Philaenus spumarius* (L.) (Homoptera: Cercopidae). Turkish J Zool 24:447–459

Yurtsever S (2001) Multiple paternity in the meadow spittlebug *Philaenus spumarius* [L.] [Homoptera: Cercopidae]. Acta Zool Cracoviensia 44:173–184

Vibrational communication and mating behaviour of the meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae)

Sabina Avosani, Elissa Daher, Pietro Franceschi, Marco Ciolli, Vincenzo Verrastro and Valerio Mazzoni

Entomologia Generalis. **2020**. 40(3), 307-321

DOI: 10.1127/entomologia/2020/0983

Abstract

Vibrational pest control techniques have been recently developed for several Hemiptera, suggesting that similar strategies could be applied to other species that emit vibrational signals. To evaluate the applicability of a similar control method for *Philaenus spumarius*, the vector of *Xylella fastidiosa* in olive, we investigated its mating behaviour and characterized the associated substrate-borne signals. Males and females arranged in singles, pairs or trios were released on a plant and the emitted signals were recorded with a laser vibrometer from the surface of a leaf. Playback trials on males were conducted to assess the role of a pre-recorded either male or female signal. The latter was tested both at the beginning and end of the season. We described the male and female repertoires, which both consisted of three vibrational signals with distinct features. Pair formation begun with the calling signal of a receptive female and mating was accomplished after a vibrational duet was established and maintained until the male reached the female on the plant. Female calls and duets occurred at the end of the season (September), while earlier in the season females emitted rejection signals to courting males. The number of male signals emitted in response to the female playback was not significantly different between the tested periods. However, there was difference in the type of signals emitted when males were stimulated by either the female or male playback. Further experiments with playbacks are warranted to identify candidate vibrational signals to be used for manipulating *P. spumarius* behaviour and develop a future control method.

Keywords: pest control; behavioural manipulation; vibrational signals; meadow spittlebug; *Xylella fastidiosa*

1. Introduction

Due to the increasing awareness about the deleterious impact of pesticides on human health and environment, there is an ongoing effort to design alternative methods for pest management in agriculture. In general, most strategies (i.e., push-and-pull and lure-and-kill tactics, mating disruption) involve the manipulation of the pest's behaviour by means of different external stimuli. To be effective, these techniques must consider the pest's physiological state and associated behaviours such as the process of pair formation (Foster and Harris 1997; Polajnar et al. 2015). In animals, mating is achieved through exchange of species-specific signals between a female and a male (Bailey 2003), which provides relevant information about identity, position and even quality of a potential mate (Endler 1992). It has been estimated that 92% of the insect species interacts by means of vibrational substrate-borne signals (Cocroft and Rodriguez 2005) and in most Hemiptera these signals are crucial to accomplish mating (reviewed in Čokl and Virant-Doberlet 2003; Polajnar et al. 2014). Typically, pair formation starts with the emission of an advertisement call by one of the two potential partners, to which a sexually mature male or female replies with its own signal. Establishment of a duet is critical to achieve mating in that it allows mate localization and copulation (Mazzoni et al. 2009b; De Groot et al. 2012). Interruption of the duet implies the interruption of the mating process. As such, in some insect species rival males evolved specific disruptive signals to prevent other males to mate (Booij 1982; Heady et al. 1986; Bailey et al. 2006; Miranda 2006). In the grapevine pest *Scaphoideus titanus*, a rival male can emit a masking signal that when transmitted as a playback in laboratory trials caused interruption of the duet (Mazzoni et al. 2009a, b). In further experiments conducted in semi-field trials, the continuous transmission of this signal into grapevine plants by electromagnetic transducers significantly reduced mating success of *S. titanus* (Eriksson et al. 2012; Polajnar et al. 2016). A similar method has been tested in laboratory (Gordon et al. 2017; Mazzoni et al. 2017a) and field (Krugner and Gordon 2018) to interfere with the mating communication of the glassy-winged sharpshooter, *Homalodisca vitripennis*, vector of the bacterium *Xylella fastidiosa* in California (Gordon et al. 2017), and the green grapevine leafhopper *Empoasca vitis* (Nieri and Mazzoni 2018). Besides mating disruption, vibrational signals could be exploited as lures to attract the target insect into a trap. For example, this approach is driving the development of vibrational traps for the brown marmorated stink bug *Halyomorpha halys* (Mazzoni et al. 2017b), and the Asian citrus psyllid *Diaphorina citri* (Hartman et al. 2017). In summary, the rapid and encouraging improvements in this field permit scientists to explore the use of vibrational signal playback as a novel tool for pest control.

The meadow spittlebug, *Philaenus spumarius* (Hemiptera: Aphrophoridae), is an abundant and world widespread species that occurs in almost all terrestrial habitats (Halkka and Halkka 1990; Stewart and Lees 1996). It is a univoltine insect that overwinters as egg and can feed on hundreds of plant species (Weaver and King 1954; Cornara et al. 2018). Recently, this species became a threat for European agriculture because it is the main vector of *X. fastidiosa* in Southern Italy (Saponari et al. 2014; Cornara et al. 2019a), where the bacterium causes the Olive Quick Decline Syndrome (OQDS) (Saponari et al. 2017). Currently, *X. fastidiosa* has been found throughout the Mediterranean (Denance et al. 2017; Olmo et al. 2017). Therefore, an effective and sustainable management strategy for *P. spumarius* has become of high priority in the European Union. In the frame of a potential behavioural manipulation approach, knowledge of the pair formation process and the associated signals of *P. spumarius* are essential prerequisites to develop a control method (i.e., to repel, disrupt mating, attract) (Pedigo and Rice 2014; Pertot et al. 2017). Several aspects of the biology (Weaver and King 1954; Chmiel et al. 1979), genetics (Halkka et al. 1973; Maryańska-Nadachowska et al. 2012; Rodrigues et al. 2014), ecology (Santoiemma et al. 2019; Bodino et al. 2019) and feeding behavior (Cornara et al. 2018b; Cornara et al. 2019b; Markhaiser et al. 2020) of *P. spumarius* have been studied, but its sexual behavior has been poorly described. Even if egg maturation occurs only late in the summer due to an ovarian parapause (Witsack 1973), previous investigations have reported that multiple matings could occur soon after adult emergence in spring (Cornara et al. 2018; Morente et al. 2018). Although there is evidence of the emission of vibrational signals by *P. spumarius* males (Tishechkin 2003), their ethological function in conspecific interactions has not been investigated, while neither the emission of female's signals nor of duets have been reported.

The objective of this study was to characterize *P. spumarius* vibrational signals, describe their role in the pair formation process, and provide information for further research and development of vibration-based sustainable control techniques.

2. Materials and methods

2.1. Insects

Fourth and fifth instar nymphs of *P. spumarius* were collected in April 2017 and May 2018 from meadows in Riva Del Garda (Trentino, Italy). Host plants carrying spittle were cut and transported using a refrigerated bag to a glasshouse at the Fondazione Edmund Mach. Insects were then reared in mesh cages (Bugdorm-6620, 60x60x120 cm, MegaView Science Co., Ltd., Taiwan) under controlled conditions (25 ± 2 °C, L16:D8, RH 75 ± 5%). Plants of *Helianthus annuus*, *Vicia faba*, *Trifolium repens* and *Rumex* spp. were placed inside each cage as food source. Nymphs were checked daily, and the newly emerged adults were removed and isolated by gender to obtain virgin individual to be used for the trials. The adults that emerged in the same day were placed on a plant of *H. annuus*, which was enclosed by a cylindrical tube made of plastic net (5 cm diameter x 15 cm height). A nylon stocking on the top of the tube permitted air ventilation. Adult emergence ranged from the end of April and lasted until the 6th of June.

2.2. Recordings

Recordings were carried out in a sound insulated chamber of the laboratory of Biotremology at Fondazione Edmund Mach (Italy), at a room temperature of 22 ± 1 °C and RH of 65%. The experimental arena was a Plexiglass cage (30x50x30 cm) with a circular opening on the top (diameter 6 cm), which was placed on an anti-vibrational table (Astel s.a.s., Ivrea, Italy). A host plant (*H. annuus* or *V. faba*, average height 12 cm, 2-4 leaves) was placed into the cage. Insects were released on the plant after 15 minutes of acclimation in the room. Vibrational signals were recorded from a small piece of reflective tape attached on the lamina of the upper leaf by a laser Doppler vibrometer (Ometron VQ-500-D-V), and digitized with Pulse (Brüel and Kjær Sound & Vibration A/S, Nærum, 104 Denmark) at a 44.1 kHz sample rate and 16-bit depth resolution through a data acquisition device (LAN XI type 3050-B-040, Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark), then stored onto a hard drive of a computer (HP, EliteBook 8460 p). Trials were recorded with a video-camera (mod. HTC-TM700, Panasonic, Japan) to associate the emission of vibrational signals with the corresponding behaviour.

2.3. Bioassays

Three experiments of spontaneous behaviour and three of playbacks to induce behavioural responses were conducted. In pair and trio trials, insects were released in a random order on the plant and their behaviour was observed for 30 minutes or until copulation occurred.

2.3.1. Bioassay 1. Spontaneous behaviour

Single males and females. Males (n = 105) and females (n = 32) were singly placed on a *H. annuus* or *V. faba* plant and vibrational signals were recorded for 30 minutes to identify spontaneous calling signals. Because males emitted signals soon after adult emergence, they were tested from the end of April until the end of June 2017 to study the daily pattern of calling activity. For statistical purposes, recordings were grouped in five 2-h periods from 08:00h to 19:00h. Calling activity of 21 males for each time-period was recorded. Single females were tested from the end of April to the end of June 2017 (n = 15) and from the end of July to the end of September 2017 (n = 17). Further recordings of males (n = 121) and females (n = 201) were performed from August to October 2018 on a small plant of *Vitis vinifera* (height 10-15 cm) to increase the number of observations during the most active period (in terms of mating communication) of the insect life cycle as emerged from the pairs' test (see results).

Pairs of a male and a female (n = 188) were tested to describe the pair formation process. Trials were conducted from the end of April to the end of September 2017. For statistical analysis, recordings were grouped in the following 10 periods of 15 days: (1) from the end of April to mid-May (n = 17), (2) from mid-May to end of May (n = 20), (3) from the beginning of June to mid-June (n = 16), (4) from mid-June to the beginning of July (n = 17), (5) from the beginning of July to mid-July (n = 18), (6) from mid-July to the beginning of August (n = 20), (7) from the beginning of August to mid-August (n = 19), (8) from mid-August to the end of August (n = 25), (9) from the beginning of September to mid-September (n = 22) and (10) from mid-September to the end of September (n = 14). Insects were released on a *H. annuus* or *V. faba* plant.

Trios (n = 47) of two males and a female were released on a *H. annuus* or *V. faba* plant from mid-July to the end of August 2017. Behaviours and associated vibrational signals were recorded to evaluate intraspecific interactions and potential rivalry behaviours between males.

2.3.2. Bioassay 2. Playback trials

Males of *P. spumarius* were stimulated with playbacks of pre-recorded male and female calling signals (MCS and FCS, respectively, see results), to evaluate the possible role of these signals in the pair formation process. Playback signals were transmitted to a *V. faba* plant using a mini shaker (Type 4810; Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark) in direct contact with a leaf via a conical rod. The background noise was filtered, and signals were modified using the audio software Adobe Audition 3.0 (Adobe Systems, Inc., San Jose, CA, USA), while the amplitude adjusted to resemble the insect natural amplitude from previous recordings. To determine whether the transmission of the female signal could evoke different behaviours in males, we tested the female playback in two different periods of the *P. spumarius* adult season: October 2017 and June 2018. Conversely, the male signal was tested as playback only in June 2018 to ascertain possible effects in a period when only males emit vibrational signals, while females are silent (see results). Insect behaviours were followed using a video-camera and vibrational signals were recorded as described above.

October 2017. Three different female calling signals (FCS) were randomly transmitted to stimulate males (n = 42). The FCSs were composed of 20-25 chirps/signal, with a total duration of 6.5 s and a repetition time of 0.25-0.30 s. A male was released on a leaf of the plant and after 3 minutes of acclimatization the playback was played seven times with one minute of interval between each signal. The test lasted for 10 minutes or until the insect jumped off.

June 2018. Males (n = 40) were stimulated with the same three FCSs used in October 2017, while other males (n = 20) were tested with three male calling signals (MCS) consisting of 40 chirps/signal. The duration of each MCS was 48 s, and the repetition time between chirps ranged between 0.20 s and 0.25 s. After 2 minutes of acclimatization, males were stimulated with either the female or the male playbacks, which were transmitted with 30 seconds of gap between consecutive signals. A third group of males (n = 20) was not stimulated as control.

2.4. Terminology

Vibrational signals were labelled according to their behavioural context.

Calling signals (or calls). Spontaneous emission of a signal by isolated insects (Booiji 1982).

Pulse. Unitary homogeneous parcel of sound of finite duration (Broughton 1963) and composed of a brief succession of sine waves (Alexander 1967).

Pulse train. Series of pulses arranged into temporally distinct groups.

Chirp. Continuous sound characterized by a fundamental frequency and a clear harmonic structure.

Syllable. Pulses and a chirp arranged into repeatable and temporally distinct groups, where a chirp is emitted after a brief train of pulses.

2.5. Statistical analyses

Spectral and temporal analyses of the recorded signals were performed with Raven 1.4 (The Cornell Lab of Ornithology, 151 Ithaca, NY) using Fast Fourier Transform (FFT) type Hann, with window length of 512 samples and 75% overlap. Recordings of males and females were used for analysis of temporal and spectral parameters of the vibrational signals; only signals recorded at 2-5 cm from the laser sticker were used to reduce the effect of different plants on signal transmission. Results are presented as means, ranges, and standard deviation (SD) together with the numbers of signals or elements within a signal analysed for each individual (N) and number of individuals (n) from which signals were obtained. The following parameters, when applicable, were measured for the signals: calling rate (intended as number of calling signals from the onset of the first one until the end of the trial), signalling latency (time from placement on the plant to the emission of the first vibrational signal), duration of the calls, duration and fundamental frequency (Ff) of the chirp, pulse/chirp repetition time (measured as the distance between the onsets of two consecutive sound elements), and duration and dominant frequency (Df) of the pulses. Additional parameters were measured for the Male Courtship Song (MCrS) (Table 2.2) and the male-female duet (Table 2.6), since those were composed of sections with a distinctive pattern. We analysed only the duets that led to copulation (5 out of 6 duets). The number of signalling males in each period of the day was taken as measure of calling activity, and the G-test for contingency table (using log-likelihood ratios) after Williams' correction (Zaar 1999) was used to compare calling activity between the periods of the day. Wilcoxon-Mann-Whitney test (Zaar 1999) was used to determine whether there was significant difference in the spectral and temporal features between the male and female signals. For playback trials, the percentage of males that emitted a signal after the stimulation were compared between trials in October 2017 and June 2018 as well as between stimulation and control, using chi-square in contingency Table (2x2 and 2x3, respectively). The same test was used to determine whether playbacks had a repellent effect on insects by comparing the percentage of males that jumped off the plant after the stimulation between trials in October 2017 and June 2018 (2x2) and between control and stimulation trials (2x3). The type of signal evoked in the tested males was evaluated to associate each signal to a possible behavioural function. To ascertain that vibrational signals emitted by the tested males were due to the playbacks, the signalling latency of the stimulated group and the control was compared using a Wilcoxon-Mann-Whitney test (Zaar 1999). Data were analysed using Past 3 (Hammer & Harper, Øyvind Hammer, Natural History Museum, and University of Oslo).

A statistical analysis of the signals was performed using R (R core team 2019) and visualized by *ggplot* (Wickham 2016) and *tidyverse* (Wickham 2017). Each measurement was characterized in terms of chirp duration, chirp fundamental frequency and repetition time between chirps. We did not perform any comparative analysis on the pulses since those were emitted mainly by males. To account for possible non-normality in the distribution of the parameters, the signals recorded from each individual were summarized by their median. An overall data inspection was performed to visualize the distribution of the log-transformed individual-specific signals (Q2) in the three-dimensional space of the parameters by using the plot3D package (Soetaert 2017). The presence of a significant clustering among the signals was assessed by a permanova approach (Anderson 2008), implemented in the vegan package (Oksanen et al. 2019). A Wilcoxon-Mann-Whitney test (Zaar 1999) was finally applied to compare male and female signals.

3. Results

3.1. Bioassays 1. Spontaneous trials

Vibrational signals and mating behaviour. Vibrational signals of *P. spumarius* are composed of two different sound elements, which are defined as separate subunits: (i) a harmonic element (“chirp”, fundamental frequency around 180.8 ± 20.6 Hz) and (ii) a pulse element (“pulse”, dominant frequency 150 ± 52.9 Hz). The two subunits when emitted in sequence constitute a syllable, where a chirp follows a brief pulse train (0.13 ± 0.02 s). The chirp is used by both males and females to compose their respective signals, while female pulses were rarely recorded (2% of the females of the pair trials). The male repertoire consists of three different types of vibrational signals: Male Calling Signal (MCS, Fig 2.1A), Male Courtship Signal (MCrS, Fig 2.1B), and Male-Male Signal (MMS, Fig 2.1C). Similarly, females emit three types of signals: Female Calling Signal (FCS, Fig 2.1D), Female Rejection Signal (FRjS, Fig 2.1E), and Female Response Signal (FRsS, Fig 2.1F). MCS (Figure 2.1A) and FCS (Figure 2.1D) are both composed of a train of short chirps. In addition, the MCS can be preceded by pulse trains or include syllables. The MCrS consists of chirp and pulses as well but with a more complex structure than the MCS (Table 2.1, Figure 2.1A) since it can be divided in two sections, which are alternately emitted (Table 2.2, Figure 2.1B). Section 1 consists of a long train of pulses with a rather variable repetition time (Table 2.2, Figure 2.1B) and it is emitted while the male is walking on the plant. Section 2 occurs when the male is stationary and has a more regular pattern, being composed of a series of syllables of relatively constant duration and repetition time (Table 2.2, Figure 2.1B). The MMS consists of a series of chirp elements with variable duration and repetition time (Table 2.3, Figure 2.1C). Similarly, the FRjS consists in a continuous, long chirp element (Table 2.5, Figure 2.1E) emitted alone or in trains with irregular repetition time. The FRsS is a short chirp emitted by a female in the time window between two consecutive male syllables during the duet (Table 2.6, Figure 2.1F).

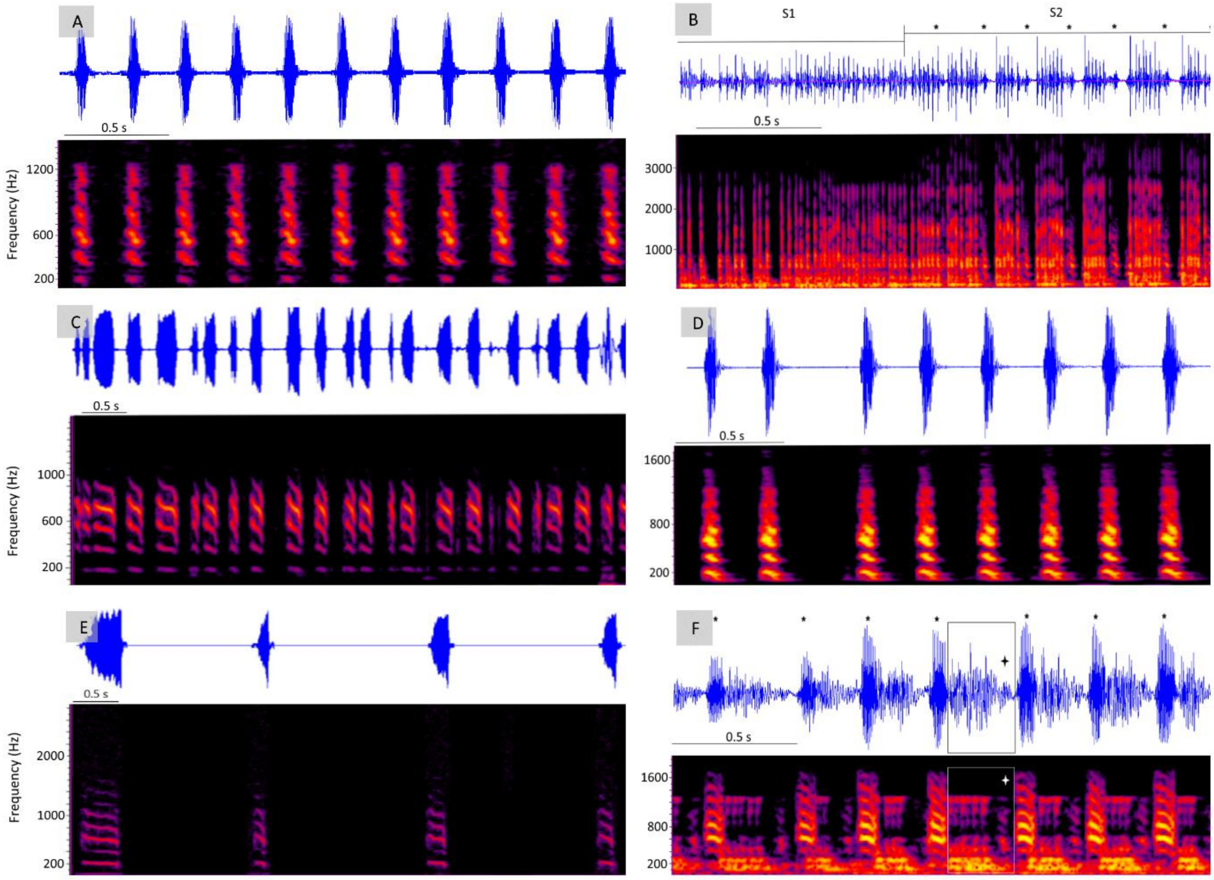

Figure 2.1. Oscillograms (above) and spectrograms (below) of the vibrational signals of *P. spumarius*. A) Male Calling Signal (MCS) composed of chirp trains. B) Male Courtship Signal (MCrS), which is composed of two sections (S1 and S2). S1 consists of pulses, while S2 of pulses and chirps arranged in syllables. Asterisks indicate the chirps. C) Male-Male Signal (MMS). D) Female Calling Signal (FCS). E) Female Rejection Signal (FRjS). F) Portion of a male-female duet; the frame highlights the male contribution, the syllable, which is composed of a short pulse train followed by a harmonic element (chirp, pointed out by the star). The asterisks indicate the Female Response Signal (FRsS), also consisting of a chirp element.

Table 2.1. Temporal and spectral properties of the Male Calling Signal (MCS) of *P. spumarius*. N = number of measured elements; n = number of individuals; chirp = harmonic element; Ff = fundamental frequency; Df = dominant frequency.

Signal parameter	Mean ± SD	Min.	Max.	n	N
Calling Latency (s)	774.71 ± 493.18	6.65	1767.43	23	1
Call Rate (%)	4.13 ± 2.97	0.24	13.86	23	See note below
Duration of MCS (s)	8.71 ± 4.00	3.27	19.74	26	4
Duration of chirp (s)	0.063 ± 0.007	0.049	0.076	26	30
Repetition time between chirps (s)	0.270 ± 0.031	0.216	0.367	26	30
Ff of chirp (Hz)	177.40 ± 21.30	140.27	217.67	26	30
Repetition time between pulse trains (s)	0.31 ± 0.05	0.23	0.36	14	5
Duration of pulse trains (s)	0.07 ± 0.02	0.06	0.10	14	5

Df of pulse trains (Hz)	161.37 ± 34.37	131.43	218.29	14	5

note = for measuring the call rate, the number of signals emitted by each individual within the recording was used

Table 2.2. Temporal and spectral properties of the Male Courtship Signal (MCrS) of *P. spumarius.* S1 and S2, sections 1 and section 2 of the MCrS; N = number of measured elements; n = number of individuals; chirp = harmonic element; Ff = fundamental frequency; Df = dominant frequency.

Signal parameter	Mean ± SD	Min	Max	n	N
Duration of MCrS (s)	601.48 ± 524.44	31.83	1371.64	8	1
Duration of S1 (s)	12.91 ± 6.08	5.74	23.76	8	5
Duration of S2 (s)	8.29 ± 2.55	5.95	13.43	8	5
Duration of syllable (s)	0.21 ± 0.02	0.20	0.25	8	30
Repetition time between syllables (s)	0.26 ± 0.02	0.24	0.30	8	30
Duration of chirp within the syllable (s)	0.06 ± 0.01	0.05	0.073	8	30
Ff of chirp within the syllable (Hz)	197.87 ± 12.01	181.33	216.27	8	30
Duration of pulse trains in S1 (s)	4.46 ± 12.17	0.12	34.59	8	30
Duration of pulse trains in S2 (s)	0.13 ± 0.02	0.10	0.17	8	30
Df of pulse trains in S1 (Hz)	147.87 ± 66.09	82.67	277.33	8	30
Df of pulse trains in S2 (Hz)	144 ± 63.10	96	276.27	8	30
Number of S1	26.50 ± 19.76	7	56	8	1
Number of S2	25.88 ± 19.52	8	55	8	1

Table 2.3. Temporal and spectral properties of the Male-Male Signal (MMS) of *P. spumarius.* N = number of measured elements; n = number of individuals; chirp = harmonic element; Ff = fundamental frequency

Signal parameter	Mean ± SD	Min.	Max.	n	N
Duration of chirp (s)	0.14 ± 0.01	0.12	0.16	8	30
Repetition time between chirps (s)	0.50 ± 0.25	0.28	0.95	8	30
Ff of chirp (Hz)	180.97 ± 20.80	153.07	206.67	8	30

Table 2.4. Temporal and spectral properties of the Female Calling Signal (FCS) of *P. spumarius.* N = number of measured elements; n = number of individuals; chirp = harmonic element; Ff = fundamental frequency.

Signal parameter	Mean ± SD	Min.	Max.	n	N
Calling Latency (s)	183.39 ± 213.15	0.128	870.799	71	1
Duration of FCS (s)	2.89 ± 1.88	2.34	9.42	36	20
Duration of chirp (s)	0.079 ± 0.014	0.056	0.106	36	30
Repetition time between chirps (s)	0.313 ± 0.055	0.252	0.478	36	30
Ff of chirp (Hz)	180.81 ± 13.82	150.93	182.13	36	30

Table 2.5. Temporal and spectral properties of the Female Rejection Signal (FRjS) of *P. spumarius.* N = number of measured elements; n = number of individuals; chirp = harmonic element; Ff = fundamental frequency.

Signal parameter	Mean ± SD	Min.	Max	n	N
Duration of chirp (s)	0.16 ± 0.04	0.12	0.25	8	30
Repetition time between chirps(s)	1.05 ± 0.67	0.33	2	8	30
Ff of chirp (Hz)	185.76 ± 18.14	167	222	8	30

Table 2.6. Temporal and spectral properties of the vibrational signals emitted during the male-female duet. S1 and S2, sections 1 and 2 of the Male Courtship Signal; N = number of measured elements; n = number of individuals; chirp = harmonic element; Ff = fundamental frequency; Df = Dominant frequency.

Signal parameter	Mean ± SD	Min.	Max.	n	N
Duration of duet (s)	245.68 ± 197.46	2.83	483.61	6	1
Duration of S1 (s)	9.05 ± 2.48	6.23	12.07	5	5
Duration of S2 (s)	7.04 ± 1.31	5.90	9.17	5	5
Duration of syllables (s)	0.19 ± 0.03	0.16	0.22	5	30
Duration of pulse trains in S2 (s)	0.16 ± 0.10	0.08	0.34	5	30
Repetition time syllables (s)	0.25 ± 0.01	0.24	0.26	5	30
Number of syllables/S2	32.41 ± 6.36	25.00	39.00	5	5
Ff of chirp within the syllable (Hz)	193.87 ± 9.41	184	206	5	30
Df of pulse trains S2 (Hz)	112.75 ± 31.40	84.27	165.87	5	30
Df of pulse trains within the syllable in S2 (Hz)	108.80 ± 14.30	95.47	130.67	5	30
Df of pulse trains in S1 (Hz)	643.72 ± 818.23	0.10	1922.99	5	30
Duration of Female Response Signal (s)	0.08 ± 0.01	0.07	0.09	5	30
Ff of Female Response Signal (Hz)	167.36 ± 10.20	158.93	182.40	5	30
Female response latency (s)	70.13 ± 91.21	0.00	223.24	7	1
Female response rate in S1 (%)	0.14 ± 0.17	0.00	0.44	7	1
Female response rate in S2 (%)	0.71 ± 0.20	0.43	0.95	7	1
Number of S1	23.2 ± 14.08	7	45	5	all
Number of S2	22.6 ± 14.47	6	45	5	all

The signals were divided in three classes according to their structure: CS, the male and female calling signals, composed of regularly repeated short chirps; RS, rejection signals consisting of long and irregularly distributed chirps; CRS, male courtship signal. Plots of the analysed parameters shows that the classes of signals have different chirp duration and repetition time, which, however, could not be used to clearly separate either calling or rejection signals between males and females (Figure 2.2). The different properties of the three classes of signals are highlighted in the 3D plots (Figure 2.3), which clearly show the partitioning of the parameter space. The RS signals could be clearly distinguished since the repetition time and the duration of the chirps were more distant from the values measured for the other two classes. Permanova shows that the signals were significantly different in the considered three parameter space (CRS-RS: $p = 0.001$) (CS-CRS: $p = 0.003$) (CS-RS: $p = 0.001$). Moreover, male and female calling signals are significantly different in chirp duration (dbl = 796, Wilcoxon rank, two-sided $p < 0.001$) and in repetition time between chirps (dbl = 730, Wilcoxon rank two-sided $p < 0.001$), while the fundamental frequencies are rather similar (dbl = 222, Wilcoxon rank, two-sided p = 1). The male and female rejection signals (the MMS and the FRjS, respectively) are significantly different in the repetition time between chirps (Wilcoxon rank, dbl = 45, two-sided p < 0.001), while the chirp duration (Wilcoxon rank, dbl = 37.5, two-sided p = 1) and the fundamental frequency (Wilcoxon rank, dbl = 33.5, two-sided p = 1) are similar between the signals. The Wilcoxon-Mann-Whitney test confirms the significant differences in the spectral and temporal features between the calling and rejection signals (Table 2.7).

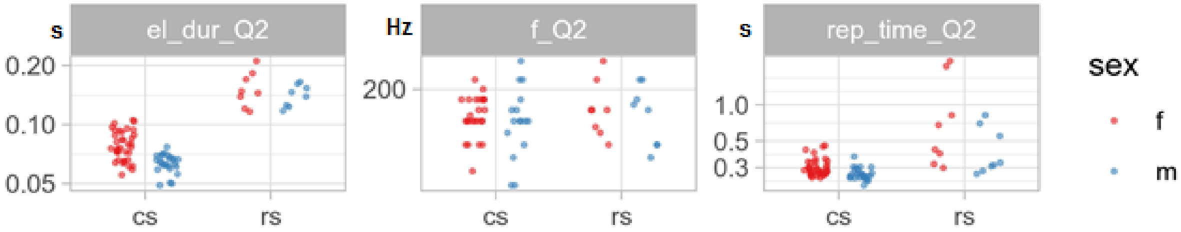

Figure 2.2. Distribution of the analysed parameters for calling and rejection signals of *P. spumarius*. Each point represents the median of the signals measured for each individual, different colours (red and blue) indicate females and males, respectively. El_dur = chirp duration; f = chirp fundamental frequency; rep_time = chirp repetition time; cs = calling signals; rs = rejection signals.

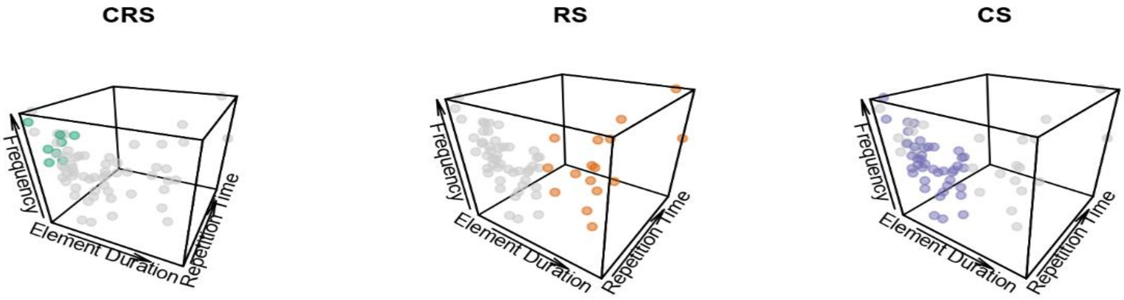

Figure 2.3. 3D plots representing the distribution of the three class of signals in relation to the others. Classes are defined as: courtship (CRS), rejection (RS), and calling (CS). Element duration = duration of chirps; CRS = male courtship signal; RS = female rejection signals and male-male signal; CS = female and male calling signals (MCS and FCS).

Table 2.7. Temporal and spectral parameters of different male and female signals after a Wilcoxon-Mann-Whitney U test. FCS = Female Calling Signal, FRjS = Female Rejection Signal; MCS = Male Calling Signal; MMS = Male-Male Signal; chirp = harmonic element; Ff = fundamental frequency; n = number of individuals.

Comparison	Parameter	U	n	p
FCS vs FRjS	Chirp duration (s)	26	8	NS
MCS vs MMS	Chirp duration (s)	0	8	< 0.001
FCS vs FRjS	Chirp repetition time (s)	8	8	0.011
MCS vs MMS	Chirp repetition time (s)	6	8	0.004
FCS vs FRjS	Chirp Ff (Hz)	8	8	0.010
MCS vs MMS	Chirp Ff (Hz)	18	8	NS

Single trials. Both males and females of *P. spumarius* emitted vibrational signals spontaneously within 30 minutes from placement on the plant. Among the males tested from April to the end of June 2017, 22% (n = 105) emitted at least one MCS, which was never recorded from individuals younger than four days from adult emergence. The MCS was usually composed of chirps only (Table 2.1, Figure 2.1A), except for eight males who emitted also short (0.07 ± 0.02 s) trains of pulses either before or within the MCS. The chirp repetition time was relatively constant (Table 2.1), while the calling latency was rather variable (Table 2.1) and there was not a significant difference in male calling activity between different periods of the day (G test; G = 3.53, df = 4, p = 0.47). In some cases, males (56.5%, n = 23) jumped from the point of signal emission after the MCS, and the number of calling signals varied greatly among different insects (4.8 ± 5.5, minimum = 1; maximum = 23). None of the females tested during the first period of trials from April to the end of June (n = 15) emitted a signal, while 52.9% of the females tested in September (n = 17) emitted a Female Calling Signal (FCS), which consisted in a chirp train with relatively constant repetition time (Table 2.4, Figure 2.1D). Females called from different parts of the leaf, and the number of FCS varied among different individuals (9.41 ± 5.8; minimum = 3; maximum = 17). In contrast with males, females did not leave the plant after calling.

Pair trials. Pair formation started with the emission of the FCS, which elicited (n=6) emission of MCrS and thus, the onset of a male-female duet (Table 2.6, Figure 2.1F). The latter was established when a female replied to the male with emission of FRsS in the time window between two consecutive male syllables within the S2 of the MCrS, although occasional FRsS occurred also during S1. The female response triggered the search of the male, which continued to emit the MCrS also while walking. The exchange of signals led to the localization of the stationary female by the male and, eventually, to copulation (mating success of duetting males: 83%). Duets were observed only in September (Table 2.8), while prior to this period, females were refractory to mating by emitting a rejection signal (FRjS) to an approaching male that tried to mount her. In contrast, a duetting female neither rejected a male nor expressed acceptance with emission of a vibrational signal. During mating, which lasted for 3-4 hours, both insects emitted copulatory vibrational signals consisting of randomly arranged and overlapped chirps. Few males (6 out of 188 tested) achieved mating without the emission of any vibrational signal but directly mounting on the female, which struggled to escape and jumped off the plant with the male attached. These forced copulations lasted for hours and occurred occasionally in our trials, throughout the season even when females were not acoustically active. Contrarily to the FCS, the MCS was emitted with slightly variable frequency throughout the experimental period (Table 2.8), while the MCrS was recorded starting from the 6th period (Table 2.8).

Table 2.8. Signals emitted by either a female or a male of *P. spumarius* during the pair trials (at least one signal per individual). FCS = Female Calling Signal, FRjS = Female Rejection Signal; MCS = Male Calling Signal; MCrS = Male Courtship Signal; chirp = harmonic element; n = number of pairs tested for each period; (1) end of April to mid-May, (2) mid-May to end of May, (3) beginning of June to mid-June, (4) mid-June to beginning of July, (5) beginning of July to mid-July, (6) mid-July to begging of August, (7) beginning of August to mid-August, (8) mid-August to end of August, (9) beginning of September to mid-September, (10) mid- September to end of September.

Period	N	Females emitting at least one	Males emitting at least one	Onset of

		FCS (%)	FRjS (%)	MCS (%) (chirps)	MCS (%) (chirp+pulses)	MCrS (%)	a duet (%)
1	17	0	17.6	11.8	11.8	0	0
2	20	0	30	15	10	0	0
3	16	0	6.3	0	0	0	0
4	17	0	17.6	5.9	5.9	0	0
5	18	0	11.1	16.7	5.6	0	0
6	20	0	30	5	5	20	0
7	19	0	0	15.8	10.5	0	0
8	25	0	4	12	12	16	0
9	22	18.2	0	22.7	4.5	9.1	9.1
10	14	28.6	0	0	7.1	28.6	28.6

Trio trials. MCS either with (14.9%, n = 47) or without pulses (6.4%, n = 47) and MCrS (4.3 %, n = 47) were recorded during trio trials as well. However, only the males of the trio trials emitted a Male-Male Signal (MMS), which was observed after interaction between the two males (17%, n = 47). In particular, MMS was mostly emitted after physical contact, after emission of MCS (with or without pulses), or after emission of MCrS by the other male in close proximity. The MMS could either partially (50%, n = 8) or completely (50%, n = 8) overlap the signals emitted by the other male, until one of them ceased to reply or, in one case, jumped off the plant. In two cases, one of the males mounted on the other. Similar to pair trials, when a male physically interacted with a female the latter emitted the FRjS (21.3%, n = 47), then ran away and/or jumped off the plant (14.9%, n = 47). The FRjS could be evoked by the MCrS of a relatively nearby male, whereas neither a FCS nor a duet were ever observed.

3.2. Bioassays 2: Stimulation trials with playbacks

Males stimulated with playbacks responded by emitting different vibrational signals (n = 42, 28.6%, Figure 2.4). The number of signalling males after the transmission of the female playback (Figure 2.4) was numerically lower in June 2018 (n = 40, 5.8%) than in October 2017, but there was no significant difference between the two periods (Chi-squared test; χ^2 = 3.220, df = 1, p = 0.073). In absence of any external vibrational stimulus, 20% of the males (n = 20) emitted at least one MCS (Figure 2.4).

Comparing the percentages of signalling males tested in the two periods, we did not find a statistical difference between the control group and the groups stimulated with playbacks of male or female signals (Chi-squared test; χ^2 =0.949, df = 2, p = 0.622). Similarly, the percentages of males that jumped off the plant did not significantly differ between the stimulated groups in October 2017 and June 2018 (Chi-squared test; χ^2 =2.052, df = 1, p = 0.152) or between the stimulated and the control groups (Chi-squared test; χ^2 = 0.739, df = 2, p = 0.691). Nonetheless, the number of jumping males in the control (n=20; 25%) was numerically higher than the group stimulated either with the female (n = 42 in October; 7.1% and n = 40 in June; 5.8%) and the male playbacks (n = 20; 15%). There was statistical difference in the signalling latency between the stimulated and the control groups (Wilcoxon-Mann-Whitney test: p = 0.0021).

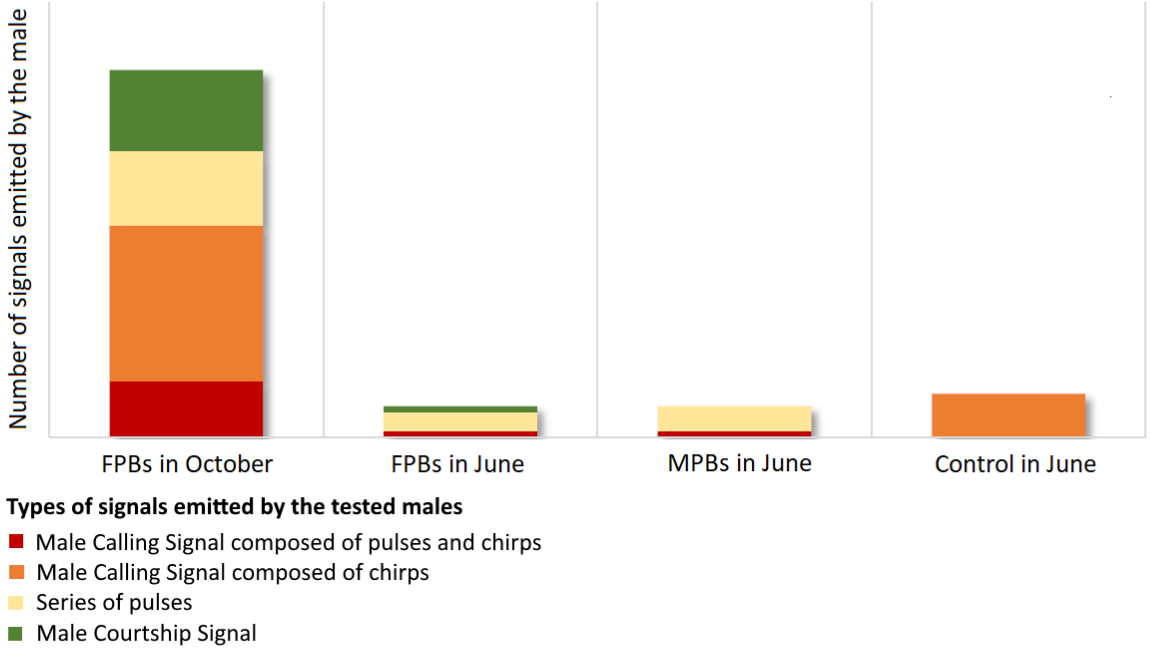

Figure 2.4. Types of signals emitted by males tested in the playback trials. FPBs = Female Playbacks; MPBs = Male Playbacks; chirp = harmonic element.

4. Discussion

The present study provides the first detailed description of the vibrational substrate-borne signals produced by *P. spumarius* and the associated mating behaviour. Similar to other species in Hemiptera (Čokl and Virant-Doberlet 2003), inter-sexual communication relies on vibrational signals and, overall, *P. spumarius* mating behaviour resembled the general scheme known for other Auchenorrhyncha, albeit with some differences.

Phenology. In auchenorrhynchans, females typically need more time than males to reach reproductive maturity (Krugner 2010; Mazzoni et al. 2009b). Accordingly, *P. spumarius* males tried to accomplish mating throughout the season in laboratory conditions, while females called and then established duets with males only from September. Because egg maturation begins in September and continues until the female dies (Weaver and King 1954), calls and vibrational duets likely occur when the female carries mature eggs in the ovaries. In fact, female calling activity is an indicator of reproductive maturity and receptiveness to mating (Hoch and Howarth 1993), and a similar relationship between presence of mature eggs and female calling has been suggested in *H. vitripennis* (Nieri et al. 2017), where the pre-oviposition period ranged from 9 to 285 days (Krugner 2010). *P. spumarius* females called in September even if we reared the insects under controlled conditions (temperature and photoperiod), and this fact is in strong disagreement with the literature (Witsack 1973; Morente et al. 2018). Since we did not performed oviposition trials with the tested females, we could not evaluate whether the ovarian parapause was broken. Nevertheless, photoperiod is potentially not the only factor that drives the development of *P. spumarius* ovaries, thus further trials should be addressed to unveil which factors promote ovarian development and the potential association between the presence of eggs and female calling activity. Evidence suggests that in the field *P. spumarius* mate soon after adult emergence (Yurtsever 2001), and in our trials some males silently approached and mounted the female. This behaviour could give a reproductive advantage to the male if the female preserves the sperm until egg maturation occurs, considering that the spermatheca allows a delayed fertilization (Robertson and Gibbs 1937). Since polyandry has been reported in *P. spumarius* (Yurstever 2001), the female could potentially fertilize eggs using newly acquired sperm from additional mates. In particular, she could use the sperm of the male that engaged a duet with her and eventually localized her. In this perspective, the sperm acquired after a forced copulation does not give a genetic contribution if the female uses the sperm of the last mate. According to this, last-male sperm precedence is a widespread reproductive strategy among insects (Gwynne 1987) and has been proposed for *P. spumarius* (Yurstsever 2001).

Pair formation process. During the initial phase of mate finding, the female calling signal (FCS) provided the indication of the availability of a reproductive mature female and triggered emission of the MCrS. The rapid inversion of female leading/male replying roles resulted in males guiding subsequent duets to locate the female, whose signal was reduced to a short chirp (the female response signal, FRsS). Mating success presumably depended on the male ability to maintain the female acceptance and to find her on the plant, while the decreased signalling activity of the female could be a strategy to save energy and/or reduce risks of predators eavesdropping (Virant-Doberlet et al. 2014). A similar behaviour was reported in *H. vitripennis* (Nieri et al. 2017) and in the planthopper *Hyalesthes obsoletus*, where females call to form a duet and then males lead the following phases of communication (Mazzoni et al. 2010). During mate identification and localization, males usually tend to optimize the energy consumption (Kuhelj et al. 2015); therefore, due to the degradation of the spectral characteristics of the signals along plants, which act as frequency filters (Michelsen et al. 1982), an important part of the mate choice likely occurs when the male is close enough to the female. Accordingly, in many Hemiptera, the male emits a specific and likely more complex signal when he gets enough close to the female to increase her acceptance (i.e, Mazzoni et al. 2010; Polajnar et al 2014). On the other hand, *P. spumarius* males use a specific courtship signal, the MCrS, unvaried throughout the duet until copulation. Since it consists of alternately emitted sections with different structures, these could have different roles and require a different involvement of resources from the male. In particular, the pulse train of the first section could serve to maintain the female interest during localization, while the female replies in the second section could provide the directional cues to the male while he is stationary. The second section is probably the most energetically costly subpart of the male repertoire, suggesting that mate choice could occur during this phase. Moreover, this could be the communication phase more susceptible to external interference by playing back disruptive signals (i.e., the FRjS and the MMS) with the aim to decrease the receptivity or even mask the female signals.

Vibrational communication and role of the signals. Chirps and pulses are emitted in a stereotyped sequence to produce a repertoire of signals, which are associated with different behavioral contexts. Series of short chirps underlie advertisement signals such as the MCS and the FCS, while longer chirps constitute a class of signals (FRjS and MMS) that denote distress after interaction with a conspecific. The FRjS, for instance, was emitted by females to reject a male after physical contact or during male signalling, a behaviour also observed in the stink bug *Nezara viridula* (Čokl et al. 2000) and in the leafhopper *Scaphoideus titanus* (Mazzoni et al. 2009a). Because MMS consisted of an exchange of signals between interacting males, its biological meaning could be of dominance, interference towards other males, expression of distress, or defining territory. In this regard, rivalry signals are present in the repertoire of many Auchenorrhyncha and their function is to disturb or mask male–female duets (Booij 1982). For example, in the treehopper *Ennya chrisura*

(Hemiptera: Membracidae) a rival signal is emitted by both competitors when in close proximity (Miranda 2006), while in *S. titanus* males can emit a disturbance noise that aims at masking the female reply (Mazzoni et al. 2009a). Further trials are therefore needed to unveil the role of this signal in male-male interactions. The biological meaning of the MCS remained unclear as well. This signal was recorded in almost all the recording periods throughout the season, but it neither elicited the emission of a FCS nor took part in the duet. We excluded that the purpose was to chase away a rival male because the playback of the MCS did not have a repellent effect on the tested males. Despite having spectral characteristics similar to FCS, a potential role of female mimicry was also excluded due to a different temporal pattern. Thus, while the FCS is clearly associated with mating and female receptivity, the MCS could be a territoriality signal that advertises the sender in terms of actual presence and, possibly, quality. In *H. obsoletus*, the male emits a calling signal with temporal and spectral features similar to the female signal (Mazzoni et al. 2010), but the behavioural meaning for this signal remains unclear. However, the MCS of *P. spumarius* (when composed of chirps and pulses) partially resembles the MCrS, therefore it could be either a form of “preparatory” courtship signal or potentially trigger the emission of a FCS from a distant female. Interestingly, the presence of a silent female was sufficient to trigger emission of MCrS in trio trials. Finally, the vibrational signals emitted during copulations could either prevent female movements or induce oviposition (Eberhard 1991). Another interesting hypothesis is that copulatory signals allow females to evaluate the mate after copulation has begun and choose whether use the sperm from successive males. This behaviour has been described as cryptic female choice by Eberhard (1991) and seems to be widespread in insects and likely relevant in the evolution of genitalia as well (Eberhard 1991).

5. Conclusions and perspectives

To conclude, *P. spumarius* communication is characterized by emission of vibrational signals, which have specific roles within the mating behaviour. The sexual behaviour of *P. spumarius* shares similarities with several aspects of the stereotyped scheme known for other Auchenorrhyncha (Mazzoni et al. 2009b; De Groot et al. 2012) although many traits resemble more planthoppers (Fulgoromorpha) and sharpshooters (Cicadomorpha: Cicadellidae: Cicadellinae) than leafhoppers (Cicadomorpha: Cicadellidae other subfamilies). In particular, when individually placed on plants both males and females emitted spontaneous calling signals, like in many planthoppers (Ichikawa and Ishii 1974; Booij 1982; Virant-Doberlet and Žežlina 2007; Mazzoni et al. 2010) and in sharpshooters (Nieri et al. 2017), whereas in most leafhoppers (i.e., subfamilies Typhlocybinae and Deltocephalinae) only males call spontaneously while females emit replying signals (i.e. Mazzoni et al. 2009b; Derlink et al. 2014; Nieri and Mazzoni 2018). However, if the continuous emission of the MCS increase the interest of a not completely sexually mature female, the calling signals of *P. spumarius* would resemble the one described in cicadas and leafhoppers (Sueur 2002; Čokl and Virant-Doberlet 2003; Mazzoni et al. 2014). Nonetheless, *P. spumarius* females emitted FCSs from different positions of the same plant, behaviour observed also in *H. obsoletus* (Mazzoni et al. 2010). The similarities and differences in the behavioural pattern, however, could be a result of an ecological convergence rather than phylogeny divergence between these species. In fact, it is important to remember that *P. spumarius*, as well as *H. obsoletus* and *H. vitripennis*, is polyphagous and has an aggregated population structure.

Eventually, we consider possible the manipulation of *P. spumarius* by means of artificial playbacks and thus, future development of low environmental impact control practices. However, further research is needed to identify an efficient signal and the most suitable strategy for application in the field. One interesting approach could be to attract males into a trap using a specific vibrational signal, preferably before the adults move from the herbaceous vegetation to the olive tree canopy (Cornara et al. 2016). Since mating is achieved by exchange of signals between partners, it would be difficult to use the female response signal (FRsS) as directional cue to guide male into a trap. Nonetheless, in *Halyomorpha halys* the female calling signal was used with good efficacy even if male and female use to duet during the pair formation process (Mazzoni et al. 2017b). In this species, the male signal could increase the female motivation and induce acceptance; however, particularly motivated females likely do not require special "attention" and therefore males could reach them without emitting any signal, like in the case of some Apoidea species (Eickwort and Ginsberg 1980). For this reason, it is worthy to test a *P. spumarius* female playback to lead nearby males into a trap, while another option is to use a pre-recorded male-female duet as attractive signal, exploiting a potential "satellite behaviour", which was described in *S. titanus*, where the male could silently localize a duetting female (Mazzoni et al. 2009a; Virant-Doberlet et al. 2014). Finally, further experiments should be conducted to investigate the role of MMS and MCS and, indeed, playback trials should assess if the continuous transmission of a specific signal could disrupt an ongoing duet or cause repellence/stress in individuals that eventually would tend to leave the plants.

References

Alexander RD (1967) Acoustical communication in arthropods. Annu Rev Entomol 12:495–526

Anderson, MJ (2008) A new method for non-parametric multivariate analysis of variance https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Bailey WJ (2003) Insect duets: underlying mechanisms and their evolution. *Physiol Entomol* 28:157–174

Bailey WJ, Macleay C, Gordon T (2006) Acoustic mimicry and disruptive alternative calling tactics in an Australian bushcricket (Caedicia; Phaneropterinae; Tettigoniidae; Orthoptera): does mating influence male calling tactic? Physiol Entomol 31:201–210

Bodino N, Cavalieri V, Dongiovanni C, et al (2019) Phenology, seasonal abundance and stage-structure of spittlebug (Hemiptera: Aphrophoridae) populations in olive groves in Italy. Sci Rep 9:1–17. https://doi.org/10.1038/s41598-019-54279-8

Booij CJH (1982) Biosystematics of the Muellerianella complex (Homoptera, Delphacidae), interspecific and geographic variation in acoustic behaviour. Z Tierpsychol 58:31–52

Broughton WB (1963) Method in bioacoustic terminology. In: Acoustic behaviour of animals. R. G. Busnel (eds.) Elsevier Publishing, Amsterdam, London, New York. pp. 324

Chmiel SM, Wilson MC (1979) Estimation of the lower and upper developmental threshold temperatures and duration of the nymphal stages of the meadow spittlebug, *Philaenus spumarius*. Environmental Entomology 8.4: 682-685

Cocroft RB, Rodriguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323–334

Čokl A, Virant-Doberlet M (2003) Communication with substrate-borne signals in small plant-dwelling insects. Annu Rev Entomol 48(1):29-50

Čokl A, Virant-Doberlet M, Stritih N (2000) The structure and function of songs emitted by southern green stink bugs from Brazil, Florida, Italy and Slovenia. Physiological Entomology 25:196–205

Cornara D, Bosco D, Fereres A (2018a) *Philaenus spumarius*: when an old acquaintance becomes a new threat to European agriculture. Journal of pest science 91(3):957-72

Cornara D, Garzo E, Morente M, et al (2018b) EPG combined with micro-CT and video recording reveals new insights on the feeding behavior of *Philaenus spumarius*. PLoS One 13:1–20. https://doi.org/10.1371/journal.pone.0199154

Cornara D, Morente M, Markheiser A, et al (2019a) An overview on the worldwide vectors of *Xylella fastidiosa.* Entomol Gen 39:157–181. https://doi.org/10.1127/entomologia/2019/0811

Cornara D, Ripamonti M, Morente M, et al (2019b) Artificial diet delivery system for *Philaenus spumarius*, the European vector of *Xylella fastidiosa*. J Appl Entomol 143:882–892. https://doi.org/10.1111/jen.12655

Cornara D, Sicard A, Zeilinger AR, Porcelli F, Purcell AH, Almeida, RPP (2016) Transmission of *Xylella fastidiosa* to grapevine by the meadow spittlebug. Phytopathology 106(11):1285-1290

De Groot M, Derlink M, Pavlovčič P, Prešern J, Čokl A, Virant-Doberlet M. (2012) Duetting behaviour in the leafhopper *Aphrodes makarovi* (Hemiptera: Cicadellidae). J Insect Behav 25:419–440

Denancé N, Legendre B, Briand M, Olivier V, De Boisseson C, Poliakoff F, Jacques MA (2017) Several subspecies and sequence types are associated with the emergence of *Xylella fastidiosa* in natural settings in France. Plant Pathology 66(7):1054-1064

Derlink M, Pavlovčič P, Stewart AJ, Virant-Doberlet M (2014) Mate recognition in duetting species: the role of male and female vibrational signals. Animal behaviour 90:181-193

Eberhard WG (1991) Copulatory courtship and cryptic female choice in insects. Biol Rev Camb Philos Soc 66:1–31. https://doi.org/10.1111/j.1469-185x.1991.tb01133.x

Eickwort GC, Ginsberg HS (1980) Foraging and mating behavior in Apoidea. Annual review of entomology 25(1):421-446

Endler JA (1992) Signals, signal conditions, and the direction of evolution. The American Naturalist 139:125-153.

Eriksson A, Anfora G, Lucchi A, Lanzo F, Virant-Doberlet M, Mazzoni V (2012) Exploitation of insect vibrational signals reveals a new method of pest management. PLoS ONE 7:e32954

Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annual review of entomology 42(1):123-146

Gordon SD, Sandoval N, Mazzoni V, Krugner R (2017) Mating interference of glassy-winged sharpshooters, *Homalodisca vitripennis.* Entomol Exp Appl 164:27–34

Gwynne DT (1987) Sex-biased predation and the risky mate-locating behaviour of female tick-tock cicadas (Homoptera: Cicadidae). Animal Behaviour 35:571–576

Halkka O and Halkka L (1990) Population genetics of the polymorphic meadow spittlebug, *Philaenus spumarius* (L.). Evolutionary biology (USA) 24:149–91

Halkka O, Halkka L, Raatikainen M, Hovinen R. (1973) The genetic basis of balanced polymorphism in *Philaenus* (Homoptera). Hereditas 74(1):69-80

Hartman E, Rohde B, Lujo S, Dixon M, McNeill S, Mankin RW (2017) Behavioral responses of male *Diaphorina citri* (Hemiptera: Liviidae) to mating communication signals from vibration traps in citrus (Sapindales: Rutaceae) trees. Florida Entomologist 100(4):767-771.

Heady SE, Nault LR, Shambaugh GF, Fairchild L (1986) Acoustic and mating behavior of *Dalbulus* leaf hoppers (Homoptera: Cicadellidae*).* Ann Entomol Soc Am 79:727–736

Hoch H, Howarth FG (1993) Evolutionary dynamics of behavioral divergence among populations of the Hawaiian cave-dwelling planthopper *Oliarus polyphemus* (Homoptera: Fulgoroidea: Cixiidae). Pacific Science 47(4): 303-318

Ichikawa T, Ishii S (1974) Mating signal of the brown planthopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae): vibration of the substrate. Appl Entomol Zool 9:196–198

Krugner R (2010) Differential reproductive maturity between geographically separated populations of *Homalodisca vitripennis* (Germar) in California. Crop Prot 29:1521–1528

Krugner R, Gordon SD (2018) Mating disruption of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) by playback of vibrational signals in vineyard trellis. Pest Manag. Sci. 74:2013-2019.

Kuhelj A, De Groot M, Pajk F, Simčič T, Virant-Doberlet M (2015) Energetic cost of vibrational signalling in a leafhopper. Behavioral Ecology and Sociobiology 69(5):815-828

Markheiser A, Cornara D, Fereres A, Maixner M (2020) Analysis of vector behavior as a tool to predict *Xylella fastidiosa* patterns of spread. Entomol Gen 40: 1-13

Maryańska-Nadachowska A, Kajtoch Ł, Lachowska D (2012) Genetic diversity of *Philaenus spumarius* and *P. tesselatus* (Hemiptera, Aphrophoridae): implications for evolution and taxonomy. Systematic Entomology 37(1):55-64

Mazzoni V, Eriksson A, Anfora G, Lucchi A, Virant-Doberlet M (2014) Active space and the role of amplitude in plant-borne vibrational communication. In Studying vibrational communication. Springer, New York. pp. 125-145

Mazzoni V, Gordon SD, Nieri R, Krugner R (2017a) Design of a candidate vibrational signal for mating disruption against the glassy-winged sharpshooter, *Homalodisca vitripennis*. Pest Manag. Sci. 73:2328-2333

Mazzoni V, Lucchi A, Čokl A, Prešern J, Virant-Doberlet M (2009a) Disruption of the reproductive behaviour of *Scaphoideus titanus* by playback of vibrational signals. Entomol Exp Appl 133:174–185

Mazzoni V, Lucchi A, Ioriatti C, Virant-Doberlet M, Anfora A (2010) Mating behavior of *Hyalesthes obsoletus* (Hemiptera: Cixiidae). Ann Entomol Soc Am 103:813–822

Mazzoni V, Polajnar J, Baldini M, Rossi Stacconi MV, Anfora G, Guidetti R et al, (2017b) Use of substrate-borne vibrational signals to attract the Brown Marmorated Stink Bug, *Halyomorpha halys*. J Pest Sci 90:1–11

Mazzoni V, Prešern J, Lucchi A, Virant-Doberlet M (2009b) Reproductive strategy of the Nearctic leafhopper *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae). Bull Entomol Res 99:401–413

Michelsen A, Fink F, Gogala M et al. (1982) Plants as transmission channels for insect vibrational songs. Behav Ecol Sociobiol 11, 269–281 https://doi.org/10.1007/BF00299304

Miranda X (2006) Substrate-borne signal repertoire and courtship jamming by adults of *Ennya chrysura* (Hemiptera: Membracidae). Ann Entomol Soc Am 99:374–386

Morente M, Cornara D, Moreno A, Fereres A (2018) Continuous indoor rearing of *Philaenus spumarius*, the main European vector of *Xylella fastidiosa*. J Appl Entomol 142:901–904. https://doi.org/10.1111/jen.12553

Nieri R, Mazzoni V (2018) The reproductive strategy and the vibrational duet of the leafhopper *Empoasca vitis.* Insect science 25(5):869-882

Nieri R, Mazzoni V, Gordon SD, Krugner R (2017) Mating behavior and vibrational mimicry in the glassy-winged sharpshooter, *Homalodisca vitripennis.* Journal of Pest Science 90(3):887-899

Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H (2019) vegan: Community Ecology Package. R package version 2.5-5. https://CRAN.R-project.org/package=vegan

Olmo D, Nieto A, Adrover F, Urbano A, Beidas O, Juan A, Marco-Noales E, López MM, Navarro I, Monterde A, Montes-Borrego M (2017) First Detection of *Xylella fastidiosa* Infecting Cherry (*Prunus avium*) and *Polygala myrtifolia* plants, in Mallorca Island, Spain. Plant Disease 101(10):1820-1820

Pedigo LP, ME Rice (2014) Entomology and pest management, 6th edn. Waveland, Long Grove

Pertot I, Caffi T, Rossi V, Mugnai L, Hoffmann C, Grando MS, Gary C, Lafond D, Duso C, Thiery D, Mazzoni V (2017) A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Protection 97:70-84

Polajnar J, Eriksson A, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2015) Manipulating behaviour with substrate-borne vibrations – Potential for insect pest control. Pest Management Science 71:15–23

Polajnar J, Eriksson A, Rossi Stacconi MV, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2014) The process of pair formation mediated by substrate-borne vibrations in a small insect. Behav Process 107:68–78

Polajnar J, Eriksson A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. Journal of pest science 89(4):909-921

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Robertson A, Gibbs AJ (1937) Spermatogenesis and fertilization in *Philaenus spumarius* Fallen*.* Journal of Tropical Medicine and Hygiene 40:257-262

Rodrigues AS, Silva SE, Marabuto E, Silva DN, Wilson MR, Thompson V, Yurtsever S, Halkka A, Borges PA, Quartau JA and Paulo OS (2014) New mitochondrial and nuclear evidences support recent demographic expansion and an atypical phylogeographic pattern in the spittlebug *Philaenus spumarius* (Hemiptera, Aphrophoridae). PLoS One, 9(6):p.e98375

Santoiemma G, Tamburini G, Sanna F, et al (2019) Landscape composition predicts the distribution of *Philaenus spumarius*, vector of *Xylella fastidiosa*, in olive groves. J Pest Sci 92:1101–1109. https://doi.org/10.1007/s10340-019-01095-8

Saponari M, Boscia D, Altamura G, et al (2017) Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. Sci Rep 7:1–13. https://doi.org/10.1038/s41598-017-17957-z

Saponari M, Loconsole G, Cornara D, Yokomi RK, De Stradis A, Boscia D, Bosco D, Martelli GP, Krugner, Porcelli F (2014) Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy. Journal of economic entomology 107(4):1316-1319

Soetaert K (2017) plot3D: Plotting Multi-Dimensional Data. R package version 1.1.1.https://CRAN.R-project.org/package=plot3D

Stewart AJ, Lees DR (1996) The colour/pattern polymorphism of *Philaenus spumarius* (L.) (Homoptera: Cercopidae) in England and Wales. Phil. Trans. R. Soc. Lond. B 351(1335):69-89

Sueur J (2002) Cicada acoustic communication: potential sound partitioning in a multispecies community from Mexico (Hemiptera: Cicadomorpha: Cicadidae). Biological Journal of the Linnean Society 75(3):379-394

Tishechkin DY (2003) Vibrational communication in Cercopoidea and Fulgoroidea (Homoptera: Cicadina) with notes on classification of higher taxa. Russian Entomological Journal, 12(2):127-181

Virant-Doberlet M, Žežlina I (2007) Vibrational communication of *Metcalfa pruinosa* (Hemiptera: Fulgoroidea: Flatidae). Annals of the Entomological Society of America 100(1):73-82

Virant-Doberlet M et al. (2014) Vibrational Communication Networks: Eavesdropping and Biotic Noise. In: Cocroft R., Gogala M., Hill P., Wessel A. (eds) Studying Vibrational Communication. Animal Signals and Communication, vol 3. Springer, Berlin, Heidelberg

Weaver CR, King D.R (1954) Meadow spittlebug, *Philaenus leucophthalmus* (L.). Research Bulletin 741

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Wickham H (2017) tidyverse: Easily Install and Load the 'Tidyverse'. R package version 1.2.1. https://CRAN.R-project.org/package=tidyverse

Witsack W (1973) Experimental and ecological investigations on forms of dormancy in homoptera-cicadina (Auchenorrhyncha). On ovarian parapause and obligatory embryonic diapause in *Philaenus spumarius* (L.) (Aphrophoridae) Zoologische Jahrbücher: Abteilung für Systematik, Okologie und Geographie der Tiere 100:517–562

Yurtsever S (2001) Multiple paternity in the meadow spittlebug *Philaenus spumarius* (L.) (Homoptera: Cercopidae). Acta zoologica cracoviensia 44(3):173-84

Zaar JH (1999) Biostatistical analysis pp. 663

Chapter 3

Vibrational playbacks and microscopy to study the signalling behaviour and female physiology of *Philaenus spumarius*

Sabina Avosani, Pietro Franceschi, Marco Ciolli, Vincenzo Verrastro and Valerio Mazzoni

DOI: 10.1111/jen.12874

Abstract

The meadow spittlebug *Philaenus spumarius*, vector of the bacterium *Xylella fastidiosa*, relies on vibrational communication to accomplish mating: the female calls to establish a duet with a male. A deeper knowledge of the species' reproductive biology and behaviour would provide useful information for developing control techniques based on principles of "biotremology", which studies the vibrational behaviour of animals. Playback tests were conducted on single females and male-female pairs of *P. spumarius* from June to October 2018, and the features of the recorded calling signals were analysed using a wavelet decomposition. Dissections were performed on females to evaluate the relationship between calling activity and ovarioles development. From August onwards, females started to emit calling signals and to develop ovarioles. Female calling activity, duration of their chirps and their responsiveness to mating increased as the season progressed, and they were correlated with ovarioles' development and presence of mature eggs. Hence, the ovarian maturation represents a key factor in association with the development of the sexual behaviour of *P. spumarius* females. Conversely, males produced advertisement signals soon after adult eclosion in May, but these signals were not involved in the pair formation process. Mating was achieved only when males produced courtship signals in response to female calling signals and established with them vibrational duets starting from August. Here, we provide new information regarding the *P. spumarius'* ethology and hypothesize that potential mating disruption techniques should consider the insect physiology and be applied when both sexes are responsive to mating signals.

Keywords: meadow spittlebug, biotremology, female calling behaviour, ovarian development, laser vibrometer, playbacks

1. Introduction

In animals, mating depends on successive phases and reciprocal interactions between the male and the female (Beach 1976). The first step is the emission of a calling signal by either the male or the female that could evoke a sexual response by a potential mate (Endler 1993). The nature of the signals (i.e., chemical, acoustic, visual, or substrate-borne) varies according to the species, and often multiple signal modalities are involved in sexual communication (Higham and Hebets 2013). Vibrational communication is probably the most common of these modalities in arthropods, with more than 200,000 insect species that use vibrational signals during pair formation process (Hill 2001; Virant-Doberlet and Cokl 2004). In this regard, it is rather common that mating is achieved if both partners maintain the vibrational interaction during the whole pair formation process. Female receptivity and motivation to respond to the male's stimulation are therefore crucial in determining male mating success (Beach 1976).

In many insects, the sexual receptivity of females is influenced by a set of both internal and external conditions and, among these, the development of the reproductive system is a key factor affecting the female receptivity and signalling activity (Engelmann 1970; Ringo 1996). The development and growth of the oocytes occurs in the ovarioles, which indeed play a main role in female behaviour (Klowden 2013). For instance, maturation of eggs can trigger the female sexual interest, as reported for the leafhopper *Empoasca devastans* (Hemiptera: Cicadellidae), which becomes receptive to mating only if the ovarioles are partially mature, whilst immature females reject courting males (Kumar and Saxena 1978). The period between female emergence as adult and acceptance to mate can vary, according to the species, from zero (i.e., immediate readiness to mate) to many months, when depending on specific growth factors such as food intake (Minelli et al. 2006). In this regard, immature females tend to show a rejective behaviour that can be expressed as simple refusal to mate or as intimidating behaviour such as the emission of stress signals (Avosani et al. 2020).

Rejective behaviour has been observed in the meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae), whose mating behaviour mostly relies on substrate-borne vibrations (Avosani et al. 2020). *P. spumarius* is an univoltine insect species and can be found in both the Palearctic and Nearctic regions, as well as most of the temperate areas of earth and oceanic islands (Cornara et al. 2018). Although this species has not been considered a pest in Europe, it recently became a serious threat for European agriculture, as the main vector of the bacterium *Xylella fastidiosa* from olive to olive and to other plants (Martelli et al. 2016; Saponari et al. 2017; Cornara et al. 2018). After the first outbreak in Southern Italy, different strains of *X. fastidiosa* were found in other Mediterranean countries (EPPO 2018; Sicard et al. 2018). As a consequence, research is underway to develop sustainable pest management strategies against its vector (Martelli et al. 2016; Cornara et al. 2017, 2018). In most of the Palearctic area, *P. spumarius* females emerge as adults in late spring (April-May), but they start to emit calling signals only at the end of the summer, while prior to this period, only males are acoustically active (Witsack 1973; Avosani et al. 2020). In late summer, pair formation starts with the female calling signal, which is followed by the male courtship signal, which is a long and complex signal consisting of two sections that are alternatively emitted. The duet is crucial for partner localization (i.e., males search for the females, which remain stationary on the plant) and a vibrational duet is established only if the female alternates her response signals with the male courtship signal. When males happen to be in close proximity to females earlier in the season, they try to mate, and copulations are observed in the field and in the laboratory (Cornara et al. 2018;

Avosani et al. 2020). However, these early matings in the spring appear imposed by the males, since immature females, at this time of the year, produce a specific rejection signal or try to escape (Avosani et al. 2020).

In *P. spumarius,* egg maturation starts from August and oviposition from September (Weaver and King 1954; Cornara et al. 2018; Morente et al. 2018), but the relationship between ovarian development and receptivity to mating (in terms of emission of vibrational signals to duet with males) of *P. spumarius* females has never been investigated. Increasing our knowledge in this respect would contribute to a better comprehension of the species phenology and of the potential to apply biotremology techniques to control this pest. This emerging field of discipline aims at using vibrational signals to modify the behaviour of insect pests, as shown in the field against the grapevine leafhopper *Scaphoideus titanus* (Hemiptera: Cicadellidae), and in the lab against other two species, *Empoasca vitis* (Hemiptera: Cicadellidae) and *Homalodisca vitripennis* (Hemiptera: Cicadellidae) (Mazzoni et al. 2009; Gordon and Krugner 2019; Nieri and Mazzoni 2019).

The main scope of this study is to assess whether the calling activity and responsiveness of females are strictly correlated to the ovarian development. In addition, by evaluating both the interactions between tested males and females and their responses to a pre-recorded male signal playback, we aim at providing further insights regarding the intra-specific communication of *P. spumarius.*

2. Materials and methods

2.1. Insect collection and rearing

Fourth and fifth instars nymphs of *P. spumarius* were collected between May and June 2018 from meadows in Riva Del Garda and San Michele all'Adige (Trentino, Italy). The collected individuals were transported to a glasshouse at the Fondazione Edmund Mach and reared in mesh cages, following the rearing protocol reported by Avosani et al. (2020). The rearing was checked every day to isolate and separate by gender the newly emerged adults, which were reared in two separate cages placed outdoors to mimic natural conditions (i.e., photoperiod, temperature, and humidity). The last adult emergence occurred at the end of June, while the adult rearing lasted from May until the end of the trials, in October.

Each cage was constituted of a cubic iron frame, with five surfaces (three lateral sides, pavement, and ceiling) covered with nylon mesh to permit air ventilation, while one side consisted of a Plexiglass door with a hole (30 cm) in the centre. A plastic cylinder (diameter 30 cm) was screwed into the hole and closed by a nylon mesh to permit both manipulation of the insects and plant watering. Wooden boxes covered by a dark plastic fabric were put on the top side of the cages to protect the rearing from rainfall and excessive sunlight. A plastic board was placed on the bottom side of the cube and pierced with a screw in numerous points to allow water to runoff. Insects were removed at the day of the trial using a mouth aspirator and transferred to the Biotremology laboratory inside 150 ml centrifuge tubes.

2.2. Recordings

Recordings were conducted in the Biotremology laboratory at Fondazione Edmund Mach (Italy), inside a sound insulated chamber maintained at a temperature of 22 ± 1 °C and 65% RH. The experimental arena was a rectangular Plexiglas cage (50x30x30 cm) with a circular opening on the top (diameter 6 cm) covered by a net, which was placed on an anti-vibrational table (Astel s.a.s., Ivrea, Italy). For acclimation, the insects were left inside the centrifuge tubes for at least 15 minutes. Vibrations were recorded from small pieces of reflective tape using two laser Doppler vibrometers (Ometron VQ-500-D-V and Polytec PDV 100) pointed, respectively, on the apical and the basal leaves of a potted grapevine plant (height: 20 cm) placed into the cage. Signals were digitized with the software Pulse 21 (Brüel and Kjær Sound & Vibration A/S, Nærum, 104 Denmark) at a 44.1 kHz sample rate and 16-bit depth resolution through a data acquisition device (LAN XI type 3050-B-040, Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark), then stored onto a hard drive of a computer (HP, EliteBook 8460 p). All trials were recorded with a video-camera (mod. HTC-TM700, Panasonic, Japan) to associate the emission of vibrational signals to the corresponding behaviour.

2.3. Playback trials

To evaluate when the female becomes receptive to mating, we performed playback trials on single females. We also tested pairs consisting of a male and a female to assess if the physical presence of a male could influence the female calling activity and/or her responsiveness to the playback. The latter was a 2.7 min pre-recorded male courtship signal (Avosani et al. 2020) and was transmitted to the plant by means of a mini shaker (Type 4810; Brüel and Kjær Sound & Vibration A/S, Nærum, Denmark) in direct contact with a leaf via a conical rod. The male courtship signal was modified using the audio software Adobe Audition 3.0 (Adobe Systems, Inc., San Jose, CA, USA). The background noise was removed, while the signal's spectral features were adjusted to the insect's natural amplitude and frequency spectrum (Avosani et al. 2020). To ascertain that the playback could elicit the emission of female calling signals and/or female response signals (Avosani et al., 2020), the playback was previously tested on single females in October 2017, while test 1 was conducted from May to October 2018.

For test 1, each month was split into two halves to obtain 10 periods (Table 3.1). Twenty females were tested per time period, resulting in a total of 200 tested females. Pairs consisting of a female and a male (n = 15) were tested from the 2nd to the 9th time period (135 tested pairs in total). After 15 minutes of silence waiting for the spontaneous emission of a female calling signal, the male courtship signal was played once, and the recording was stopped 5 minutes after the end of the playback. In the case of a calling female, the male courtship signal was played 1 minute after the end of the first female calling signal, to prevent the female from leaving the plant or a potential decrease of her interest in mating. In the pair trials, if the female and the male established a vibrational duet, 15 minutes were provided to the male from the onset of the duet to find the replying female.

Table 3.1. Periods and corresponding dates for both females tested singly and females tested in pairs with males.

Period	Dates	n of females tested singly	n of females tested in pairs with a male
1	23-31 May	20	0
2	8-15 June	20	15
3	22-30 June	20	15
4	8-16 July	20	15
5	21-27 July	20	15
6	02-13 August	20	15
7	20-27 August	20	15

8	2-13 September	20	15
9	17-24 September	20	15
10	1-5 October	20	0

2.3.1. Analysed parameters: female behaviour

Per each time period, the following parameters were measured: the percentages of calling females (females that called spontaneously), the percentages of replying females (females that emitted a female calling signal or few random response signals following the playback), the number of duets (females that emitted regular response signals coordinated with the playback, thus establishing a duet) and the number of females that called after the playback. Similarly, in pair trials, the following parameters were measured: the percentages of calling females, replying females and the number of duets (females that established a duet with the playback and/or the male).

2.3.2. Analysed parameters: male behaviour

To better understand the sexual behaviour of *P. spumarius,* we measured the male-female interactions (i.e., number of females that rejected the male and number of matings). The male signals (i.e., type of signal emitted, occurrence of signals during the test) were analysed to investigate the male behaviour and the role of vibrational signals in response to the male courtship signal of the playback, which represented a potential mating rival. The male calling signal consisted of a series of elements, which could comprise an alternation of pulses (unitary homogeneous parcel of sound of finite duration (Alexander 1967) and chirps (continuous sound characterized by a fundamental frequency and a clear harmonic structure), or only chirps (Avosani et al. 2020).

2.4. Ovary morphological development

Per each time period, half (n = 10) of the females tested with the playback (n = 20) were transferred to the Entomology laboratory and dissected in order to measure the developmental stage of the ovarioles and the number of developing/fully developed chorionated oocytes (eggs). To kill the insects and preserve the integrity of the tissues, each specimen was enclosed into a centrifuge tube (150 ml) containing a small piece of cotton, which was soaked with 2-3 drops of ethyl acetate. The abdomen was opened, and the ovaries were extracted under a light microscope (Nikon SMZ800, Nikon Corporation, Tokyo, Japan) on a glass depression slide. A drop of phosphate buffered saline (10%) was used to maintain the tissues fresh and hydrated during the observation. Ovarioles' developmental stage was ranked as: immature = no oocyte development; under maturation = presence of developing and chorionated oocytes (eggs) in some ovarioles; mature = all ovarioles bear at least one chorionated oocyte. Per each time period, we counted the average number of eggs (mean ± SD) and the maximum number of eggs per dissected female.

2.5. Statistical analysis

The Spearman rank correlation (rs) was used to estimate the strength of correlations between the percentages of calling females and females with developing ovarioles. A Mann-Whitney U-test with Monte Carlo permutation was used to compare the percentages of calling, replying, and duetting females between the single females and pair groups. Statistical analyses and graphic design were done using R software (packages *ggpubr*, *ggplot2*, *tidyverse*, *dpylr*, *lattice*) (Grothendieck 2008; Wickham 2011, 2014; Team 2017; Wickham et al. 2019). To evaluate whether female motivation, in terms of calling activity, differs through the season, the calling latency (the time from insect release to the first calling signal) was compared between the different time periods in the single female group. For the comparison, a non-parametric Kruskal-Wallis test, Bonferroni corrected, and followed by a pairwise Mann-Whitney U-test as post hoc was performed using the software Past (Hammer et al. 2001).

A wavelet analysis was performed on the signals, since this approach allows a high temporal and spatial resolution by introducing a finite combination of scaling functions (Rach et al. 2013). To assess whether the features of the chirps of the calling signals produced by females and males change throughout the season, a collection of 10 chirps per calling signal was sampled and analysed using the *WaveletComp* R package (Rösch and Schmidbauer 2018). The chirps were collected from 57 female calling signals and 30 male calling signals. The step-by-step procedure can be found in Supporting Information.

3. Results

3.1. Playback trials

3.1.1. Analysed parameters: female behaviour

Single females emitted no spontaneous calling signal nor calling/response signal to the playback during the first four time periods (Figure 3.1A and 3.1B). Similarly, none of them established a duet with the male courtship signals of the playback (Figure 3.1C). Only one female, in the pair group, emitted a calling signal in the 5th period (Figure 3.1A). From the 6th time period, more females emitted calling signals spontaneously and/or in response to the playback (Figure 3.1A and 3.1B), mostly 1-2 seconds after the stimulus has started. Most of the females engaged a duet with the playback, regularly emitting response signals within the male courtship signal (Figure 3.1C). They also emitted calling signals when the playback ceased (Figure 3.1D). The number of females that called and interacted with the playback progressively increased through the season, reaching a peak in the 8th and 9th periods (Figure 3.1A and 3.1B). The Mann-Whitney U-test showed that the percentages of calling, replying, and duetting females for each of the periods were not statistically different between the single females and pair groups (Table 3.2). In the single female group, the calling latency was statistically different between the five periods in which we recorded female calls (Kruskal-Wallis test for equal medians; H (χ^2) = 8,7; p-value = 0.049). However, the post hoc test showed that the difference was significant only between the 6th and the 8th periods (Figure 3.2).

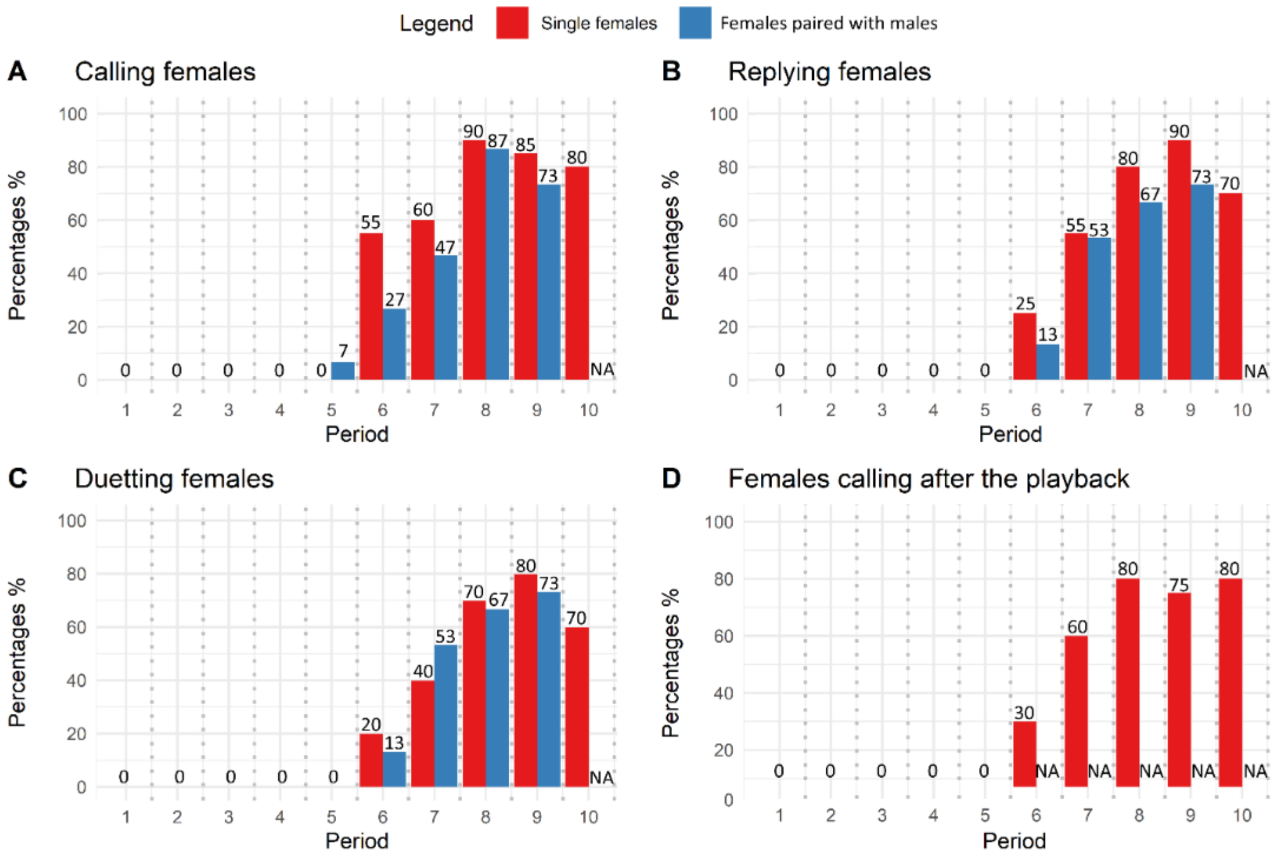

Figure 3.1. Percentages of calling, replying, and duetting females in the two tested groups. Per each period were tested: single females (n = 20) and females paired with males (n = 15). See Table 3.1 for definition of the periods.

Table 3.2. Comparison between the percentages of calling, replying, and duetting females between females tested singly and females tested in pairs with males. Mann-Whitney U-test, with Monte Carlo permutation for the comparison.

Percentages of	n females	U	p-value
Calling females	5	10	0.68
Replying females	5	9.5	0.60
Duetting females	5	11.5	0.92

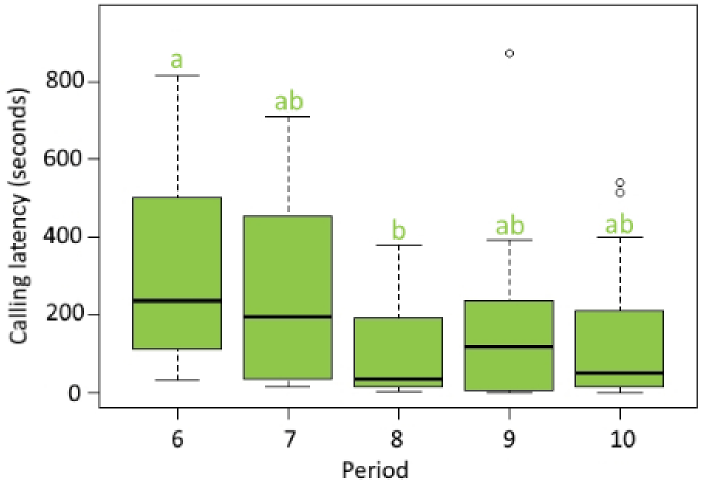

Figure 3.2. Post hoc test of the calling latencies between different periods in the singly tested females group. Different letters indicate statistically significant differences (Mann-Whitney, Bonferroni corrected, p = 0.04). See Table 3.1 for definition of the periods.

3.1.2. Analysed parameters: male behaviour

In contrast to females, males of *P. spumarius* emitted calling signals throughout all the tested periods. Some males (n = 10) produced calling signals spontaneously, before the transmission of the playback and in absence of any stimulus (Figure 3.3A). Nonetheless, male calling signals were emitted after physical interaction with a female (n = 5), as well. Some males produced calling signals in response to the playback (n = 23), and these signals were composed of only chirps (21.7%, n = 23) or pulses arranged with chirps (78.3%, n = 23) (Table 3.3). The male courtship signal was emitted by males only in response to female calling signals (64.3%, n = 15) or to the playback (35.7%, n = 15), and was recorded mostly in the last three time periods (Figure 3.3B). Both male calling and male courtship signals were produced during and after the transmission of the playback, while trains of pulses were emitted after the end of the stimulus (Table 3.3).

Some males established physical and vibrational interactions with the female, whose reply depended on her receptiveness to mating. In fact, in the early periods (Table 3.1), females remained silent, and two females replied to male calling signals with the emission of rejection signals. From the 6th period, females responded to male courtship signals of the playback and/or of the male with female response signals (Figure 3.1B). Eventually, females established vibrational duets with both the playback and the male (Figure 3.3D). Even in the presence of a calling female, some males did not emit a courtship signal and, consequently, did not establish a duet. The number of female calling signals exceeded the number of male courtship signals in the last four periods of the season (Figure 3.3B and 3.3C). Mating occurred starting from the 7th period (Figure 3E) and mostly required the establishment of the male-female duet (Figure 3.3D). Considering the total number of tested pairs through the season (n=121), nine out of twelve duetting pairs achieved mating, while three males did not find the female before the end of the test. Nonetheless, in the 8th period, a male silently found the female while she was duetting with the playback, while in the 3rd period (Figure 3.3E), a male silently approached and mounted a female, although she was neither calling nor responsive to the playback.

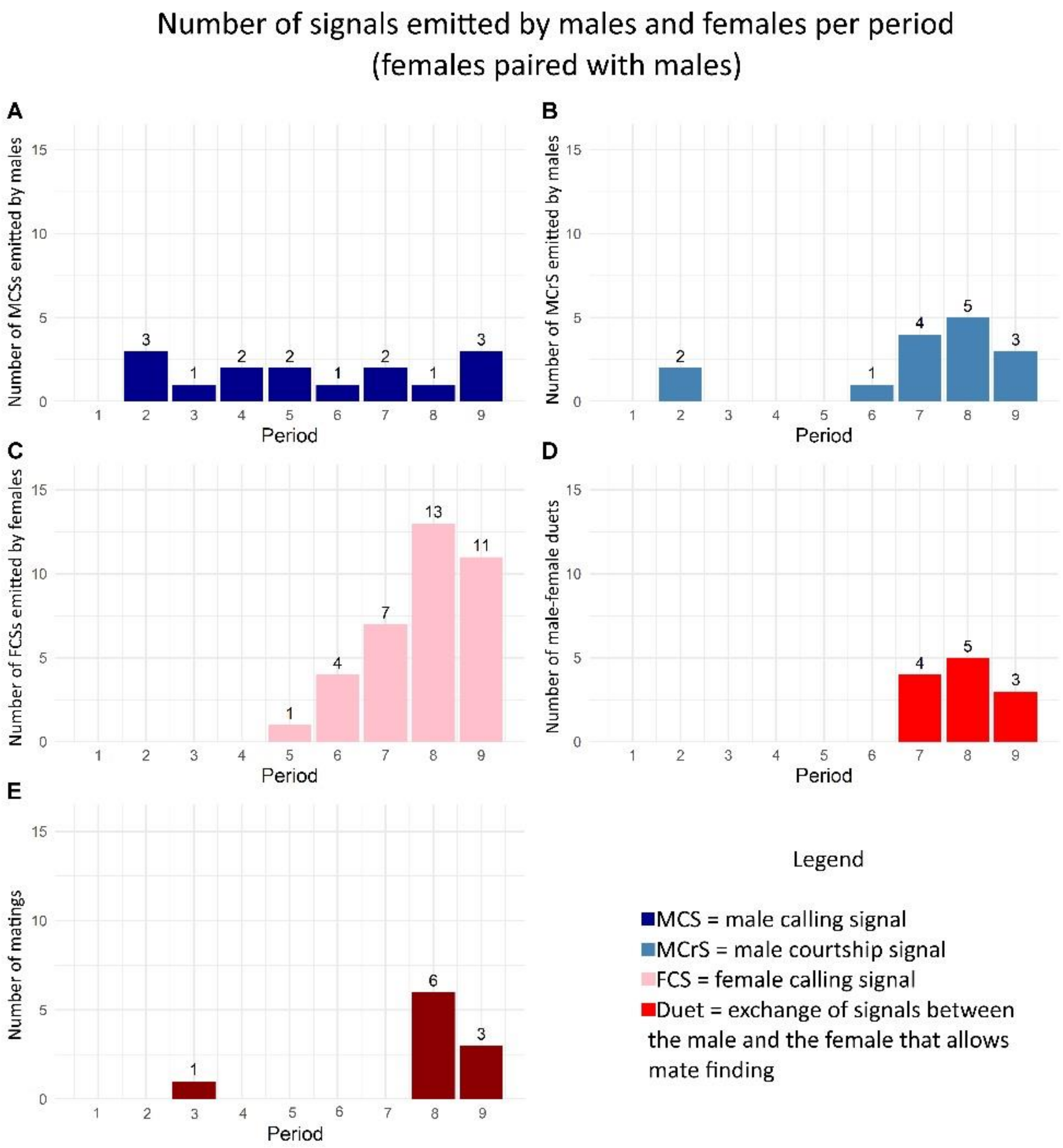

Figure 3.3. Signals emitted by *P. spumarius* males and females, and mating events observed for each of the tested periods (n = 15 pairs tested/period). See Table 3.1 for definition of the periods. The number above each bar indicates the number of signals or mating events recorded for each period, although the male calling signals elicited by the playback are not included in the figure.

Table 3.3. Type and number (n) of signals emitted by *Philaenus spumarius* males in response to the playback throughout the tested period (see Table 3.1). Signals occurred both during and after the transmission of the playback.

Type of response	n	Signals occurring during the playback	Signals occurring after the playback
Male courtship signal	5	5	4
Male calling signal (only chirps)	5	4	3
Male calling signal (pulses + chirps)	18	16	7
Train of pulses	3	0	3

3.2. Ovary morphological development

None of the females dissected in the first five periods carried developing oocytes in their ovarioles, which were completely immature (Figure 3.4A). Starting from the 6th period, the number of developing oocytes and chorionated eggs (Figure 3.4B) progressively increased, reaching a peak between the 8th and 9th periods (Figure 3.5A). The number of females that carried completely mature ovarioles (Figure 3.4C) was higher in the 9th period then in earlier periods (Figure 3.5A). Similar trends of females with immature, developing and mature ovarioles across the periods are shown in Figure 3.5A. Mature eggs were found in the female abdomen from the 6th period, and the number rapidly increased in the subsequent periods, reaching a peak in the 9th period (Table 3.4).

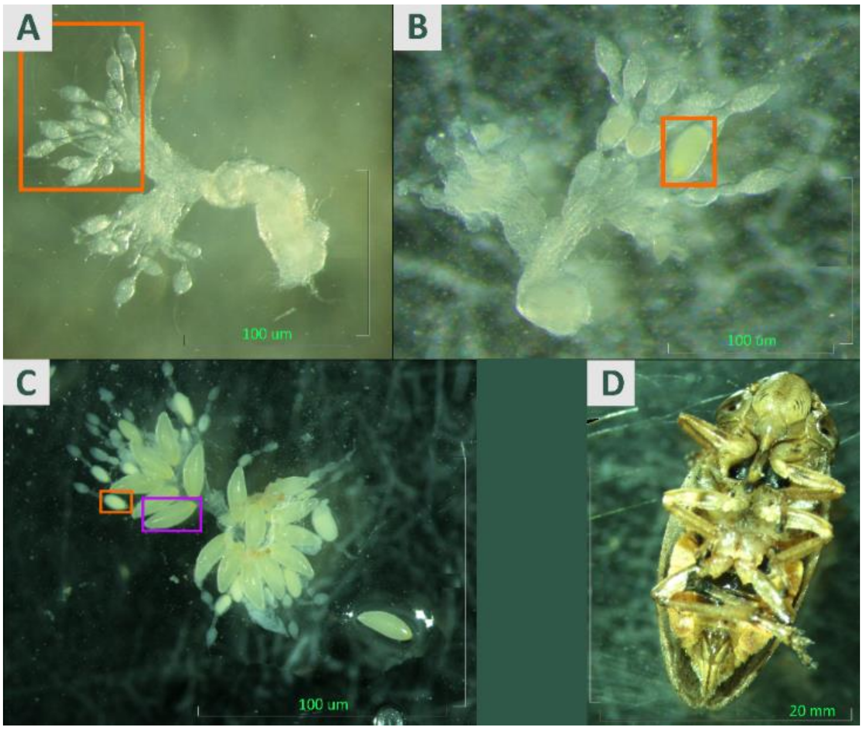

Figure 3.4. Panel showing the different stages of egg development in *P. spumarius*. A) Ovaries of a female tested in July; the orange square highlights the immature ovarioles of one of the ovaries. B) Ovaries of a female tested in August; the orange square indicates an oocyte that is developing inside an ovariole. C) Ovaries of a female tested in September; the orange square indicates an oocyte under development, while the purple square shows a chorionated oocyte (egg) inside the ovariole. These ovaries were ranked as ‘mature’ since every ovariole carried at least an egg. D) Female before being dissected, ventral side.

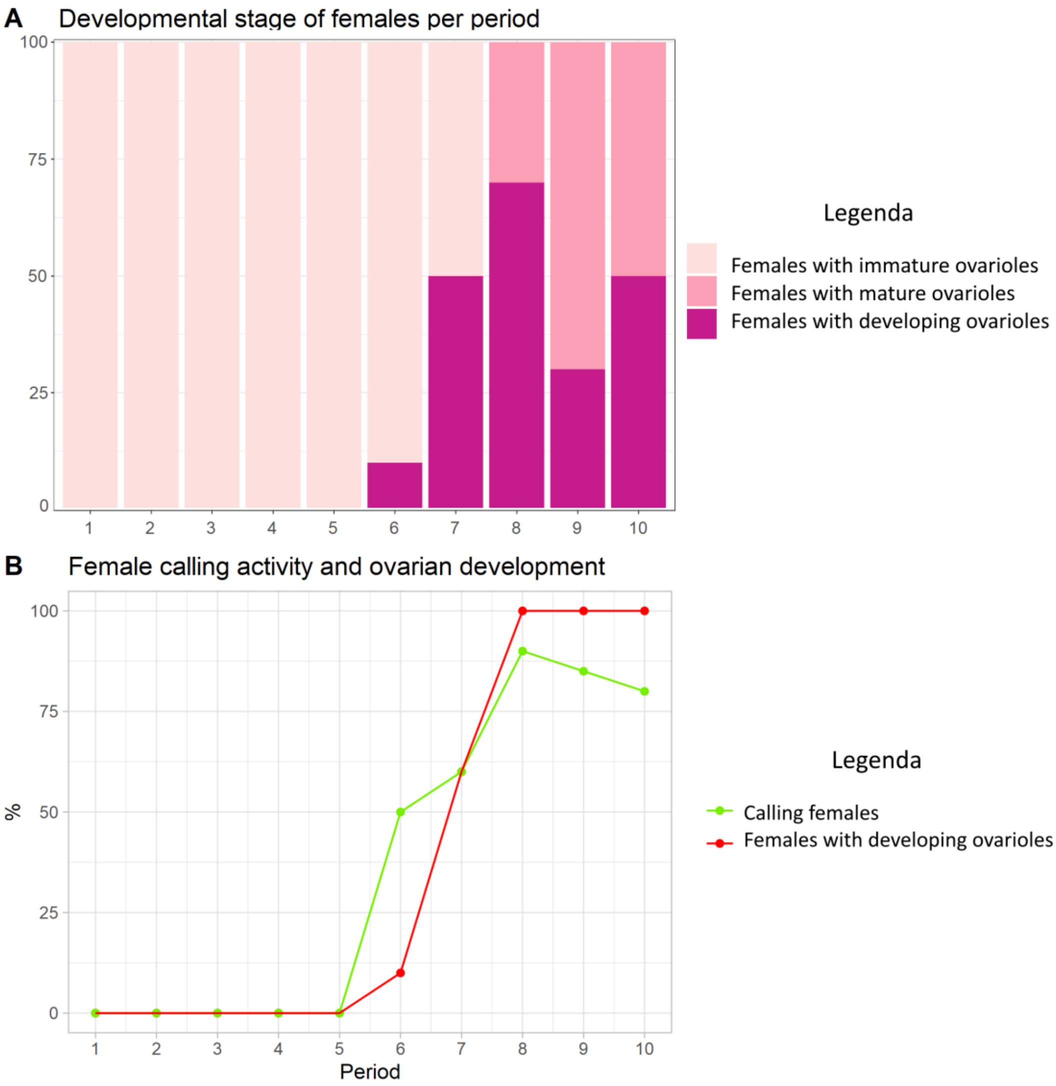

Figure 3.5. A) Developmental stage of the ovarioles of the females tested (n=10) for each period. Stacked chart of the percentage of females that carried either immature or under maturation or completely mature ovarioles. B) Percentages of calling females (green line) and females with developing ovarioles (red line). See Table 3.1 for definition of the periods.

Table 3.4. Mean ± standard deviation (SD) and the maximum number of eggs per female per time period (see Table 3.1).

Period	Number of tested females	Number of eggs (mean ± SD)	Maximum number of eggs per female
From 1 to 5	50	0	0
6	10	1.0	1
7	10	2.0 ± 0	2
8	10	9.7 ± 6.8	21
9	10	22.9 ± 9.2	41
10	10	18.1 ± 11.2	43

3.3. Synchrony between female developmental stages and calling activity

The Spearman rank correlation test showed that the percentage of calling females was correlated with the percentage of females whose ovarioles was under maturation (Spearman test; Statistic = 373, mean = 302.5, variance = 567.91, rho corrected = 0.99, p = 0.0008). A similar trend between the percentage of calling females and females with ovarioles under development across the periods is shown in Figure 3.5B. Both the percentages of calling females and females with ovarioles under development reach their peaks between the 8th and the 9th period (Figure 3.5B). Wavelet analysis demonstrated increasing female chirp duration as the season progressed, a trend not evident in males (Figure 3.6). The regression was statistically significant for females (Multiple R-squared = 0.2422, adjusted R-squared = 0.2409, F-statistic: 178.4 on 1 and 558 DF, p-value = 2.2e-16), but not significant for males (Multiple R-squared = 0.01808, adjusted R-squared = 0.008992, F-statistic: 1.989 on 1 and 108 DF, p-value: 0.1613).

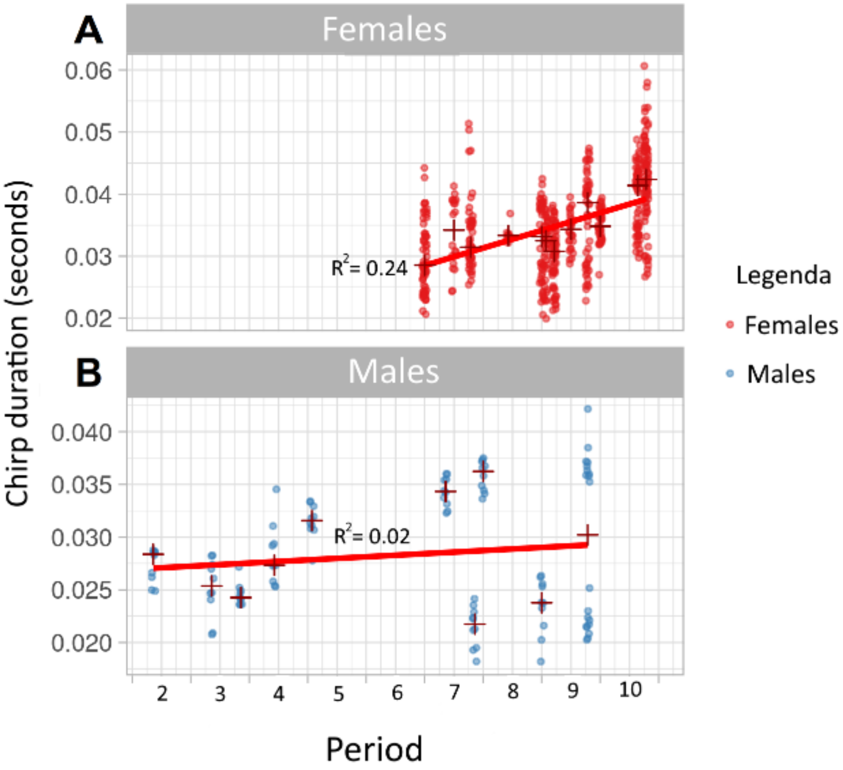

Figure 3.6. Comparison of the duration of the chirps of the female calling signal (A, above) and the male calling signal (B, below). The slope of the regression line suggests that the female chirps increase in duration along the season (A, above, p = 2.2e-16), while the same trend is not observed for males (B, below, p = 0.1613). The red crosses represent the median values. See Table 3.1 for definition of the periods.

4. Discussion

The present study demonstrated that, in the *P. spumarius,* female calling activity and receptivity to mating is associated with ovarian development and presence of mature eggs.

Prior to August, females did not have eggs or oocytes under maturation in their ovaries and did not emit calling signals. Some females produced calling signals in August, when they carried partially or fully mature oocytes, while the peak of calling females was reached at the end of September, when most of the females' ovarioles were completely developed and carried mature eggs. Similarly, calling latency (the time required to emit the first call) decreased from early August to September, suggesting a progressive interest of females in finding a mate. Nonetheless, males can achieve mating also at the beginning of the summer (Cornara et al. 2018). Forced matings are common among insects, and likely give a tactical advantage to males over females (Markow 2000; De Mello 2007; Vahed and Carron 2008). By silently approaching a female, a male could save energy and decrease the probability of detection by rivals and eavesdropping predators (Snedden and Sakaluk 1992; Virant-Doberlet et al. 2019). *Philaenus spumarius* females forced to mate likely store the sperm in their spermatheca even when there are no mature oocytes (Robertson and Gibbs 1937; Yurtsever 2001). However, from evaluating the distinctive colour patterns in crossing experiments, Yurtsever (2001) estimated that almost 90% of a *P. spumarius* female's offspring seems to be fathered from the last mating male. If the latter is a male chosen after the establishment of a vibrational duet, the female would use his sperm, while the sperm obtained after a previous coercive mating would be discarded. Molecular studies are needed to confirm this hypothesis. Besides sperm competition, sperm survival should be investigated to better understand the reproductive biology of *P. spumarius*. The spermatheca allows sperm storing, but not necessarily its long-term survival. In some insect species, the female reproductive tract can reduce the viability of the sperm (i.e., by storing the male contribution in multiple spermathecae or by means of spermicide (Hellriegel and Ward 1998; Greeff and Parker 2000). Similar strategies enable females to promote male competition (Bernasconi and Keller 2001), neutralize substances in the sperm that could affect female fitness (Andrés and Arnqvist 2001), or influence paternity (Greeff and Parker 2000). However, in honeybees, females can produce secretions aimed at increasing the sperm viability and storage in the spermatheca (Den Boer et al. 2009b). Similar strategies are common among other eusocial hymenopteran, where queens mate once and live for several years (Den Boer et al. 2009a), while likely rare among univoltine species such as *P. spumarius*. A deeper knowledge of female sperm usage and sperm survival in the spermatheca could suggest if mating disruption could be a worthy control strategy, given that techniques aimed at interfering with mate finding would scarcely be effective in suppressing *P. spumarius* populations, if the female uses the sperm acquired in spring. The presence of the male did not trigger the female calling behaviour, since the percentage of calling and duetting females was similar between the group of single females and pairs. This outcome confirms that a key factor determining female sexual behaviour and receptivity to mating in *P. spumarius* is the reproductive maturity of the ovarioles. A similar relationship between sexual behaviour and development of reproductive organs has been reported for many insect species with lapsed oviposition (Schal and Chiang 1995; Teal et al. 2000). At the end of the summer, *P. spumarius* females start to lay between 18 and 51 eggs each (Weaver and King 1954; Witsack 1973; Cornara et al. 2018; Morente et al. 2018). We found an average of 22.9 ± 9.2 eggs inside the female abdomen at the end of September, with a maximum of 41 eggs, while in earlier periods we found significantly fewer mature eggs. This delayed oocyte maturation likely allows the females to synchronize the development of all eggs, which are fertilized and then laid in the same period, when the conditions are more favourable for eggs' survival, i.e., less dry, and hot (Wiegert 1964; Maramorosch 2012). The coordination between the calling activity and the reproductive maturity could be therefore an adaptation that maximizes the probability of a successful fertilization, resulting in an increased female fitness. By delaying their calling signal to later in the season, females could also save energy (Teal et al. 2000). Vibrational signalling is energetically demanding, as demonstrated for the leafhopper *Aphrodes makarovi,* in which the number of emitted calling signals and the time of calling in early life negatively affect insect survival (Kuhelj et al. 2015). Besides energy expenditure, a delayed calling activity could decrease the probability of being detected by eavesdropping predators. Recent studies demonstrated that arthropod predators use the vibrational signals emitted by other insects to make foraging decisions and to locate potential preys (Virant-Doberlet et al. 2019).

Our study also demonstrated that the length of the chirps of the female calling signal increased as the season progressed, in contrast to males' calling signals. The longer chirps produced in October could be due to the presence of mature eggs, which likely caused an increased abdomen size compared to August and early September (author's personal observation). This hypothesis could also explain why the length of the male chirps did not change during the season. Alterations of the abdomen size could result in differences in the signal features, if the insect uses the muscles and/or other structures located in the abdomen to produce vibrations, as proposed for some planthoppers (Davranoglou et al. 2019). If the presence of mature eggs in *P. spumarius* influences the movement of these structures, the features of the emitted signals could be affected. Nonetheless, considering that only virgin females were tested, it would be worthy to compare the chirp length of both virgin and mated females also considering those that have already oviposited, to assess if the chirp length might depend on the female interest in mating. Although the wavelet-based approach is commonly used for signal recognition (Selin et al. 2007; Rach et al. 2013; Korinšek et al. 2019), it can also be applied to identify possible changes in the features of an insect signal between different phenological or mating status, as we demonstrated in this work.

Our work provided insights concerning intra-sexual communication in *P. spumarius*. As previously reported, regardless of the period of the season, males emitted calling signals, which do not seem to be mating signals becausethey are not used to identify and locate mates (Avosani et al. 2020). Male courtship signals were recorded starting from August, in response to female calling signals and/or to male playback and resulted for most males in starting a duet and achieving mating. In some insect species, males use features of the rivals' signals (i.e., call rate, length) to predict the rival quality, and they may choose to leave the plant if they cannot compete with the other male (Greenfield and Minckley 1993). For instance, in the grasshopper *Ligurotettix coquilletti*, high population densities were correlated with a higher number of non-signalling males compared to low-density territories. In this and other Orthopteran species, males express dominance by signalling, while inactive males occupy a subordinate role (Greenfield & Shelly, 1985). Some *P. spumarius* males did not produce courtship signals, even when females were calling, potentially because these males were not interested in competing with the playback. However, many males reacted to the stimulus by emitting calling signals, potentially to mask the rival and/or the female signals, or to stop the rival male courtship and to gain a reproductive advantage upon the competitor. In the treehopper *Tylopelta gibbera*, males produce masking signals to prevent rivals from finding the replying female, while the leafhopper *Scaphoideus titanus* males emit disturbance/masking signals to reduce female response rate (Mazzoni et al. 2009; Legendre et al. 2012). Since the male disturbance signal was used to develop vibrational mating disruption strategies against *S. titanus*, further research should assess whether the male calling signal in *P. spumarius* represents a competitive masking signal and, if so, how it could be exploited to manipulate the insect behaviour (Mazzoni et al. 2009; Polajnar et al. 2016). On the other hand, the male calling signal could underlie mutual assessment, given that animals display collective signalling behaviours to assess local populations, regulate their density during their lifespan (Wynne-Edwards 1962) or avoid conflicts over a territory (Hill 2009). The aim of our work was not to assess the role of the male calling signal in insect spacing and, potentially, aggression. Future experiments focussed on male-male interaction could suggest whether and how the male calling signal could be exploited to manipulate the behaviour of *P. spumarius*.

To conclude, our work demonstrates that *P. spumarius'* female receptivity to mating is correlated to the development of the ovarioles and the presence of mature eggs. Data regarding the female sexual receptivity is crucial before designing a mating disruption tactic for the control of this insect, since male-female communication could be affected only when both genders are sexually mature. Further research should investigate sperm survival and the occurrence of sperm competition in *P. spumarius*, as this information could determine whether mating disruption could be an effective control strategy.

References

Alexander RD (1967) Acoustical communication in arthropods. Annu Rev Entomol 12:495–526

Andrés JA, Arnqvist G (2001) Genetic divergence of the seminal signal-receptor system in houseflies: The footprints of sexually antagonistic coevolution? Proc R Soc B Biol Sci 268:399–405. https://doi.org/10.1098/rspb.2000.1392

Avosani S, Daher E, Franceschi P, et al (2020) Vibrational communication and mating behavior of the meadow spittlebug *Philaenus spumarius*. Entomol Gen 40:307–321. https://doi.org/10.1127/entomologia/2020/0983

Beach FA (1976) Sexual attractivity, proceptivity, and receptivity in female mammals. Hormones and behavior 7(1):105-138.

Bernasconi G, Keller L (2001) Female polyandry affects their sons' reproductive success in the red flour beetle *Tribolium castaneum*. J Evol Biol 14:186–193. https://doi.org/10.1046/j.1420-9101.2001.00247.x

Cornara D, Bosco D, Fereres A (2018) *Philaenus spumarius*: when an old acquaintance becomes a new threat to European agriculture. J Pest Sci (2004) 91:957–972. https://doi.org/10.1007/s10340-018-0966-0

Cornara D, Saponari M, Zeilinger AR, et al (2017) Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. J Pest Sci (2004) 90:521–530. https://doi.org/10.1007/s10340-016-0793-0

Davranoglou LR, Cicirello A, Taylor GK, Mortimer B (2019) Planthopper bugs use a fast, cyclic elastic recoil mechanism for effective vibrational communication at small body size. PLoS Biol 17:. https://doi.org/10.1371/journal.pbio.3000155

De Mello FDAG (2007) Female monopolization and paternity assurance in South American crickets (Orthoptera, Grylloidea): Mating plugs, extra claspers and forced copulation. Pap Avulsos Zool 47:245–257. https://doi.org/10.1590/S0031-10492007002000001

Den Boer SPA, Baer B, Dreier S, et al (2009a) Prudent sperm use by leaf-cutter ant queens. Proc R Soc B Biol Sci 276:3945–3953. https://doi.org/10.1098/rspb.2009.1184

Den Boer SPA, Boomsma JJ, Baer B (2009b) Honey bee males and queens use glandular secretions to enhance sperm viability before and after storage. J Insect Physiol 55:538–543. https://doi.org/10.1016/j.jinsphys.2009.01.012

Endler JA (1993) Some general comments on the evolution and design of animal communication systems. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences, 340(1292):215-225.

Engelmann F (1970) The physiology of insect reproduction. Pergamon Press. New York 692pp

EPPO (2018) EPPO global database. Eur Mediterr Plant Prot Organ

Gordon SD, Krugner R (2019) Mating Disruption by Vibrational Signals: Applications for Management of the Glassy-Winged Sharpshooter. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 355–373

Greeff JM, Parker GA (2000) Spermicide by females: What should males do? Proc R Soc B Biol Sci 267:1759–1763. https://doi.org/10.1098/rspb.2000.1207

Greenfield MD, Minckley RL (1993) Acoustic dueling in tarbush grasshoppers: Settlement of Territorial contests via alternation of reliable signals. Ethology 95:309–326. https://doi.org/10.1111/j.1439-0310.1993.tb00480.x

Grothendieck G (2008) lattice: Multivariate Data Visualization with R . Springer Science & Business Media

Hammer Ø, Harper DAT, Ryan PD (2001) Past: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4:9

Hellriegel B, Ward PI (1998) Complex female reproductive tract morphology: Its possible use in postcopulatory female choice. J Theor Biol 190:179–186. https://doi.org/10.1006/jtbi.1997.0546

Higham JP, Hebets EA (2013) An introduction to multimodal communication. Behav Ecol Sociobiol 67:1381–1388. https://doi.org/10.1007/s00265-013-1590-x

Hill PSM (2001) Vibration and animal communication: A review. Am Zool 41:1135–1142. https://doi.org/10.1668/0003-1569(2001)041[1135:VAACAR]2.0.CO;2

Hill PSM (2009) How do animals use substrate-borne vibrations as an information source? Naturwissenschaften 96:1355–1371. https://doi.org/10.1007/s00114-009-0588-8

Klowden MJ (2013) Reproductive Systems. Physiological Systems in Insects. Academic Press, London & San Diego, 198-251

Korinšek G, Tuma T, Virant-Doberlet M (2019) Automated Vibrational Signal Recognition and Playback. In: Biotremology: Studying Vibrational Behavior. Springer, pp 149–173

Kuhelj A, de Groot M, Pajk F, et al (2015) Energetic cost of vibrational signalling in a leafhopper. Behav Ecol Sociobiol 69:815–828. https://doi.org/10.1007/s00265-015-1898-9

Kumar H, Saxena KN (1978) Mating behavior of the cotton leafhopper, *Empoasca devastans*, in relation to its age, ovarian development, diurnal cycle, and CO2 treatment. Ann Entomol Soc Am 71:108–110. https://doi.org/10.1093/aesa/71.1.108

Legendre F, Marting PR, Cocroft RB (2012) Competitive masking of vibrational signals during mate searching in a treehopper. Anim Behav 83:361–368. https://doi.org/10.1016/j.anbehav.2011.11.003

Maramorosch K (2012) Leafhopper vectors and plant disease agents. Elsevier

Markow TA (2000) Forced matings in natural populations of Drosophila. Am Nat 156:100–103. https://doi.org/10.1086/303368

Martelli GP, Boscia D, Porcelli F, Saponari M (2016) The olive quick decline syndrome in south-east Italy: a threatening phytosanitary emergency. Eur J Plant Pathol 144:235–243.

https://doi.org/10.1007/s10658-015-0784-7

Mazzoni V, Lucchi A, Čokl A, et al (2009) Disruption of the reproductive behaviour of *Scaphoideus titanus* by playback of vibrational signals. Entomol Exp Appl 133:174–185. https://doi.org/10.1111/j.1570-7458.2009.00911.x

Minelli A, Brena C, Deflorian G, et al (2006) From embryo to adult - Beyond the conventional periodization of arthropod development. Dev Genes Evol 216:373–383. https://doi.org/10.1007/s00427-006-0075-6

Morente M, Cornara D, Moreno A, Fereres A (2018) Continuous indoor rearing of *Philaenus spumarius*, the main European vector of *Xylella fastidiosa*. J Appl Entomol 142:901–904. https://doi.org/10.1111/jen.12553

Nieri R, Mazzoni V (2019) Vibrational mating disruption of *Empoasca vitis* by natural or artificial disturbance noises. Pest Manag Sci 75:1065–1073. https://doi.org/10.1002/ps.5216

Polajnar J, Eriksson A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. J Pest Sci (2004) 89:909–921. https://doi.org/10.1007/s10340-015-0726-3

Rach MM, Gomis HM, Granado OL, et al (2013) On the design of a bioacoustic sensor for the early detection of the red palm weevil. Sensors (Switzerland) 13:1706–1729. https://doi.org/10.3390/s130201706

Ringo J (1996) Sexual receptivity in insects. Annu Rev Entomol Vol 41 473–494. https://doi.org/10.1146/annurev.ento.41.1.473

Robertson A, Gibbs AJ (1937) Spermatogenesis and fertilization in *Philaenus spumarius* Fallen. J Trop Med Hyg 40:257–262

Rösch A, Schmidbauer H (2018) WaveletComp: Computational Wavelet Analysis. R package version 1.1. 1–38

Saponari M, Boscia D, Altamura G, et al (2017) Isolation and pathogenicity of *Xylella fastidiosa* associated to the olive quick decline syndrome in southern Italy. Sci Rep 7:1–13. https://doi.org/10.1038/s41598-017-17957-z

Schal C, Chiang A-S (1995) Hormonal control of sexual receptivity in cockroaches. Experientia 51:994–998. https://doi.org/10.1007/BF01921755

Selin A, Turunen J, Tanttu JT (2007) Wavelets in recognition of bird sounds. EURASIP J Adv Signal Process https://doi.org/10.1155/2007/51806

Sicard A, Zeilinger AR, Vanhove M, et al (2018) *Xylella fastidiosa* : Insights into an emerging plant pathogen. Annu Rev Phytopathol 56:181–202. https://doi.org/10.1146/annurev-phyto-080417-045849

Snedden WA, Sakaluk SK (1992) Acoustic signalling and its relation to male mating success in sagebrush crickets. Anim Behav 44:633–639. https://doi.org/10.1016/S0003-3472(05)80291-X

Teal PEA, Gomez-Simuta Y, Proveaux AT (2000) Mating experience and juvenile hormone enhance sexual signaling and mating in male Caribbean fruit flies. Proc Natl Acad Sci U S A 97:3708–3712. https://doi.org/10.1073/pnas.97.7.3708

Team RC (2017) R Foundation for Statistical Computing; Vienna, Austria: 2016. R: A language and environment for statistical computing. URL http//www R-project org/[Google Scholar

Vahed K, Carron G (2008) Comparison of forced mating behaviour in four taxa of Anonconotus, the

Alpine bushcricket. J Zool 276:313–321. https://doi.org/10.1111/j.1469-7998.2008.00492.x

Virant-Doberlet M, Cokl A (2004) Vibrational communication in insects. Neotrop Entomol 33:121–134. https://doi.org/10.1590/s1519-566x2004000200001

Virant-Doberlet M, Kuhelj A, Polajnar J, Šturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol 7:1–15. https://doi.org/10.3389/fevo.2019.00203

Weaver C, King D (1954) Meadow spittlebug, *Philaenus leucophthalmus* (L.). Ohio Agric Exp Station Res Bull 741:1–99

Wickham H (2011) ggplot2. Wiley Interdiscip Rev Comput Stat 3:180–185

Wickham H (2014) Dplyr. In: useR! Conference

Wickham H, Averick M, Bryan J, et al (2019) Welcome to the Tidyverse. J Open Source Softw 4:1686. https://doi.org/10.21105/joss.01686

Wiegert RG (1964) Population energetics of meadow spittlebugs (*Philaenus spumarius* L.) as affected by migration and habitat. Ecol Monogr 34:217–241. https://doi.org/10.2307/1948501

Witsack W (1973) Experimentell-okologische Untersuchungen uber Dormanz-Formen von Zikaden (Homoptera, Auchenorrhyncha). 2. zur Ovarial-Parapause und obligatorischen Embryonal-Diapause von *Philaenus spumarius* (L.) (Aphrophoridae). Zool Jahrbücher Abteilung für Syst Okol und Geogr der Tiere 100:517–562

Wynne-Edwards VC (1962) Animal dispersion: in relation to social behaviour

Yurtsever S (2001) Multiple paternity in the meadow spittlebug *Philaenus spumarius* [L.] [Homoptera: Cercopidae]. Acta Zool Cracoviensia 44:173–184

Chapter 4

Occupancy and detection of agricultural threats: the case of *Philaenus spumarius*, European vector of *Xylella fastidiosa*

Sabina Avosani, Clara Tattoni, Valerio Mazzoni and Marco Ciolli

Agriculture, Ecosystems & Environment. **Submitted for publication, under review**

Abstract

Occupancy models estimate presence and detection probability of species of interest such as agricultural pests of which it can provide useful information regarding distribution, detectability and also contribute to decision making. In this work, we propose an approach to demonstrate the applicability of occupancy models in insect monitoring having as targets the spittlebug *Philaenus spumarius*, the main European vector of *Xylella fastidiosa,* and other Auchenorrhyncha species. Surveys were performed in two different agricultural settings (olive orchards and vineyards) in Trentino (northern Italy), where the association between *P. spumarius* and host plants was also investigated. *Philaenus spumarius* was present in all surveyed sites and its detectability was significantly influenced by site covariates (forest and vineyard coverage, hours of sunlight) and plot covariates (weed height). Site covariates also influenced the detection probability of other Auchenorrhyncha, while the co-occurrence of different species did not affect *P. spumarius* occupancy. *Philaenus spumarius* nymphs were found mainly on Asteraceae and Lamiaceae, while Fabaceae were less selected. Only adults were found on olive and grapevine canopies. The influence of covariates on detectability of *P. spumarius* and other potential *X. fastidiosa* vectors is discussed, as well as the importance of shaping plant composition at those sites susceptible to the bacterium. Our results suggest that an introduction of *X. fastidiosa* in Trentino would be hard to control given the widespread occurrence, in different habitats of the agroecosystem, of its main vector in this territory.

Keywords: Auchenorrhyncha, meadow spittlebug, presence, detectability, host plant association

1. Introduction

Understanding environmental drivers that explain presence and absence of a given species in a site is the basis of conservation and management of populations of a certain organism (Kellner and Swihart 2014). Species distribution models can predict the occurrence of species in a site and support studies dealing with biogeography, invasive species ecology, and natural resource management (Duarte et al. 2019; Malek et al. 2018; Piyapong et al. 2020). These models enable researchers to disentangle the effects of ecological variables on species distribution (Kellner and Swihart 2014; Duarte et al. 2019). Nonetheless, species distribution models may be affected by imperfect species detection, due to observer errors, cryptic species, or environmental conditions (Kellner and Swihart 2014). As a result, the target species is missed at certain locations, even if sites are occupied.

Occupancy models have been designed to solve this issue and produce estimations on detection and occupancy probability of a species (MacKenzie et al. 2003, 2009, 2017). Ignoring imperfect detection in a survey can lead to unreliable outcomes and consequent wrong decisions for conservation and management (Guillera-Arroita et al. 2014). Moreover, current models allow to run site-occupancy models for one or several species (Dorazio et al. 2006; Bailey et al. 2014). Although the number of studies applying occupancy models showed an increasing trend in the last decades, there is a taxonomic bias towards terrestrial organisms, especially vertebrates, which are the main targets of most studies while invertebrates, such as insects, have been neglected (Devarajan et al. 2020; Mourguiart et al. 2021). Nonetheless, entomologists consider occupancy an effective tool to study single species as well as communities, given that insects pose detection issues due to ecological traits as phenology (Mourguiart et al. 2021). Single and multi-species occupancy models have been used to study insect species of different orders using field collected data (Orthoptera: Mourguiart et al., 2020; Odonata, Orthoptera, Lepidoptera: Malinowska et al., 2014; Lepidoptera: Cabeza et al., 2010; Hymenoptera and Homoptera: Sileshi, 2007; Coleoptera: Brodie et al., 2019) and museum data (Zeilinger et al. 2017). To our best knowledge, a similar method was never applied to study species of Auchenorrhyncha (Hemiptera: Cicadomorpha), which comprises important agricultural pests (Raven 1983; Katis et al. 2007). Besides species conservation projects, pest management strategies could benefit from applying occupancy models to monitor insects of agricultural interest. In case of vectors of plant pathogens, occupancy models could predict potential disease dynamics in a crop and support decision-making in the context of control strategies (Sileshi 2007). In fact, presence-absence sampling allows saving time when the target species is a small insect occurring at high densities, in particular in surveys that require multiple visits during the same season (Wilson and Room 1983; Sileshi 2007).

Notwithstanding the advantages, occupancy models have never been used to study the presence of the meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae) in crops. Studies aimed at understanding the ecological characteristics of this species exponentially increased after it was recognised as the main vector of the plant pathogen *Xylella fastidiosa* (Saponari et al. 2014; Cornara et al. 2018; Cavalieri et al. 2019). This bacterium was recently introduced in Europe and is associated with important plant diseases such as Pierce’s disease of grapevine in California (USA) and the Olive Quick Decline Syndrome (OQDS) in southern Italy, but can infect numerous other plant species (Almeida 2016; Martelli et al. 2016; Saponari et al. 2019; Desprez-Loustau et al. 2020). To collect ecological data regarding *P. spumarius* and estimate disease dynamics, extensive research was aimed at investigating its phenology, abundance, and plant preference in different European locations. Even if the sampling design of previous studies (Morente et al. 2018; Bodino et al. 2019, 2020) provided fundamental information, they did not account for imperfect detection.

Here, we propose a simple approach to demonstrate applicability of occupancy models in pest insect monitoring, by determining *P. spumarius* presence while accounting for detectability. Surveys were performed in two different agricultural settings (olive orchards and vineyards) in Trentino, which is a *X. fastidiosa*-free area. Furthermore, given that knowledge of preferential plant selection by *P. spumarius* nymphs and adults could support design of integrated pest management programs (Bodino et al. 2020), we investigate the association between spittlebugs and host plants.

2. Materials and methods

2.1. Field surveys

Field surveys were conducted from March to September 2018 in two agricultural areas of the Trentino region (northern Italy) to study the presence and host affiliation of *P. spumarius* by using occupancy models. The first area was in Riva del Garda, where five olive orchards were selected (sites rdg1, rdg2, rdg3, rdg4, rdg5; Fig. 4.1). The second area was in San Michele all'Adige, where five sites consisted of vineyards (sma1, sma2, sma3) and riparian habitat comprising vineyards (sma4, sma5) (Fig. 4.1). Each site, which was subjected to a low-input management (organic), was surveyed within a 200 m perimeter. The presence of other spittlebugs (superfamily Cercopoidea) such as *Neophilaenus* spp., *Lepyronia coleoptrata*, *Cercopis* spp. and *Aphrophora* spp. was recorded as well. These species, being also xylem-feeders (Morente et al. 2018; Bodino et al. 2019, 2020) could be potential vectors of *X. fastidiosa*. In total, each site was visited 17 times.

The quadrat sampling method was used to monitor the nymphal populations, since it is an effective method for detecting spittlebug juveniles (Whittaker 1973; Morente et al. 2018; Dongiovanni et al. 2019; Bodino et al. 2020). In particular, the sampling unit (SU) was a rectangle of 0.25 m^2 (100 x 25 cm), which was randomly positioned on the ground (20 SU per site, 100 SU per area). The soil and vegetation inside the SU were inspected for the presence of spittlebug nymphs, which were removed from the host plant with a thin paint brush and counted. Surveys were conservative, thus species identity and larval instar were determined directly in the field using the identification keys from Vilbaste (1982), and after counting, nymphs were placed on their hosts. Beside *P. spumarius*, number and stage of other spittlebug nymphs were recorded and identified, when possible. Nymph surveys were carried out biweekly from the end of March until the mid of June, when only adults were found.

Surveys aimed at detecting adults were carried out weekly from May (when first emerging adults could be found on the herbaceous cover) to the end of September. Overall, the duration of the survey was sufficiently short to exclude potential local extinctions and localisation of species (Dorazio et al. 2006). A sweeping net (diameter 30 cm, length 60 cm) was used to sample herbaceous plants and tree canopies at randomly selected points (20 per each site, 100 per area). The sweeping net was chosen due to its reliability as sampling method for spittlebug adults (Morente et al. 2018; Bodino et al. 2019). For each point, five sweeps were performed on the ground cover and five on the tree canopy (either olive or grapevine, depending on site). Given that at the sampling point occurred at random locations within a site, in some occasions, olive trees/grapevines were not present within the point. In such cases, if other woody plants were present, insects were sampled with the sweeping net from their canopies, and plants were identified at the genus level. *Philaenus spumarius* and the other sampled spittlebug species were counted, identified and sexed (when possible) directly in the field. In addition, two auchenorrhynchan species, *Metcalfa pruinosa* (Fulgoromorpha: Flatidae) and *Stictocephala bisonia* (Cicadomorpha: Membracidae), which we found abundant in the surveyed area (see results) and are known as potential crop pests (Seljak 2002; Cornara et al. 2019), were considered for the analysis.

2.2. Occupancy models

Occupancy models were chosen because they account for imperfect detection of organisms in surveys and determine the probability of true presence or absence of a species in a site, which is visited multiple times. Occupancy models are defined as an extension of Generalized Linear Mixed Effects models (GLMMs) using a Bernoulli distribution where random effects model true state of occurrence (MacKenzie et al. 2002, 2003; Duarte et al. 2019). Two different probabilities are modelled, where ψ represents the probability that a site is occupied by the target species (occupancy), and p_j is the probability of detecting the species during the j^{th} survey, given it is present (detectability) (Kéry 2011; Devarajan et al. 2020).

Our single season occupancy model contained two assumptions, according to Devarajan et al. (2020). The first assumes that the occupancy state is "closed", thus species are present at occupied sites for the duration of the sampling season and occupancy does not change at a site within the sampling season. The second assumption states that sites are independent, hence detection of a species at one site is independent of detecting it at other sites. Single- and multi-species occupancy models were run to estimate occupancy and detection probabilities for *P. spumarius* and co-occurring auchenorrhynchan species, respectively.

2.2.1. Single-species occupancy models

Occupancy and detection probability of *P. spumarius* were estimated by using occupancy models to determine true presence or absence of the insect in surveyed sites. Due to the sampling design used, two occupancy models were run at two different scales for *P. spumarius*.

Site-level scale model. This model was fed with "aggregated" data collected during 17 visits to assess a general trend for species occurrence. Data collected from each sampling unit were aggregated for each surveyed site, to obtain single presence/absence values for the n visit at the given site.

Plot-level scale model. This model was run at sampling unit-level, to assess the effects of plot-level covariates on detection. The model was fed with a 200 x 17 data matrix, where 200 was the total number of sampled units (20 per plot) and 17 was the number of visits.

2.2.2. Multi-species occupancy models

A multi-species occupancy model was run with site-level data and included second order interactions to evaluate the occupancy conditioned by other Auchenorrhyncha species, given the presence of *P. spumarius* and the effect of covariates on detectability of co-occurring species.

2.3. Model selection and statistical analyses

Single- and multi-species occupancy models were run using different linear combination of the covariates, and the best models were later selected using Akaike's information criterion (AIC). The best models were selected when Delta AIC < 1 in comparison to the first best model and were averaged to create a single best model. Statistical analysis and plots were carried out in the framework of Rstudio (Team 2020) using R (Team 2017) and the packages *Unmarked* (Fiske and Chandler 2011), *ggplot2* (Wickham 2016), *reshape2* (Wickham 2007) and *MuMIn* (Barton 2009).

2.4. Environmental covariates

In occupancy models, it is possible to define site-level covariates and plot-level covariates. While the first describe the environment using site-specific data that do not change with repeated visits (i.e., land use, forest coverage, elevation), plot-level covariates are collected at each visits and can change across sampling occasion and site (i.e., air temperature, humidity) (MacKenzie and Bailey 2004).

In our study, site-level covariates were the following: olive tree coverage (0-1), vineyard coverage (0-1), anthropic coverage (i.e., roads, houses…) (0-1), forest coverage (0-1), agricultural coverage (crops other than olive and grapevine), (0-1) average elevation (m aslm), average slope (degrees), average sun hours during the vegetative period (1st April – 31st October), and directions of the physical slopes face, namely the aspect (N, S, E, W, NW, NE, SW, SE). After creation of a buffer zone of 200 m for each site, covariates data were collected from real land use dataset PAT 2003/08 provided by "Servizio Urbanistica e Tutela del Paesaggio" of the province of Trento (https://www.sciamlab.com/opendatahub/it/dataset/p_tn_uso-del-suolo-reale-urbanistica-ed-08-2003-200509/resource/9d522d8f-68ae-46b1-a0b3-4067751873f).

The following plot-level covariates were collected from the sampling units (quadrat or sweeping net) at each visit: coverage of herbaceous cover (0-1), height of herbaceous cover (cm), composition of herbaceous cover (what family plant was prevalent within the sampling unit), composition of swept canopies (olive, grapevine, other woody hosts), and period of sampling.

2.5. Host plant affiliation

To investigate the affiliation between *P. spumarius* and its host plants, analyses were conducted using data from 588 sampling units in which at least one specimen was found, either in the quadrat placed in the herbaceous cover or in the canopies swept with the net. Relative percentage of adults and nymphs on each plant family was measured. In addition, ten plant genera preferred by *P. spumarius* nymphs in Bodino et al. (2020) were selected, and relative percentage of the nymphal instars on these hosts was calculated.

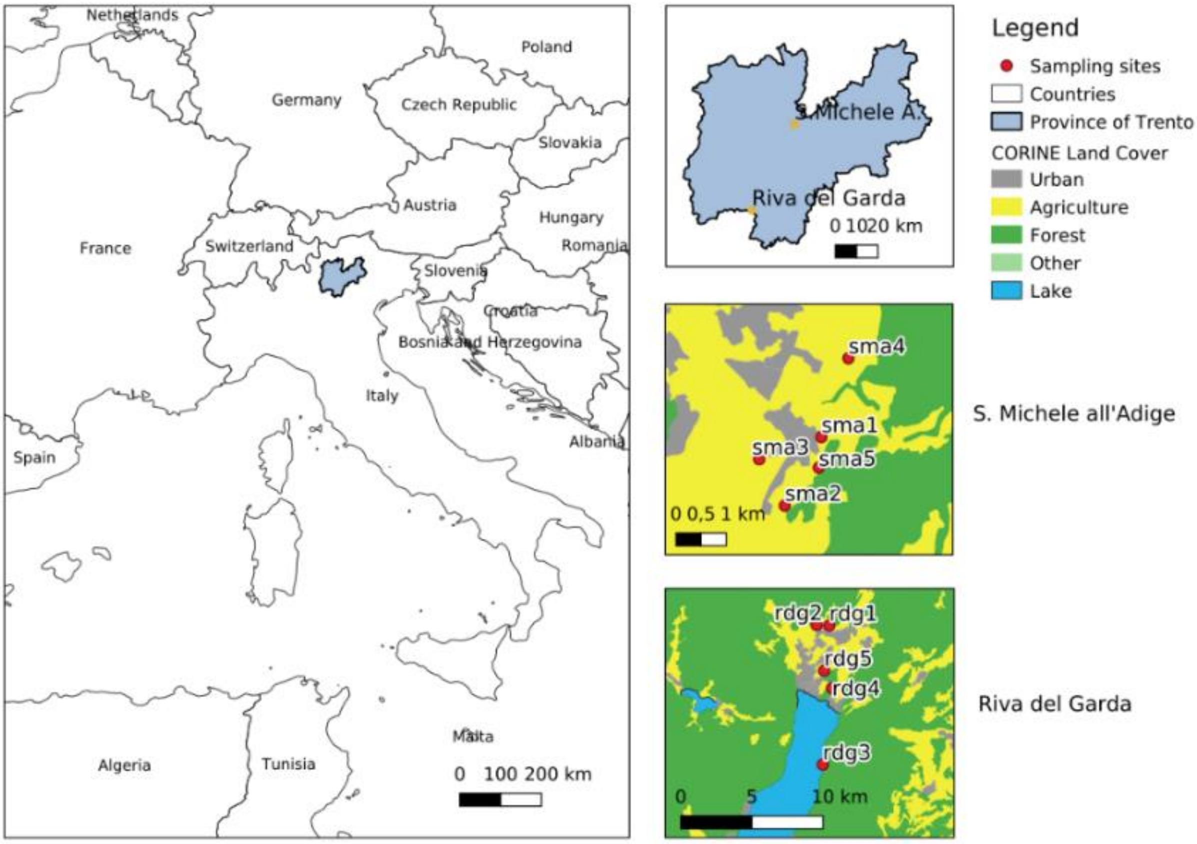

Figure 4.1 Study areas in Trentino, which were visited to investigate occupancy and detectability of *Philaenus spumarius* and other auchenorrhynchan species. Five olive orchards (rdg1, rdg2, rdg3, rdg4, rdg5) and five habitats comprising vineyards (sma1, sma2, sma3, sma4, sma5) were sampled.

3. Results

3.1. Single-species occupancy models

3.1.1. Site-level occupancy model

The presence of *P. spumarius* was detected 107 times and its absence 63 times during our survey (Fig. 4.2). Number of visits in which the species was detected ranges from a minimum of 4 (site sma1) to a maximum of 17 (recorded at all visits in site rdg3) with an average of 11 ± 4.37 detections per site. Proportion of sites that recorded at least one presence of *P. spumarius* (namely, the naïve occupancy) was equal to one, meaning that the species was present in all sites but not always detected. The model without any covariate (occupancy null model) yielded a baseline of $\psi = 0.999$, SE = 0.003 (occupancy); and $p = 0.65$, SE = 0.037 (detectability). According to this outcome, variables that affected the detectability (p) of the species were estimated.

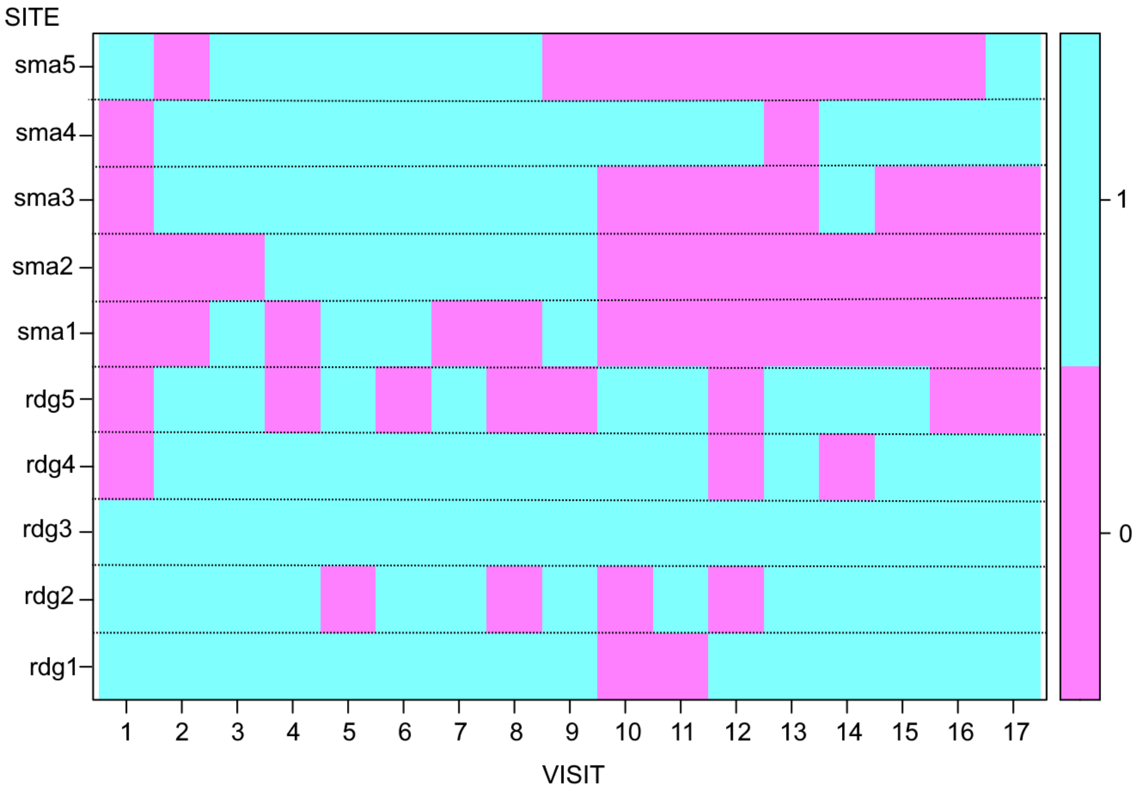

Figure 4.2 Presence (light blue squares) and absence (pink squares) of *Philaenus spumarius* per visit (x axis) across surveyed sites (y axis) from March to September 2018 in Trentino. Riva del Garda sites: rdg1, rdg2, rdg3, rdg4 and rdg5. San Michele all'Adige sites: sma1, sma2, sma3, sma4 and sma5.

In total, 1022 occupancy models were run by varying the combination of 10 site covariates in the detection part of the model. In the best models, significant covariates that explained detectability (p) were vineyard coverage (p-value < 0.001), forest coverage (p-value = 0.002) and average hours of sunlight (p-value = 0.002). Detection probability of *P. spumarius* was highest in vineyards regardless of the amount of coverage within the 200 m perimeter (Fig. 4.3A). The detectability of this species was significantly affected by the presence of forests since it steadily decreased when the forest coverage was higher than 0.4 within 200 m buffer at the sampled sites (Fig. 4.3B). In sites with an average number of sunlight hours greater than 10, detectability of *P. spumarius* decreased as well (Fig. 4.3C).

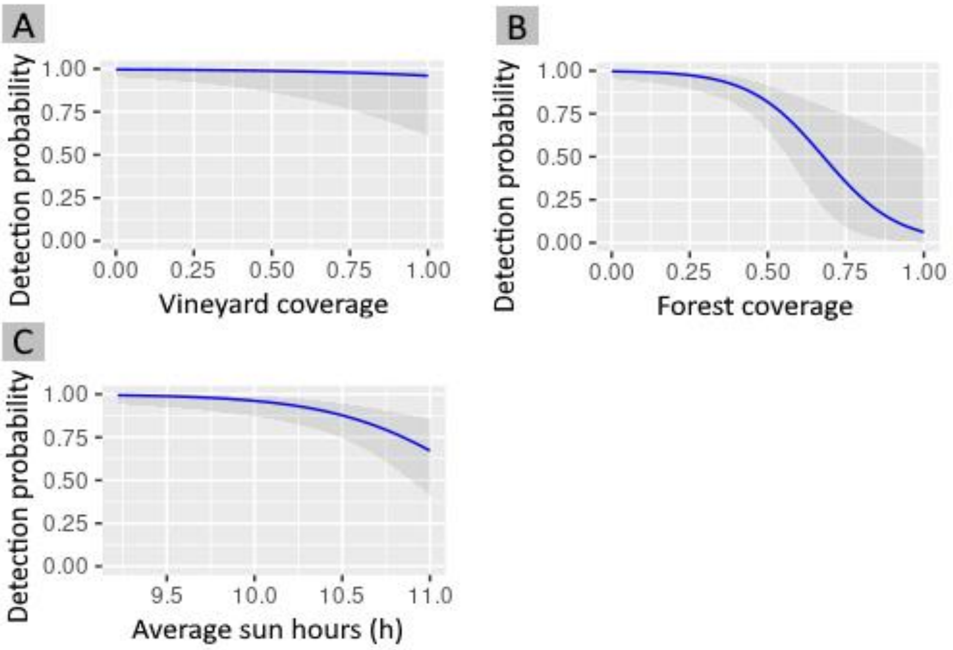

Figure 4.3 Detection probability of *Philaenus spumarius* according to different site covariates (A, B, C) in olive groves and vineyards located in Trentino. Shaded areas represent 95% confidence interval.

3.1.2. Plot-level occupancy model

The presence of *P. spumarius* was recorded 436 times, with 162 plots (out of 170 in total) in which it was detected at least one time. The average number of presences recorded per sampling unit was 2 ± 2.09 (mean ± SD). Although the *P. spumarius* presence at plot-level was more scattered than at site-level, the occupancy null model yielded a high occupancy probability (ψ =0.867, SE = 0.031) and a detection probability of 0.148, SE = 0.007. According to this, the effect of plot-level variables on *P. spumarius* detectability (p) was evaluated. In total, 169 occupancy models were run with varying combinations of five covariates in the detection part of the model. The best models retained weed height and weed coverage, but only weed height was moderately significant in explaining the detection probability (p-value = 0.0259) (Figure 4.4). The possibility to detect the spittlebug in our study area increased when weeds were taller, while weed coverage had apparently no effect.

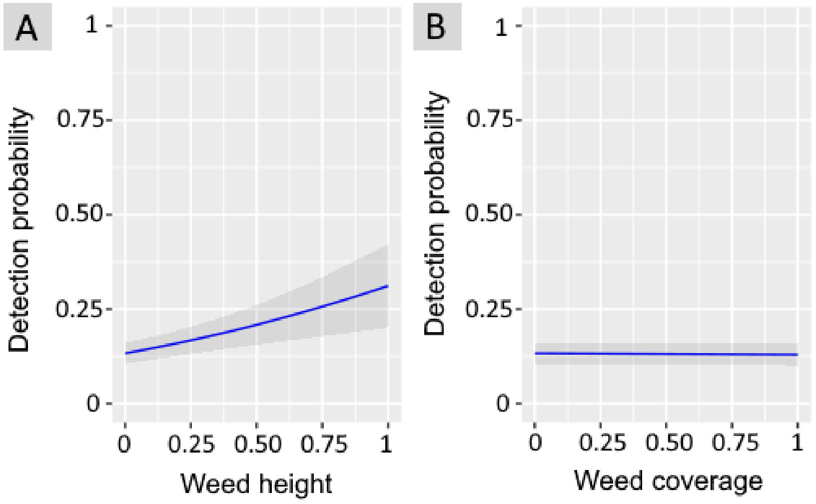

Figure 4.4 Detection probability of *Philaenus spumarius* according to two plot-level covariates (A, weed height; B, weed coverage) in olive groves and vineyards located in Trentino. Shaded areas represent 95% confidence interval.

3.2. Multi-species occupancy model

Beside *P. spumarius*, *Stictocephala bisonia* and *Metcalfa pruinosa* were recorded, together with spittlebug species belonging to the genus *Neophilaenus, Aphrophora, Lepyronia* and *Cercopis*. Given that the survey was conservative, identification was not possible at species-level in the field.

Number of sites with at least one detection, number of presences, absences, and total individuals per each species in the sites is shown in Table 4.1. The percentage of sites occupied by each species per visit is reported in Figure 4.5. Similar to Dorazio et al. (2006), a heatmap was used to display species richness and number of individuals across the surveyed sites (Fig. 4.6). Number of individuals found in each site was generally lower than 30, whilst more than 50 specimens of either *P. spumarius, Neophilenus spp.,* or *Lepyronya coleoptrata* were occasionally counted in the same site. Species were never detected all together: the highest species richness was 6 species in 3 sites at San Michele all'Adige (sma2, sma4 and sma5) with an average number of species recorded per site of 4.7 ± 1.16.

Table 4.1 Summary of the multi-species survey. For each species, number of sites with at least one detection, number of times a species was recorded as present or absent, and the total number of individuals recorded during the whole survey are reported.

Species or Genus	Sites with at least one detection	Present	Absent	Total Individuals
Philaenus spumarius	10	107	63	788
Neophilaenus spp.	6	18	152	118
Aphrophora spp.	5	19	151	40
Lepyronia coleoptrata	9	40	130	274
Cercopis spp.	7	16	154	71
Metcalfa pruinosa	7	14	156	31

Stictocephala bisonia	6	9	161	12

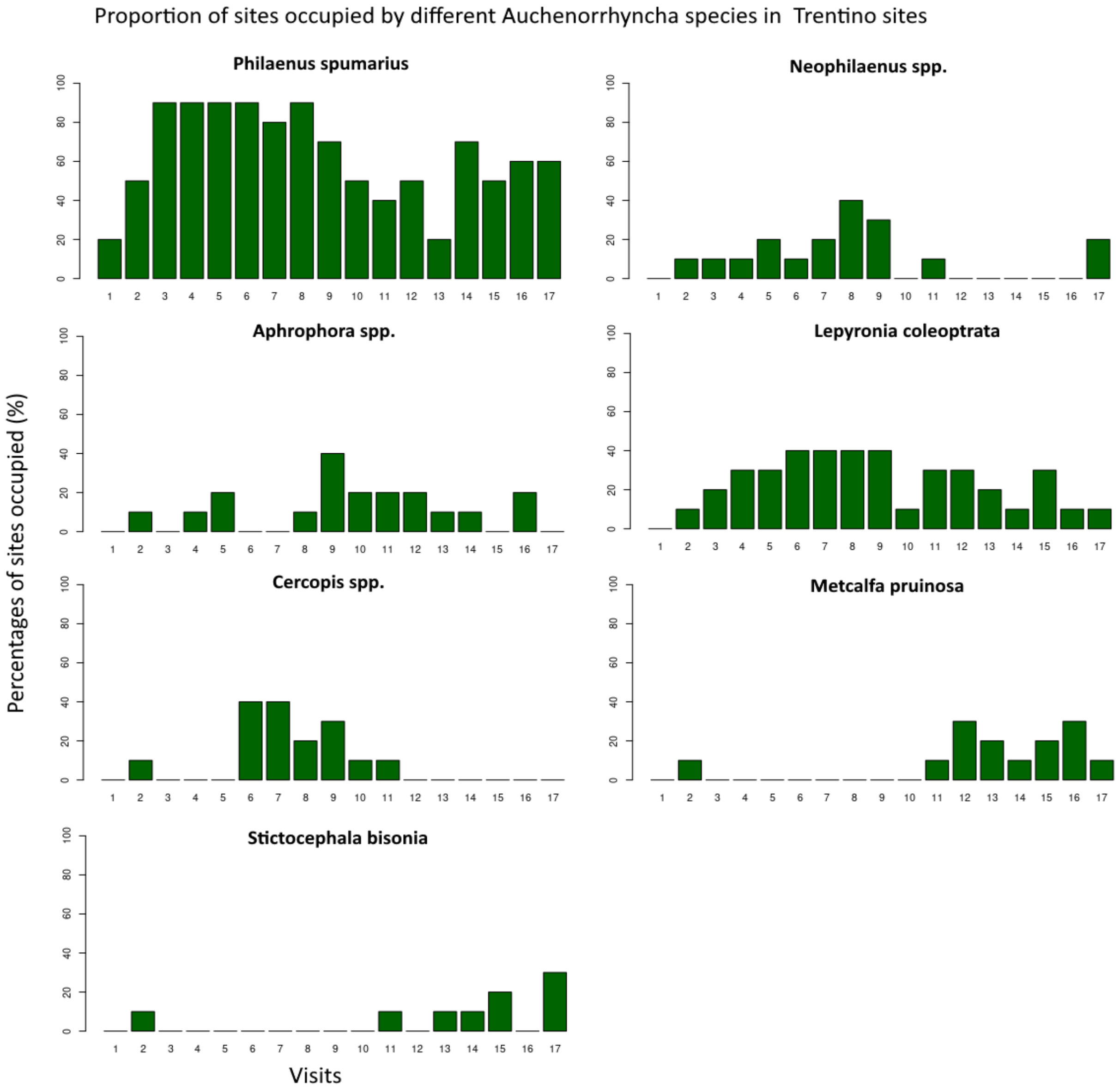

Figure 4.5 Proportion of occupied sites by Auchenorrhyncha species recorded in different sites surveyed from March to September 2018 in Trentino.

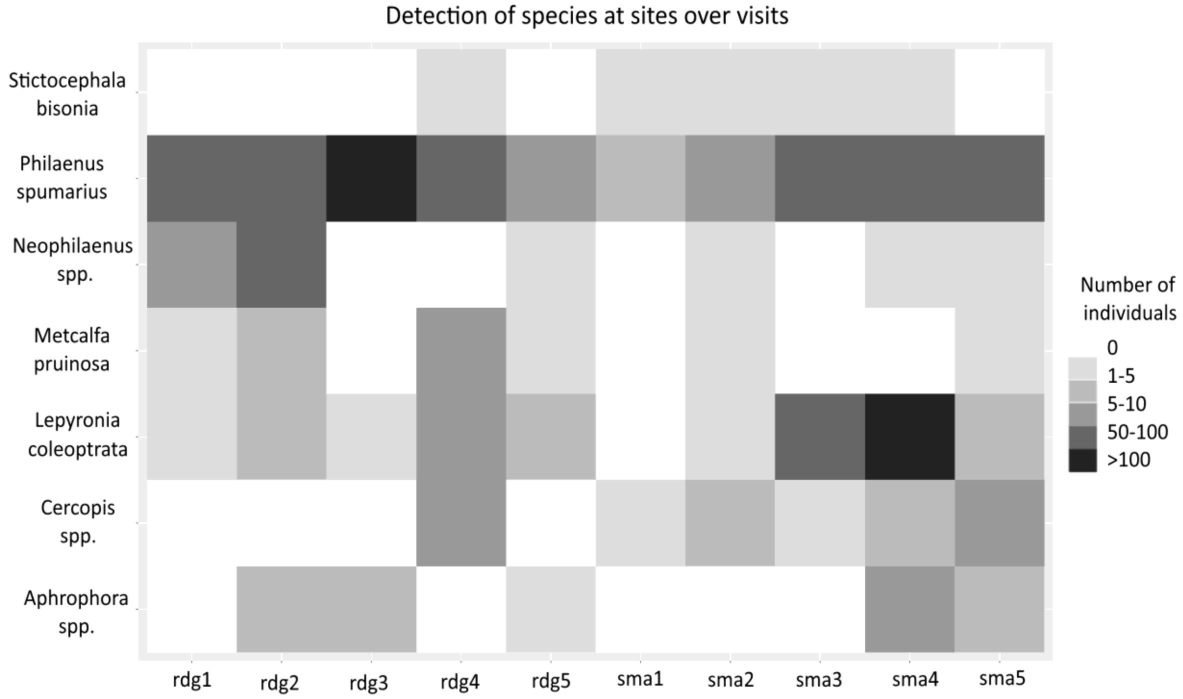

Figure 4.6 Heatmap showing the number of auchenorrhynchan individuals detected at different sites, surveyed from March to September 2018 in Trentino. Riva del Garda sites: rdg1, rdg2, rdg3, rdg4 and rdg5. San Michele all'Adige sites: sma1, sma2, sma3, sma4 and sma5.

A null model was run to compute naive probabilities of occupancy and detection for each species (Table 4.2): most species showed a very high occupancy rate of study sites, except *Aphrophora* spp. (ψ = 0.45). Naïve detection probabilities for all co-occurring species were much lower than that of *P. spumarius* (Table 4.2).

Table 4.2 Naïve occupancy and detection probabilities of auchenorrhynchan species in different sites, surveyed from March to September 2018 in Trentino.

Species or genus	Naïve occupancy	Naïve occupancy SE	Naïve detection	Naïve detection SE
Philaenus spumarius	1.000	0.003	0.629	0.037
Neophilaenus spp.	0.627	0.163	0.169	0.039
Aphrophora spp.	0.452	0.150	0.025	0.009
Lepyronia coleoptrata	0.905	0.096	0.260	0.036
Cercopis spp.	0.792	0.177	0.119	0.033
Metcalfa pruinosa	0.851	0.207	0.097	0.032
Stictocephala bisonia	0.982	0.375	0.054	0.027

A multi-species occupancy model was run with the same set of covariates as the single-species model, and effects of environmental variables on detectability (p) of the species were estimated. The variable that better explained the detectability of the species co-occurring with *P. spumarius* were the vineyard and olive tree coverages. These variables had a species-specific effect on species detectability (Fig. 4.7A and B). The coverage of olive trees steadily increased the probability of detecting an individual of *Aphrophora* spp., *Lepyronia coleoptrata, Metcalfa pruinosa* or *Neophilaenus* spp, while for *Cercopsis spp.* and *Strictocephala* bisonia this effect was very limited (Fig. 4.7B). A growing coverage of vineyards increased the detectability of *Cercopsis spp.* and *Metcalfa pruinosa* but had a negative effect on that of *Aphrophora spp., Neophilaenus spp.* and *Strictocephala bisonia* but no effect on *Lepironia coleoptrata'*s (Fig. 4.7A). The models suggested that *P. spumarius* occupancy was not statistically influenced by presence of other species (ψ = 0.99).

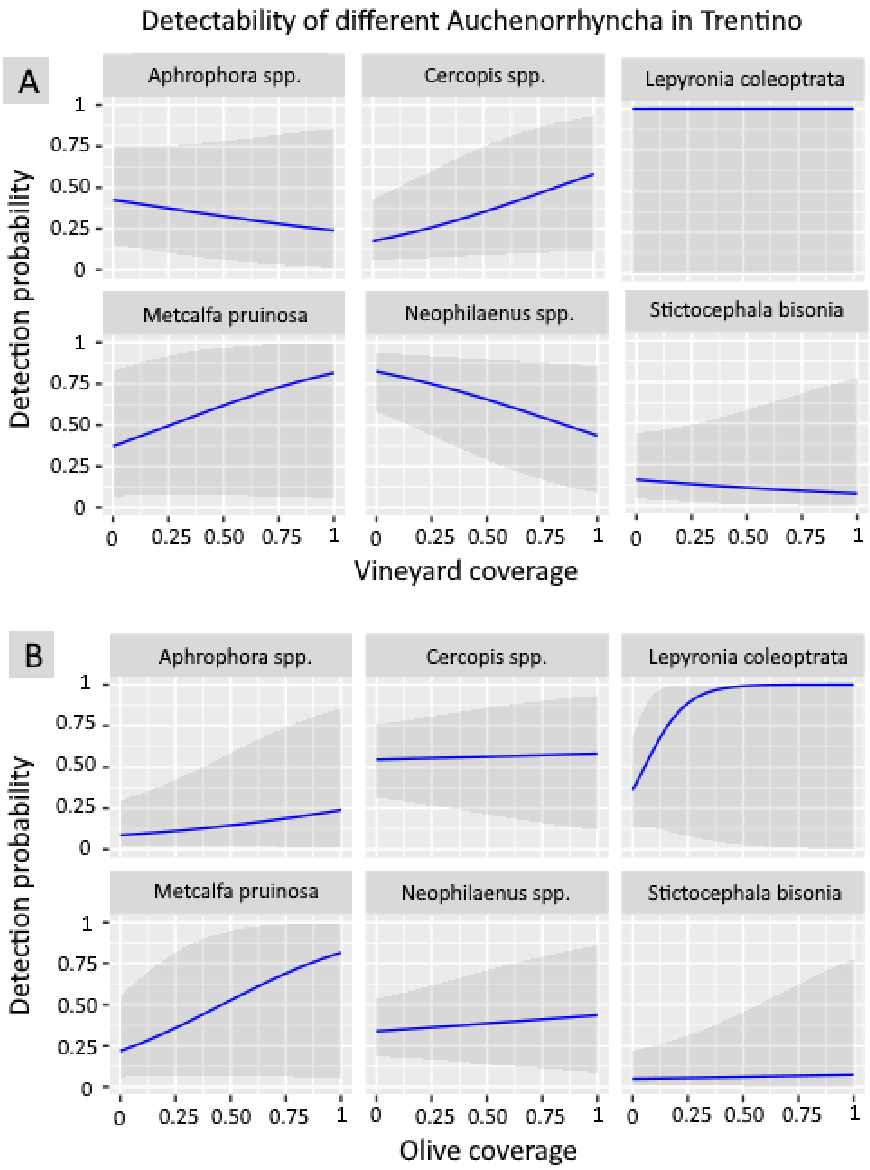

Figure 4.7 Effect of (A) vineyard or (B) olive tree coverage on detectability of different auchenorrhynchan species collected in Trentino. Shaded areas represent 95% confidence interval.

3.3. Host plant affiliation

During the sampling season, 75 adults and 513 nymphs of *P. spumarius* were recorded. Nymphs included first (26), second (70), third (112), fourth (99) and fifth (206) instars. *Philaenus spumarius* was observed on plants belonging to 25 different families. A total of 315 *P. spumarius* individuals were found on *Asteraceae*, 68 on *Lamiaceae,* 66 on *Plantaginaceae*, 58 on *Fabaceae* and

Oleaceae, 54 on *Caryophyllaceae*, 46 on *Convolvulaceae* and 25 on *Rubiaceae* (Fig. 4.8). Plant families that hosted less than 25 *P. spumarius* individuals were aggregated in a unique class (namely, "others") accounting for 98 individuals (Fig. 4.8).

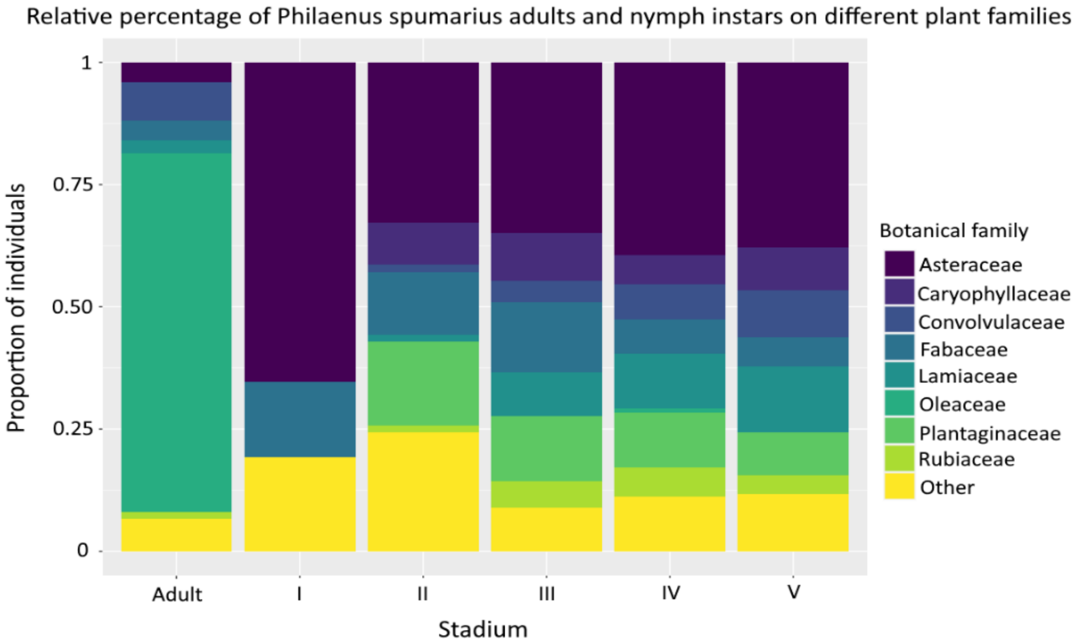

Figure 4.8 Relative percentage of adult and nymph instars of *Philaenus spumarius* sampled from different plant families in Trentino. I, II, III, IV, V = first, second, third, fourth and fifth nymphal instar, respectively. Plant families that hosted less than 25 *P. spumarius* individuals per plant are included in the group "others".

The proportion of adults and nymphs distributed per host plant is shown in Fig. 4.9. The majority of *P. spumarius* (237), collected from the quadrat sampling and net sweeping of canopies, were observed on *Conyza spp*., which hosted all stages of development (Fig. 4.9). First instars were observed only on *Trifolium spp.* and *Conyza spp.* Other nymphal stages were recorded on all selected plants except on olive trees, from which exclusively adults were collected (N = 57, Fig. 4.9).

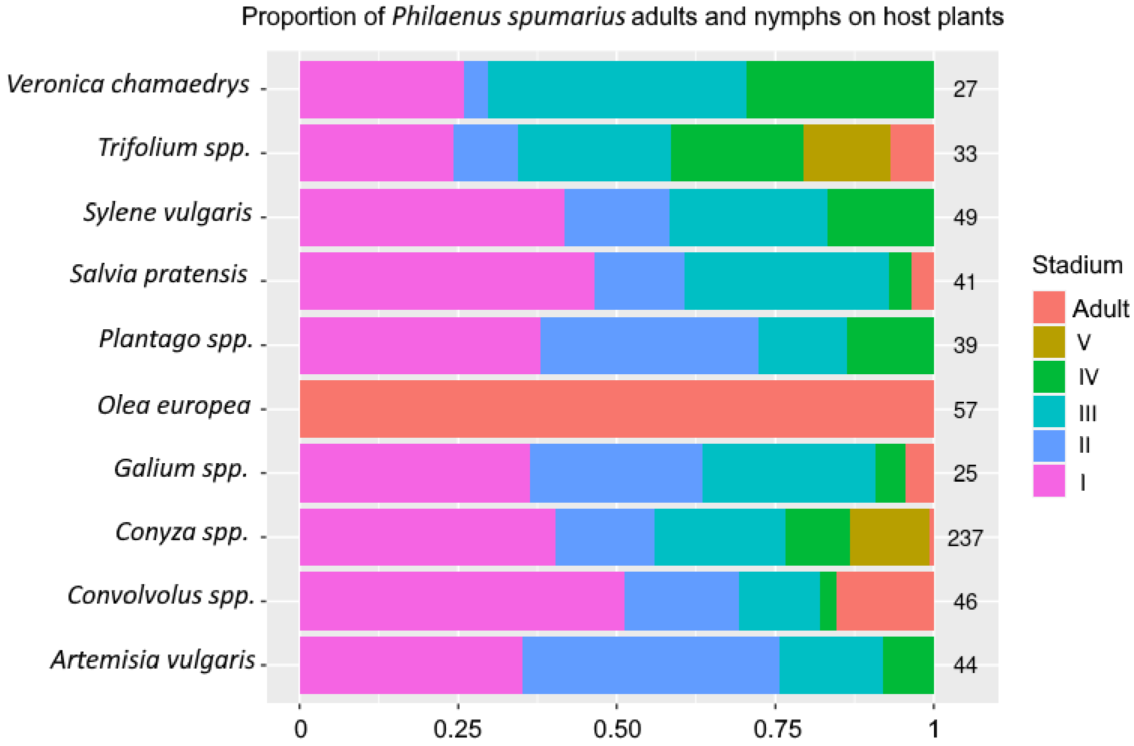

Figure 4.9 Relative percentage of adults and nymphs of *Philaenus spumarius* on 10 host-plant genera. Insects were collected from quadrat sampling and sweeping of canopies in vineyards and olive groves in Trentino. Numbers beside bars represent total number of nymphs sampled on each plant species. I, II, III, IV, V = first, second, third, fourth and fifth nymphal instar, respectively.

4. Discussion

In this study, occupancy models were used, for the first time, to estimate occupancy and detection probabilities of *P. spumarius* and other auchenorrhynchan field collected in Trentino.

Philaenus spumarius is the main vector of *X. fastidiosa* in Europe and can colonize many habitats. The definition of models of presence and/or distribution in a certain territory is therefore necessary to design effective strategies of prophylaxis or protection. In our research, although the *P. spumarius* occupancy was high in all visited sites, its detectability was influenced by features of the area, such as presence of forests, vineyard coverage, sunlight hours and plant height. In accordance with the hypothesis that “almost any species may be overlooked” (Kéry 2011; Mata et al. 2014), our results suggest that *P. spumarius*, which is the most abundant spittlebug in Europe (Cornara et al. 2018, 2019), may be missed in certain environments. This can have important repercussions on decision making (i.e., pest management), since basing an analysis only on the reported presence of a species can introduce biases. In such a context, investigations in agro-ecosystems (i.e., Bodino et al., 2020, 2019; Cornara et al., 2017; Dongiovanni et al., 2019; Morente et al., 2018; Santoiemma et al., 2019), as much as they are accurate and provide valuable knowledge, still can suffer from the omission of the detection estimation. For instance, in some Iberic olive groves, even if *P. spumarius* nymphs were not found in the herbaceous cover, adults were sampled from olive canopies (Morente et al. 2018). As much as this could be an effect of adult immigration from external sites, it could be in part due to the underestimation of nymph occupancy as defined by the landscape context. Therefore, the occurrence of estimation biases could be overcome by including detection in future models. This approach would be particularly useful whenever the final aim of a study is to provide indications for pest and crop management (Bodino et al. 2019), since they could allow reliable comparisons between crops from different areas but also better determine peaks of presence and detection probabilities in association with the features of the study sites.

In our case, high vineyard coverage was associated with an increase in the *P. spumarius* detectability. Vineyards in Trentino are trained as pergola, which protects a relative high portion of surface from direct sunlight, while plants are irrigated. These conditions seem to provide a humid microclimate that supports populations of *P. spumarius*, favouring a widespread presence and high detectability in this agroecosystem (Cornara et al. 2018; Beal et al. 2021). However, this species was found abundant on herbaceous vegetation within and surrounding vineyards also in other Italian regions that use different trellis systems, like Guyot or cordons (Aldini et al. 1998; Braccini and Pavan 2000; Pavan 2006). Farming practices can therefore influence the spittlebug presence, but rather than the trellis system, the management and the landscape seem to make the difference. In fact, large populations of *P. spumarius* were found in low-input management plantations (Kunz et al., 2010; Mazzoni, 2006; our study), whereas the spittlebug was absent in vineyards subjected to conventional management (Santoiemma et al. 2019). High input farming, notably when based on the large use of pesticides, reduces the biodiversity in agro-ecosystems (Brühl and Zaller 2019). In the case of *P. spumarius,* the use of pesticides is a common practice (and almost the only available) that farmers adopt to protect olive orchards in Southern Italy (Cornara et al. 2018). This approach offers a short-term control strategy, but its efficacy in reducing disease risks is highly variable (Purcell 1980; Madden et al. 2000; Daugherty et al. 2015). On the other hand, the reduction of biodiversity is consistent, and can have long-term countereffects, as generalist insects such as *P. spumarius* can quickly occupy emptied niches (Civitello et al. 2015; Brühl and Zaller 2019; Sánchez-Bayo and Wyckhuys 2019; Wagner et al. 2021). Our visited vineyards were subjected to low-input management, and auchenorrhynchan other than *P. spumarius* were accordingly thriving in these habitats. In particular, higher percentages of vineyards were associated with an increased probability of detecting *Cercopis* spp. and *Metcalfa pruinosa*, as they are frequently observed in humid habitats and on woody plants (Alma 2002; Mazzoni et al. 2008). On the other hand, high vineyard coverages had a negative effect on the detectability of *Aphrophora* spp., *Neophilaenus* spp. and *Stictocephala bisonia*. The scattered presence of these species and the scarce probability of detecting them is consistent with the literature, considering that they are only occasionally found within Italian vineyards (Mazzoni et al., 2008).

The olive coverage also had a positive effect on the detectability of auchenorrhynchans, more pronounced for *Aphrophora* spp., *L. coleoptrata*, *M. pruinosa* or *Neophilaenus* spp., and less for *Cercopis* spp. and *S. bisonia*. The herbaceous cover of olive groves in Trentino remains flourishing, providing a shady environment and a humid microclimate, contrarily to Mediterranean olive orchards in Southern Italy, where the ground cover dries during summer (Bodino et al., 2020). In Trentino, the rich cover of olive groves could therefore foster the presence of Auchenorrhyncha, which were thus easily detected. Although the detection probability of *P. spumarius* was not influenced by the percentage of olive coverage, this species was the predominant spittlebug in the surveyed olive groves (and vineyards), in accordance with other Italian surveys (Ben Moussa et al. 2016; Cornara et al. 2017; Bodino et al. 2019, 2020). *Philaenus spumarius* could nonetheless thrive in the same habitat with other Auchenorrhyncha, to the point of sharing the same host plant and even the same spittle mass with other spittlebug species. The presence of other species did not affect the occupancy of *P. spumarius*, likely due to a trade-off between costs and benefits of gregarious feeding. In fact, although nymphs can share the same host plant to overcome plant barriers and allocate less resources to spittle production, this strategy implies a reduced food quality, to the detriment of some individuals (Mangan and Wutz 1983; Wise et al. 2006; Mcevoy 2013).

Besides the coverage of crops, there was an important factor significantly affecting the probability of detecting *P. spumarius*: the presence of forests. In fact, relatively high forest coverage in the 200 m buffer surrounding the site was associated to a lower probability of detecting the spittlebug. This habitat likely provides a shelter for *P. spumarius*, especially adults, which may move into woodland areas when the conditions in olive orchards or vineyards are less favourable (i.e., during hot days or crop management practices). Moreover, forests can provide nutritious hosts such as actinorhizal plants, which are characterised by a reliable supply of xylem-borne organic nitrogen compounds and may be exploited by spittlebugs when plants within crops are under stress (Thompson 1999). Nevertheless, the effect of forest covers on the *P. spumarius* presence was considered of scarce relevance and thereby discarded from previous distribution models (see Santoiemma et al., 2019). Even though these studies disclosed several environmental factors driving *P. spumarius* occurrence in olive groves, our result indicate to include forests into future models. The increasing number of sunlight hours is another factor associated with a lower probability of detecting *P. spumarius*. The quantity of sun reaching a site is generally dependent on topographic factors (i.e., elevation and slope angle) and has repercussions on growing and quality of plants, as well as on crop management (Zorer et al. 2017). This factor can indeed impact the life of *P. spumarius*, whose biology strictly depends on temperature and humidity (Cornara et al., 2018). A rise in temperatures could, for instance, elicit a lower detectability due to behavioural adjustments. Firstly, as increasing air temperature generally reduces host plant quality (Lemoine et al. 2014), the feeding activity of phytophagous insects such as *P. spumarius* may be impaired. Spittlebug nymphs and adults could be therefore less detectable due to their seeking behaviour towards more nutritious plants. Secondly, spittlebug nymphs usually find shelter in hidden parts of plants to escape direct sunlight, while adults migrate during the summer period and delay oviposition until weather cools (Weaver and King 1954; Cornara et al. 2018). Similarly, by providing sheltered places, tall plants in the ground cover are expected to be associated to a low likelihood of detecting *P. spumarius*, whilst in our study we observed the exact opposite. Higher plants (up to 100 cm) increased the detectability of the spittlebug, potentially because the shade provided by taller plants led spittlebugs towards the apex of the host plant, resulting in a higher detection probability.

Another factor that was expected to influence the *P. spumarius* detectability was the percentage of herbaceous cover within the site. The first reason is that higher ground coverages were previously associated to a higher spittlebug presence in other Italian olive groves (Bodino et al., 2019, 2020). Secondly, plant distribution and density can influence the ability of an insect to find its host plants, notably engaging the insect in a search for preferred plant species. For example, some plants could “escape” from phytophagous insects by clumping in space, increasing the distance between clumps, or lowering plant density (Cain 1985). In our case, the detectability of *P. spumarius* was not dependent on the percentage of herbaceous cover, likely due to the large polyphagy of the spittlebug, which can exploit a great number of plant species regardless of the distribution. This suggests that the role of ground cover may be overestimated when detectability is omitted, because the effect of the covariate on spittlebug detection could be erroneously ascribed to its presence. In this way, we propose including occupancy models to future field surveys to provide accurate estimations of the presence of *P. spumarius* in study sites.

As mentioned above and extensively reported in the literature, *P. spumarius* is a highly polyphagous insect, which thrives on more than 500 host plants (Cornara et al. 2018; Markheiser et al. 2019). Even so, this spittlebug has some preferred hosts (Bodino et al., 2020; Cornara et al., 2018; Dongiovanni et al., 2019). In our study, it was collected from a great variety of plants, both from the ground covers of olive groves and vineyards and from trees canopies. Adults were mainly sampled from olive canopies, although they could be found even on herbs. On the other hand, nymphs were always collected from the herbaceous cover and were sampled from many different plant families, especially Asteraceae, but also Lamiaceae and Plantaginaceae, while Fabaceae were less selected, even if they were reported as a preferred host by nymphs in Southern Italy (Apulia). Considering that the availability of hosts during the season affects the *P. spumarius* plant selection (Bodino et al., 2020), regional differences are therefore due to the distribution and phenology of the host plants in different agroecosystems. Because it is suggested that *P. spumarius* chooses hosts with high xylem amino acid concentrations and for this reason it is associated with actinorhizal plants (Thompson, 1999), these hosts should be therefore considered when shaping the ground cover of crops vulnerable to *X. fastidiosa*. In this regard, the spittlebug occupancy may be reduced by removing the preferred plant species, whilst favouring non-host plants, since a lower quality of food sources could result in higher mortality of nymphs and/or lower fecundity of adults (Morente et al., 2018). Even if a deeper knowledge regarding the association between the spittlebug and plants is needed, we can conclude that ground covers of olive orchards and vineyards should be shaped to reduce the presence of *P. spumarius* in regions like Trentino, which are currently *X. fastidiosa*-free, as a prophylaxis measure to reduce the risks of disease outbreaks (Bodino et al., 2020).

Considering that the percentages of sites occupied by *P. spumarius* were constantly high during our survey, serious consequences are expected if *X. fastidiosa* is introduced in Trentino, where a renowned niche extra virgin olive oil is made and where wine production is a key economic input (Alaeddinoglu and Türker 2016; Mucci et al. 2019; Moreno-Sanz et al. 2020). In this context, a precise knowledge of the *P. spumarius* distribution and phenology all over a territory is a key element. An important flaw in this is that traditional monitoring tools such as interception sticky traps, sticky-shoot, and yellow sticky traps has been proved to be ineffective to sample *P. spumarius* in the field, especially to intercept nymphs, which are less mobile than adults (Morente et al., 2018). On the other hand, innovative monitoring tools based on vibrations has been proposed for some pest species (Hartman et al. 2017; Takanashi et al. 2019). *Philaenus spumarius* intraspecific communication relies on vibrational signals, and the use of species-specific signals could be tested for capturing individuals (Avosani et al. 2020). Furthermore, a recent study demonstrated that coupling of volunteer-collected data with traditional ecological surveys allows improvement of existing monitoring programs (Malek et al. 2018). Even if field surveys are still necessary to monitor the distribution of *P. spumarius*, researchers could involve farmers' help, since the latter could provide information regarding the spittlebug presence in their vineyards and olive groves. In particular, they could report the presence of spittle masses, while a user-friendly platform could be developed to help farmers identify and report the species. A similar tool could also allow the gathering of information regarding how (i.e., sticky trap or visual), when (date) and where (location) the spittlebug was observed. Presence/absence data collected by farmers could be then used to run occupancy models, providing estimations of both distribution and detectability of the spittlebug. By comparing data from conventional and organic crops, it could be even possible to obtain information about the exact role of the crop management in determining the *P. spumarius* occupancy (Cornara et al. 2018; Mesmin et al. 2020; Molinatto et al. 2020).

To conclude, we demonstrated that the presence of *P. spumarius* and other Auchenorrhyncha can be underestimated even when using the most efficient sampling methods for these species. We recommend including occupancy models when studying the presence of pest species, given that these models more accurately estimate species distribution by considering imperfect detection. Estimations of future *X. fastidiosa* outbreaks related to vectors' distribution could benefit from this approach as well. Moreover, our outcomes regarding the association between *P. spumarius* and its host plants in Trentino could guide the management of the herbaceous cover, to limit the colonization of spittlebugs in susceptible crops.

References

Alaeddinoglu F, Türker N (2016) From wine production to wine tourism experience: the case of Anatolia, Turkey. J Tour Gastron Stud 4:25–25. https://doi.org/10.21325/jotags.2016.40

Aldini RN, Guardiani MC, Cravedi P (1998) Faunistical notes on the hoppers (Homoptera Auchenorrhyncha) in vineyards in the province of Piacenza. Boll di Zool Agrar e di Bachic 30:61–68

Alma A (2002) Auchenorrhyncha as pests on grapevine. Denisia 176:531–538

Avosani S, Daher E, Franceschi P, et al (2020) Vibrational communication and mating behavior of the meadow spittlebug *Philaenus spumarius*. Entomol Gen 40:307–321. https://doi.org/10.1127/entomologia/2020/0983

Bailey LL, MacKenzie DI, Nichols JD (2014) Advances and applications of occupancy models. Methods Ecol Evol 5:1269–1279

Barton K (2009) MuMIn: multi-model inference, R package version 0.12. 0. http://r-forge r-project org/projects/mumin/

Beal DJ, Cooper M, Daugherty MP, et al (2021) Seasonal abundance and infectivity of *Philaenus spumarius* (Hemiptera: Aphrophoridae), a vector of *Xylella fastidiosa* in California vineyards . Environ Entomol 1–10. https://doi.org/10.1093/ee/nvaa178

Ben Moussa IE, Mazzoni V, Valentini F, et al (2016) Seasonal fluctuations of sap-feeding insect species infected by *Xylella fastidiosa* in Apulian olive groves of southern Italy . J Econ Entomol 109:1512–1518. https://doi.org/10.1093/jee/tow123

Bodino N, Cavalieri V, Dongiovanni C, et al (2020) Spittlebugs of mediterranean olive groves: Host-plant exploitation throughout the year. Insects 11:. https://doi.org/10.3390/insects11020130

Bodino N, Cavalieri V, Dongiovanni C, et al (2019) Phenology, seasonal abundance and stage-structure of spittlebug (Hemiptera: Aphrophoridae) populations in olive groves in Italy. Sci Rep 9:1–17. https://doi.org/10.1038/s41598-019-54279-8

Braccini P, Pavan F (2000) Auchenorryncha: potential vectors of phytoplasms associated with vine yellows. Inf Agrar 56:103–107

Brodie BS, Popescu VD, Iosif R, et al (2019) Non-lethal monitoring of longicorn beetle communities using generic pheromone lures and occupancy models. Ecol Indic 101:330–340. https://doi.org/10.1016/j.ecolind.2019.01.038

Brühl CA, Zaller JG (2019) Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Front Environ Sci 7:2013–2016. https://doi.org/10.3389/fenvs.2019.00177

Cabeza M, Arponen A, Jäättelä L, et al (2010) Conservation planning with insects at three different spatial scales. Ecography (Cop) 33:54–63. https://doi.org/10.1111/j.1600-0587.2009.06040.x

Cain ML (1985) Random search by herbivorous insects: a simulation model. Ecology 66:876–888. https://doi.org/10.2307/1940550

Cavalieri V, Altamura G, Fumarola G, et al (2019) Transmission of *Xylella fastidiosa* subspecies *pauca* sequence type 53 by different insect species. Insects 10(10):324 https://doi.org/10.3390/insects10100324

Civitello DJ, Cohen J, Fatima H, et al (2015) Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proc Natl Acad Sci U S A 112:8667–8671. https://doi.org/10.1073/pnas.1506279112

Cornara D, Bosco D, Fereres A (2018) *Philaenus spumarius*: when an old acquaintance becomes a new threat to European agriculture. J Pest Sci (2004) 91:957–972. https://doi.org/10.1007/s10340-018-0966-0

Cornara D, Morente M, Markheiser A, et al (2019) An overview on the worldwide vectors of *Xylella fastidiosa*. Entomol Gen 39:157–181. https://doi.org/10.1127/entomologia/2019/0811

Cornara D, Saponari M, Zeilinger AR, et al (2017) Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. J Pest Sci (2004) 90:521–530. https://doi.org/10.1007/s10340-016-0793-0

Daugherty MP, O'Neill S, Byrne F, Zeilinger A (2015) Is vector control sufficient to limit pathogen spread in vineyards? Environ Entomol 44:789–797. https://doi.org/10.1093/ee/nvv046

Devarajan K, Morelli TL, Tenan S (2020) Multi-species occupancy models: review, roadmap, and recommendations. Ecography (Cop) 43:1612–1624. https://doi.org/10.1111/ecog.04957

Dongiovanni C, Cavalieri V, Bodino N, et al (2019) Plant selection and population trend of spittlebug immatures (Hemiptera: Aphrophoridae) in olive groves of the Apulia region of Italy. J Econ Entomol 112:67–74. https://doi.org/10.1093/jee/toy289

Dorazio RM, Royle JA, Söderström B, Glimskär A (2006) Estimating species richness and accumulation by modeling species occurrence and detectability. Ecology 87:842–854. https://doi.org/10.1890/0012-9658(2006)87[842:ESRAAB]2.0.CO;2

Duarte A, Whitlock SL, Peterson JT (2019) Species distribution modeling. 10.1016/B978-0-12-409548-9.10572-X

Fiske IJ, Chandler RB (2011) Unmarked: An R package for fitting hierarchical models of wildlife occurrence and abundance. J Stat Softw 43:1–23. https://doi.org/10.18637/jss.v043.i10

Guillera-Arroita G, Lahoz-Monfort JJ, MacKenzie DI, et al (2014) Ignoring imperfect detection in biological surveys is dangerous: A response to 'Fitting and interpreting occupancy models'.' PLoS One 9:e99571. https://doi.org/10.1371/journal.pone.0099571

Hartman E, Rohde B, Lujo S, et al (2017) Behavioral responses of male *Diaphorina citri* (Hemiptera: Liviidae) to mating communication signals from vibration traps in Citrus (Sapindales: Rutaceae) Trees . Florida Entomol 100:767–771. https://doi.org/10.1653/024.100.0425

Katis NI, Tsitsipis JA, Stevens M, Powell G (2007) Transmission of plant viruses. Aphids as Crop Pests 353–390. https://doi.org/10.1079/9781780647098.0323

Kellner KF, Swihart RK (2014) Accounting for imperfect detection in ecology: A quantitative review. PLoS One 9:. https://doi.org/10.1371/journal.pone.0111436

Kéry M (2011) Towards the modelling of true species distributions. J Biogeogr 38:617–618. https://doi.org/10.1111/j.1365-2699.2011.02487.x

Kunz G, Roschatt C, Schweigkofler W (2010) Biodiversity of planthoppers (Auchenorrhyncha) in vineyards infected by the Bois noir phytoplasma. 10:89–108

Lemoine NP, Burkepile DE, Parker JD (2014) Variable effects of temperature on insect herbivory. PeerJ 2014:1–18. https://doi.org/10.7717/peerj.376

MacKenzie DI, Bailey LL (2004) Assessing the fit of site-occupancy models. J Agric Biol Environ Stat 9:300–318. https://doi.org/10.1198/108571104X3361

MacKenzie DI, Nichols JD, Hines JE, et al (2003) Estimating site occupancy, colonization, and local extinction when a species is detected imperfectly. Ecology 84:2200–2207. https://doi.org/10.1890/02-3090

MacKenzie DI, Nichols JD, Lachman GB, et al (2002) Estimating site occupancy rates when detection probabilities are less than one. Ecology 83:2248–2255. https://doi.org/10.1890/0012-9658(2002)083[2248:ESORWD]2.0.CO;2

MacKenzie DI, Nichols JD, Royle JA, et al (2017) Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence: Second Edition. Elsevier

Mackenzie DI, Nichols JD, Seamans ME, Gutiérrez RJ (2009) Modeling species occurrence dynamics with multiple states and imperfect detection. Ecology 90:823–835. https://doi.org/10.1890/08-0141.1

Madden L V., Jeger MJ, Van Den Bosch F (2000) A theoretical assessment of the effects of vector-virus transmission mechanism on plant virus disease epidemics. Phytopathology 90:576–594. https://doi.org/10.1094/PHYTO.2000.90.6.576

Malek R, Tattoni C, Ciolli M, et al (2018) Coupling traditional monitoring and citizen science to disentangle the invasion of *Halyomorpha halys*. ISPRS Int J Geo-Information 7:. https://doi.org/10.3390/ijgi7050171

Malinowska AH, Van Strien AJ, Verboom J, et al (2014) No evidence of the effect of extreme weather events on annual occurrence of four groups of ectothermic species. PLoS One 9:1–10. https://doi.org/10.1371/journal.pone.0110219

Mangan RL, Wutz A (1983) Aggregation patterns of meadow spittlebugs, *Philaenus spumarius* L. (Homoptera: Cercopidae), on old-field alfalfa plants 1. Environ Entomol 12:151–157. https://doi.org/10.1093/ee/12.1.151

Markheiser A, Cornara D, Fereres A, Maixner M (2019) Analysis of vector behavior as a tool to predict *Xylella fastidiosa* patterns of spread. Entomol Gen 40:1–13. https://doi.org/10.1127/entomologia/2019/0841

Mata L, Goula M, Hahs AK (2014) Conserving insect assemblages in urban landscapes: accounting for species-specific responses and imperfect detection. J Insect Conserv 18:885–894. https://doi.org/10.1007/s10841-014-9696-7

Mazzoni V (2006) The role of the soil coverage on the occurrence of occasional ampelophagous planthoppers and leafhoppers in vineyards. Iobc Wprs Bull 29:167

Mazzoni V, Anfora G, Ioriatti C, Lucchi A (2008) Role of winter host plants in vineyard colonization and phenology of *Zygina rhamni* (Hemiptera: Cicadellidae: Typhlocybinae). Ann Entomol Soc Am 101:1003–1009. https://doi.org/10.1603/0013-8746-101.6.1003

Mcevoy PB (2013) Niche partitioning in spittlebugs (Homoptera : Cercopidae) sharing shelters on host plants. Ecological Society of America 67:465–478. http://www.jstor.org/stable/1938590

Mesmin X, Chartois M, Genson G, et al (2020) *Ooctonus vulgatus* (Hymenoptera, Mymaridae), a potential biocontrol agent to reduce populations of *Philaenus spumarius* (Hemiptera, Aphrophoridae) the main vector of *Xylella fastidiosa* in Europe. PeerJ 2020:e8591. https://doi.org/10.7717/peerj.8591

Molinatto G, Demichelis S, Bodino N, et al (2020) Biology and prevalence in northern Italy of *Verrallia aucta* (Diptera, Pipunculidae), a parasitoid of *Philaenus spumarius* (Hemiptera, Aphrophoridae), the main vector of *Xylella fastidiosa* in Europe. Insects 11:1–16. https://doi.org/10.3390/insects11090607

Moreno-Sanz P, Lombardo L, Grando MS, et al (2020) Genetic resources of *Olea europaea* L. In the Garda trentino olive groves revealed by ancient trees genotyping and parentage analysis of drupe embryos. Genes (Basel) 11:1–28. https://doi.org/10.3390/genes11101171

Morente M, Cornara D, Plaza M, et al (2018) Distribution and relative abundance of insect vectors of *Xylella fastidiosa* in olive groves of the Iberian peninsula. Insects 9:. https://doi.org/10.3390/insects9040175

Mourguiart B, Couturier T, Braud Y, et al (2021) Multi-species occupancy models: an effective and flexible framework for studies of insect communities. Ecol Entomol 46:163–174. https://doi.org/10.1111/een.12991

Mucci M, Baldessari M, Michelotti F, et al (2019) Captures of olive fruit fly in Alto Garda Trentino with different traps. IOBC-WPRS Bull 141:147–152

Pavan F (2006) Xylem-feeding Auchenorrhyncha potentially involved in Pierce’s disease of grapevines in Europe. Boll di Zool Agrar e di Bachic 38:103–114

Piyapong C, Tattoni C, Ciolli M, et al (2020) Modelling the geographical distributions of one native and two introduced species of crayfish in the French Alps. Ecol Inform 60:101172. https://doi.org/10.1016/j.ecoinf.2020.101172

Purcell AH (1980) Environmental therapy for Pierce’s disease of grapevines. Plant Dis 64:388–390. https://doi.org/10.1094/pd-64-388

Raven JA (1983) Phytophages of xylem and phloem: A comparison of animal and plant sap-feeders. Adv Ecol Res 13:135–234. https://doi.org/10.1016/S0065-2504(08)60109-9

Sánchez-Bayo F, Wyckhuys KAG (2019) Worldwide decline of the entomofauna: A review of its drivers. Biol Conserv 232:8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Santoiemma G, Tamburini G, Sanna F, et al (2019) Landscape composition predicts the distribution of *Philaenus spumarius*, vector of *Xylella fastidiosa*, in olive groves. J Pest Sci (2004) 92:1101–1109. https://doi.org/10.1007/s10340-019-01095-8

Saponari M, Loconsole G, Cornara D, et al (2014) Infectivity and transmission of *Xylella fastidiosa* by *Philaenus spumarius* (Hemiptera: Aphrophoridae) in Apulia, Italy. J Econ Entomol 107:1316–1319. https://doi.org/10.1603/EC14142

Seljak G (2002) Non-european Auchenorrhyncha (Hemiptera) and their geographical distribution in Slovenia. Acta Entomol Slov 10:97–101

Sileshi G (2007) A method for estimating insect abundance and patch occupancy with potential applications in large-scale monitoring programmes. African Entomol 15:89–101. https://doi.org/10.4001/1021-3589-15.1.89

Takanashi T, Uechi N, Tatsuta H (2019) Vibrations in hemipteran and coleopteran insects: Behaviors and application in pest management. Appl Entomol Zool 54:21–29. https://doi.org/10.1007/s13355-018-00603-z

Team RC (2017) R Foundation for Statistical Computing; Vienna, Austria: 2016. R: A language and environment for statistical computing. URL http//www R-project org/[Google Sch

Team Rs (2020) RStudio: integrated development for R. RStudio, PBC, Boston

Thompson V (1999) Spittlebugs associated with actinorhizal host plants. In: Canadian Journal of Botany. 77(9):1387–1390. https://cdnsciencepub.com/doi/abs/10.1139/b99-058

Vilbaste J (1982) Preliminary key for the identification of the nymphus of north European Homoptera Cicadinea. II: Cicadelloidea

Wagner DL, Grames EM, Forister ML, et al (2021) Insect decline in the Anthropocene : Death by a thousand cuts. 118:1–10. https://doi.org/10.1073/pnas.2023989118

Weaver C, King D (1954) Meadow spittlebug, *Philaenus leucophthalmus* (L.). Ohio Agric Exp Station Res Bull 741:1–99

Whittaker JB (1973) Density regulation in a population of *Philaenus spumarius* (L.)(Homoptera: Cercopidae). J Anim Ecol 163–172

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. J. R. Stat. Soc. Ser. A (Statistics Soc. 174:245–246

Wickham H (2007) Reshaping data with the reshape package. J Stat Softw 21:1–20. https://doi.org/10.18637/jss.v021.i12

Wilson LT, Room PM (1983) Clumping patterns of fruit and arthropods in cotton, with implications for binomial sampling. Environ Entomol 12:50–54

Wise MJ, Kieffer DL, Abrahamson WG (2006) Costs and benefits of gregarious feeding in the meadow spittlebug, *Philaenus spumarius*. Ecol Entomol 31:548–555. https://doi.org/10.1111/j.1365-2311.2006.00814.x

Zeilinger AR, Rapacciuolo G, Turek D, et al (2017) Museum specimen data reveal emergence of a plant disease may be linked to increases in the insect vector population. Ecol Appl 27:1827–1837. https://doi.org/10.1002/eap.1569

Zorer R, Volschenk CG, Hunter JJ (2017) Integrating Geographic Information Systems and hemispherical photography in the assessment of canopy light profiles in a vineyard. Agric For Meteorol 232:672–681. https://doi.org/10.1016/j.agrformet.2016.09.011

Vibrational disruption of feeding behaviours of a vector of plant pathogen

Sabina Avosani, Alice Berardo, Nicola M. Pugno, Vincenzo Verrastro, Valerio Mazzoni, Daniele Cornara

This chapter is adapted from a manuscript accepted for publication in *Entomologia generalis* (June 2021)

Abstract

Interference with the behaviours associated to host plant recognition, and inter- and intra-specific communication of insect vectors of plant pathogens, could represent a long-term sustainable strategy to cohabit with the organisms the insects transmit. Here we show that the transmission over a suitable host plant (sunflower) of a stimulus designed based on a vibrational signal used for intra-specific communication significantly affects the probing and feeding behaviour of the spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae), the main European vector of the fastidious bacterium *Xylella fastidiosa* to olive. Specifically, ca. 30% of the individuals did not even attempt to probe the treated plants (sunflower), while the remaining showed a sex-independent reduction in ingestion of the xylem sap, i.e., *P. spumarius'* main food source, of ca. 67% compared to the control. The possible reflection of a signal-based vector behaviour disturbance on the epidemiology of *X. fastidiosa*, together with future research needs are discussed.

Keywords: *Philaenus spumarius*; Aphrophoridae; biotremology; electrical penetration graph; behavioural disruption

1. Introduction

Invasive plant pathogens and pests affect the multifunctionality of agroecosystems, in treacherous and often unpredictable ways (Simberloff et al. 2013; Ali et al. 2021). Although such impact is often estimated only in terms of yield losses, a cascade of effects is primed following introduction of an alien organism into a new ecosystem and subsequent spill over. Considering the case of the vector-borne bacterium *Xylella fastidiosa* ST53 outbreak in olive orchards in Salento (Apulia region, Southern Italy), several authors focused on the significant decrease in table olives and olive oil production as the main consequence of pathogen introduction and spread (Almeida 2016a; Saponari et al. 2019). However, losses in food provisioning are just the tip of an iceberg, with death and removal of infected olive plants recently predicted to potentially prime a cascade of events resulting in the destruction of the local biodiversity and economy (Ali et al. 2021). Moreover, the current measures aimed at containing *X. fastidiosa* outbreaks by controlling the vector may have major, and overlooked, side effects. In this regard, soil tilling against juveniles and treatments with synthetic pesticides targeting adults have been proposed to control the populations of the meadow spittlebug *Philaenus spumarius* (Hemiptera: Aphrophoridae), which is the main driver of *X. fastidiosa* secondary spread within Apulian olive orchards (Cornara et al. 2017b, 2019; EFSA et al. 2019). Extensive soil tilling, especially in dry environments as Mediterranean olive orchards, may affect soil quality, augment the risk of desertification, and reduce habitats sheltering beneficial arthropods (i.e., predators, parasitoids and pollinators) (Kairis et al. 2013; Karamaouna et al. 2019; Bodino et al. 2020; Mesmin et al. 2020; Molinatto et al. 2020). Although pesticides are generally considered as unavoidable and essential tool to protect crops and ensure good yields, they represent one of the main drivers of the current biodiversity decline (Brühl and Zaller 2019; Sánchez-Bayo and Wyckhuys 2019; Chávez-Dulanto et al. 2020). Such decline of biodiversity might promote the spread *X. fastidiosa,* which is vectored by generalist insects like *P. spumarius*, as generalists can occupy the niches left empty by species affected by the decline (Civitello et al. 2015; Sánchez-Bayo and Wyckhuys 2019). Therefore, tools aimed at reducing abundance of generalist vectors might indeed in the long-term lead to a further spread of the plant disease by favouring these species. In addition, even considering the short-term efficacy of a pesticide-based vector control strategy, chemical control offers a highly variable reduction in disease risk (Madden et al. 2000; Daugherty et al. 2015). Finally, even if reducing vector load is hypothesized to decrease the transmission probability (Purcell 1980), pesticides could not prevent feeding behaviours conducive to transmission. It is therefore mandatory to rethink about *X. fastidiosa* control strategies, developing and applying long-term sustainable tools against vectors and safeguarding ecosystem services. Manipulation of vector behaviours, such as reducing host plant finding, suitability and residency time, or interfering with insects' communication during crucial steps of their life cycle could represent an efficient and environmentally safe strategy for the containment of vector-borne plant pathogens (Fereres and Moreno 2009; Mazzoni et al. 2009; Mokrane et al. 2020).

Biotremology is the science that studies the use of substrate-borne vibrations in animal communication. Many insect groups, including spittlebugs, use vibrational signals for close-range interactions, especially as social and sexual communication, and predator-prey interactions (Avosani et al. 2020; Hill & Wessel 2016; Takanashi et al. 2019; Virant-Doberlet et al. 2019). Characterization and subsequent playback of species-specific vibrational signals on a host plant can therefore be used to disrupt relevant insect behaviors, resulting in the reduction of pest populations (Gordon and Krugner 2019; Mazzoni et al. 2019; Polajnar et al. 2015). For instance, species-specific vibrations transmitted to grapevine plants disrupt the mating behavior of the leafhopper *Scaphoideus titanus* (Hemiptera: Cicadellidae), which sexual communication relies on the exchange of vibrational signals (Mazzoni et al. 2009, 2019). Aggregation and mating signals have been exploited to develop trapping strategies for invasive pest species such as the brown marmorated stinkbug *Halyomorpha halys* (Hemiptera: Pentatomide) and the Asian citrus psyllid *Diaphorina citri* (Hemiptera: Psyllidae), respectively (Mankin 2019; Polajnar et al. 2019). However, *P. spumarius* is a highly polyphagous and abundant species, in which mating occurs throughout the season on different host plants, while oviposition happens during the fall on herbaceous plants after the breakage of the ovarian parapause (Morente et al. 2018; Witsack 1973). Therefore, applying vibrations to disrupt mating as a way to control spittlebug populations appears pointless.

Nevertheless, beside mating, vibrations can be used to affect pest behaviors, such as probing and feeding (Takanashi et al. 2019). Since insect mechano-receptors are usually tuned for specific signal proprieties (Lakes-Harlan and Strauß 2014; Virant-Doberlet and Cokl 2004), species-specific vibrational signals are more effective in interfering with behaviors than unspecific or broadband noises (Bomford & O'Brian 1990). In particular, the alarm or distress signals emitted by animals can be used as repellent stimuli and are more resistant to habituation, which commonly arise when the stimulus is an unspecific and monotonous noise (Bomford & O'Brian 1990). *Philaenus spumarius* could accordingly be sensitive to signals aimed at repelling conspecifics or expressing stress. In this regard, *P. spumarius* emits distress signals in the presence of other individuals, especially after a physical or vibrational interaction, as similarly reported for other insect species (Alexander 1957; Avosani et al. 2020). The female rejection signal is used by *P. spumarius* females to reject courting or approaching males, and likely contains temporal and/or spectral features that can affect crucial behaviors (Avosani et al. 2020). It is therefore possible that the continuous transmission of the rejection signal could disturb the spittlebugs resident on the treated plant and interfere with their activities. (i.e., impair feeding thus possibly decrease the probability of *X. fastidiosa* acquisition (Daugherty and Almeida 2009)).

In this work, we aimed at assessing whether and to which extent a vibrational stimulus based on the intra-specific signal “female-rejection” and transmitted on a suitable alternative host plant, i.e., as sunflower (*Helianthus annuus*), could interfere with spittlebug probing and feeding behavior. Here, we present the promising results gathered by coupling the real-time probing and feeding behavior observations with recordings of signal transmission and propagation on the tested plants and discuss the applicability of a vibrational control of spittlebug populations to olive plants.

2. Materials and methods

2.1. Insects and plants

Adults of *Philaenus spumarius* were collected from oak trees (*Quercus ilex*) in Valenzano (Apulia, Southern Italy, *Xylella fastidiosa*-free area) during June 2020. Collected individuals were caged in insect rearing tents (BugDorm-2120 Insect Rearing Tent, 60x60x60 cm) covered with a nylon net to protect the insects from excessive sunlight and storms and kept on a meadow in the premises of CIHEAM-Bari institute (Apulia, Southern Italy). Plants used for the rearing were sunflower (*Helianthus annuus*) and vetch (*Vicia sativa*), replaced fortnightly, and two-year old *Vitis vinifera* var. Cabernet Sauvignon cuttings; plants were watered twice per week. For the experiments, we used four-week-old sunflower plants (approximately 30 cm tall), grown inside pots (5x10x5 cm^3) filled with soil and vermiculite (6:1), and watered twice per week. Olive (*Olea europaea*) plants, var. Ogliarola salentina, were two-year old seedlings pruned in May 2020 and water-fertilized once per week (PLANTAFOL 30 10 10, VALAGRO), in order to obtain fresh shoots of approximately 30 cm by September. All the plants were reared inside a glasshouse under controlled conditions (26 ± 2°C, 60% relative humidity RH).

2.2. Vibrational stimulus design

A signal designed based on a species-specific (a synthetic interference signal, SIS, Figure 5.1) vibrational stimulus was used to investigate if the probing and feeding behaviour of *P*. *spumarius* could be affected by means of vibrations transmitted to a plant. The SIS consisted in a complex signal (Figure 5.1) assembled using the audio software Adobe Audition 3.0 (Adobe Systems, Inc., San Jose, CA, USA). The SIS was composed of harmonic elements (the chirps) derived from a female rejection signal (Avosani et al. 2020) recorded with a laser vibrometer (VQ-500-D-V, Ometron Ltd., Harpenden, UK) from a reflective sticker glued on a sunflower plant in close proximity to the insect (1 cm). The chirps were amplified (+10 dB boost) to increase the intensity of the frequency bands above 200 Hz (the resonance frequency of the mini shaker). Temporal and spectral features were modified to obtain a stimulus that could both cover the *P. spumarius* signals and be distressing. In this regard, within other insect species (i.e., stinkbugs), distress signals are emitted in response to disturbances and are characterized by high dominant frequencies and fast repetition rates (Lazzari, Manrique and Schilman 2006). To create the SIS, the repetition time between chirps was reduced (0.28 ± 0.06 s) compared to the original female signal (Avosani et al. 2020), while the chirp dominant frequency was set to 820 Hz. The signal was propagated by using the below-mentioned setup (exciter-amplifier-laptop) to plants of sunflower and olive (we did not test intensities that turned substrate-borne signals to air-borne). The signal was transmitted at two different intensities (SIS15 and SIS50, respectively), in that the volume was set from the laptop (default Windows music player), being the volume of the SIS50 three times higher than the SIS15’s one.

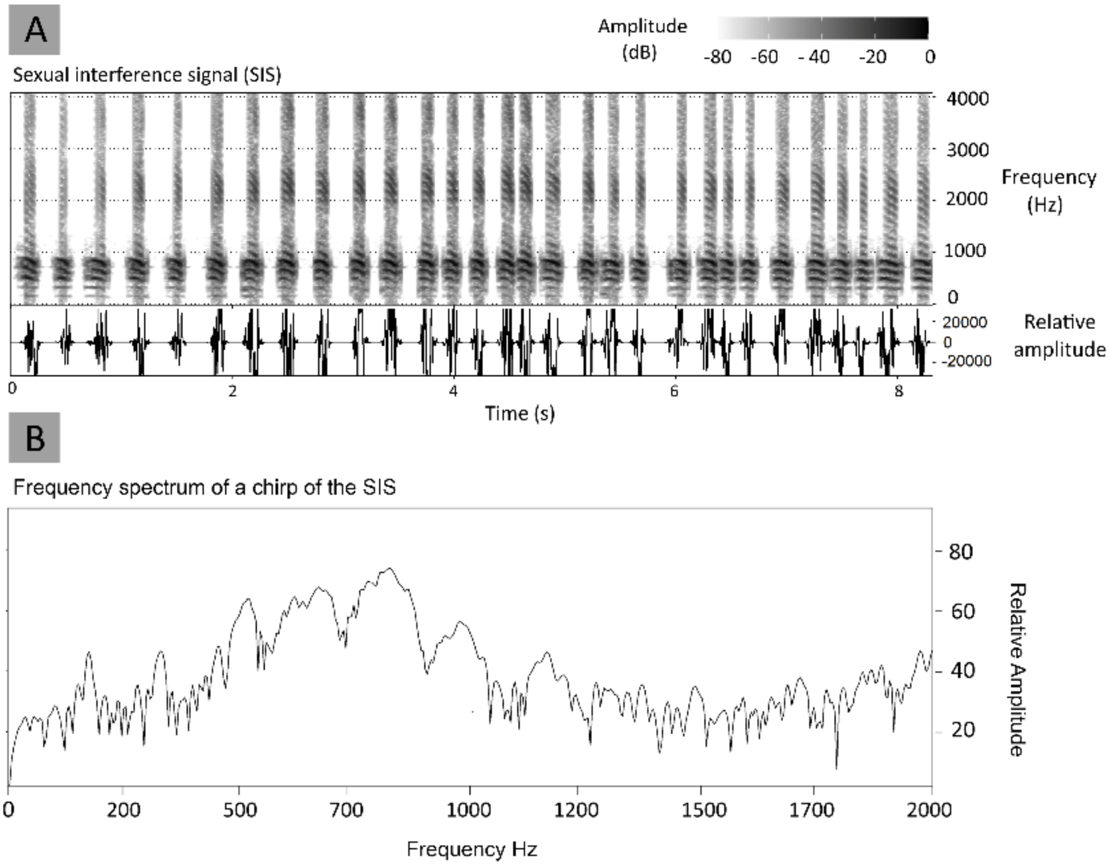

Figure 5.1. A) Spectrogram (above) and oscillogram of the synthetic interference signal (SIS). The signal was 8.3 s of duration and was composed of 30 chirps. B) Frequency spectrum of a chirp of the SIS (mean duration of 0.18 s and a dominant frequency of 820 Hz).

2.3. Vibration stimuli effect on *P. spumarius* probing and feeding behaviour

Experiments were conducted in the Electrical Penetration Graph (EPG)-lab at CIHEAM-Bari (Apulia, Southern Italy) at temperature 25 ± 1°C and RH 65% during August and September 2020. Spittlebugs were tethered and connected to the EPG amplifiers following the protocol described by Cornara et al. (2018). The feeding behaviour of the tested insects was recorded with a Giga 8-DC EPG (EPG-systems, Wageningen, The Netherlands) at 1 Giga Ohm input resistance, assembled inside a Faraday cage, under controlled conditions (24 ± 1°C, 40% RH). Output from the EPG at 100x gain was digitalized at a sample rate of 100 Hz per channel and recorded using Stylet+ software (EPG-systems, Wageningen, The Netherlands). For each test, vibrational stimuli were transmitted by means of a mini shaker (Visaton BS 76; Visaton GmbH & Co, Germany) in contact with the stem of a sunflower plant by means of a conical rod (5 cm long). The conical rod was perpendicularly pointed on the plant stem halfway between the apical and basal portion, hence ca. 15 cm from the soil. The mini shaker, kept in position using a clamp, was plugged to an amplifier (Nobsound NS-01G, Nobsound, Shenzhen Cavins Tech Ltd, China) controlled by a laptop (HPEnvy 15). The plant and the clamp with the exciter were placed inside a Faraday cage hosting the EPG.

Test on sunflower. Firstly, we performed Electrical Penetration Graph (EPG)-assisted 3h observations of the probing and feeding behaviour of *P. spumarius* males and females on sunflower plants treated with SIS at two different volumes. The signals were turned-on 20 min before the insect was placed on the plant and loop-played for the 3h EPG recordings by using the software Windows

Music Reader. For the control, we used the same set-up as for treated plants, with the conical rod in contact with the plant, but turning the amplifier off. The experimental design was completely randomized: a single treatment/signal was carried out per time (during each 3h recording a single treatment was performed, with two replicates/plants per time, with the plants at ca. 80 cm from each other) to avoid interference among the different signals (Figure 5.2). The position (channel of the EPG device used) of each treatment/signal was switched during each recording to avoid position effects. We performed each 3h EPG observation with a single combination insect/plant. Total number of recordings carried out per treatment and sex on sunflower are reported in Table 5.1. The insects were offered 5-cm apical portion of the sunflower plant, with access to stem, petiole and leaf. Probing and feeding behaviour were recorded for three hours with the EPG. For the analysis of the probing and feeding behaviour, we followed the waveforms definitions by Cornara et al. (2018) with slight modifications. We considered ten patterns (waveforms representing the different steps/behaviours performed by the insect from the insertion of stylets into the plant to their withdrawal): i) np, non-probing; ii) C, pathway; iii) Xc, xylem contact; iv) Xi, xylem ingestion (frequency > 0.1 Hz); v) LF, low frequency xylem ingestion (frequency < 0.1 Hz); vi) npN, non-pathway interruption of xylem activity; vii) pN, pathway interruption of xylem activity; viii) R, resting; ix) Xe, behavior putatively associated to *X. fastidiosa* inoculation (Cornara et al. 2020); (x) W, stylets withdrawal. Overall, we assessed the differences in probing and feeding behaviour among treatments by considering the variations in: i) non-sequential variables (WDI, waveform duration per individual; NWEI, number of waveform events per individual; WDEI, median duration of each waveform event per individual; pWDI, percentage of the total probing time spent in a certain waveform); ii) sequential variables; iii) number of probes (with or without xylem ingestion) performed by the spittlebugs. In the present work, sequential and non-sequential variables considered, and waveforms definition and supposed/ascertained biological meaning were based on previous studies (Cornara et al. 2018, 2020). We additionally took note of the number of spittlebugs per treatment spending the 3h EPG performing activities other than probing, as walking, resting, or dabbing plant tissues without inserting the stylets (behaviour observed with a x10 magnifying lens), and alive at the end of the recording; these insects are referred to as “non-probing individuals”.

“Start & Stop” test. In this test, the feeding behaviour of spittlebug males was recorded from sunflower plants treated with SIS50 (the stimulus displaying the greatest effect on probing and feeding behaviour, see results) were conducted. A spittlebug male connected to the EPG-amplifier was permitted to initiate a probe on the sunflower plant connected to the mini shaker; the signal (SIS50) was then activated and loop-played for ten minutes once the insect reached a xylem vessel and started xylem ingestion. We analysed insect probing and feeding behaviours during the ten minutes with the signal on, and during the successive ten minutes with signal off. A single combination insect/plant was used for each recording. Twenty-six replicates (26 males) were carried out.

Test on olive. According to the results on sunflower (see results), we tested the effect of SIS50 on the feeding behaviour of *P. spumarius* males on olive plants, following the same design described for sunflower plants. A total of 40 replicates, i.e., 20 males for SIS50 and 20 for the control, was carried out.

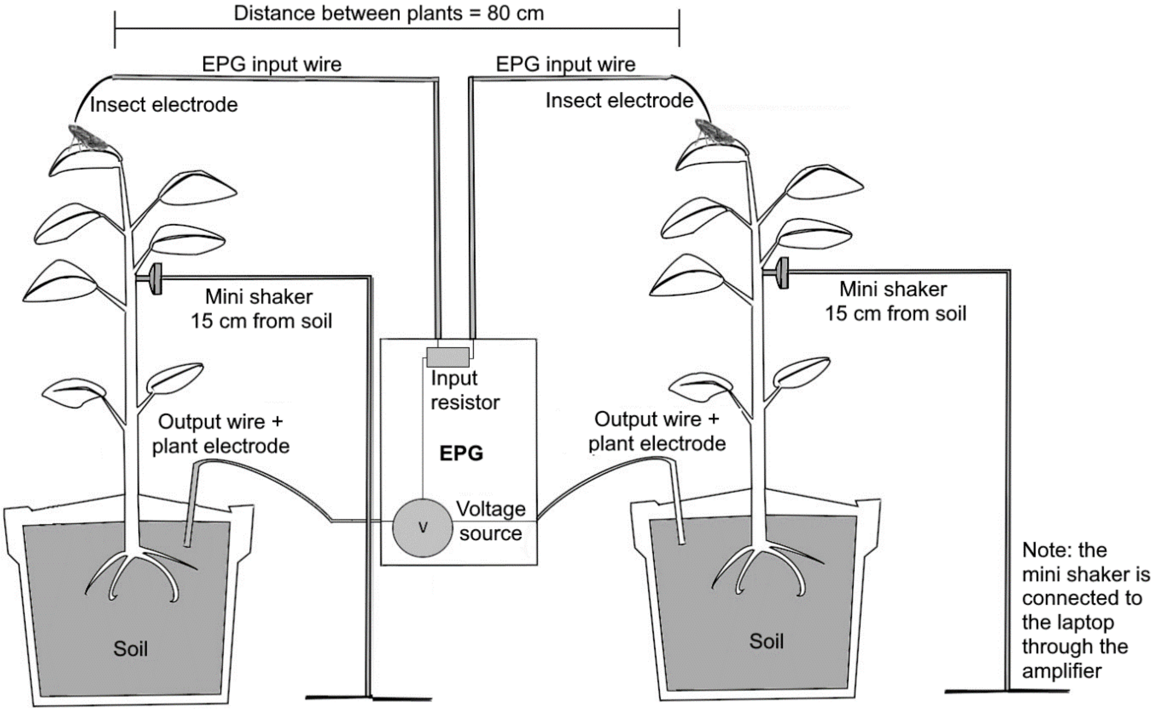

Figure 5.2: Experimental setup of Electrical Penetration Graph (EPG) tests. A specimen of *P. spumarius* connected to a channel of the EPG amplifier by a thin electrode was offered the 5-cm apical portion of the host plant, with access to stem, petioles and leaves. Vibrational stimuli were transmitted via a mini shaker, which was in direct contact with the stem of the plant. A single treatment/signal was carried out per time with two EPG channel per time.

2.4. Data analysis

We performed a G-test in a contingency table (2 × 3) followed by a Ryan multiple comparisons for proportions (Ryan 1960) to compare the number of probing individuals (either males or females) between treatments and control.

We explored the effect of vibrational signals on spittlebugs probing and feeding behaviour (i.e. WDI, NWEI, WDEI, pWDI, probes, and sequential variables) with a linear mixed-effects model (lme; “REML” method). The explanatory variables were treatments (SIS15, SIS50, and control), sex, and the interaction between these two factors. These variables showed no collinearity. Differences among treatments and control were assessed by Tukey’s test (Tukey’s “honest significant differences” (HSD) method) for pairwise comparison. The data obtained from the EPG were transformed when necessary with ln (x + 1) or √ (x + 1) to reduce heteroscedasticity and improve normal distribution. We accounted for the nested design of the study by including the mini shaker used as random factor. We additionally explored xylem ingestion duration (Xi WDI) trend during the 3h recording using Generalized Linear Mixed-Effects Models (glmer) with treatment, recording time (hour), their reciprocal interaction, and sex as explanatory variables, and mini shaker and insect identity as random factors (Poisson distribution).

All the analyses were performed in R (R Core Team, 2020). We ran the models using *nlme* and *lme4* packages (Bates et al. 2014; Pinheiro J, Bates D, DebRoy S 2016). We checked the models for residual distribution using the *car* package (Fox and Weisberg 2019). There was no evidence of either spatial or temporal autocorrelation of model residuals (analyses performed using the *ncf* and *acf* packages, respectively (Bjornstad 2013)). Graphs were generated using *ggplot2* package (Valero-Mora 2010). Only insects that performed at least a probe, namely insects that inserted the stylet into the plant tissues, were considered for statistical analysis.

2.5. Signal transmission on host plants

Observations of signal propagation and characteristics on host plant species were conducted in the biotremology laboratory at Fondazione Edmund Mach (Trentino, Northern Italy), inside a sound insulated chamber maintained at a temperature of 22 ± 1 °C and 65% RH, with plants placed on an anti-vibrational table (Astel s.a.s., Ivrea, Italy). The intensity values of the SIS were measured on the apical parts (approx. 10 cm of distance between each recording points) of sunflower (namely "H") and olive (namely "O") plants. The height (30 cm) and shape of the plants were similar to the ones used for EPG trials. The stimuli were transmitted perpendicularly to the stems of the plants by means of the same experimental design used for the EPG tests. Either two sunflower or olive plants were used for each recording session to simulate EPG conditions. A laser vibrometer (VQ-500-D-V, Ometron Ltd., Harpenden, UK and OM DS VibroGo E 52039, Polytec GmbH, Waldbronn, Germany) was pointed towards each plant. Vibrations were recorded from small pieces of reflective tape (0.5x0.5 cm^2) glued to three different points of the plants, after setting the laser sensitivity to 5 mm/s/V. The signals were acquired with a hard drive multichannel LAN-XI data acquisition device (Brüel and Kjær Sound and Vibration A/S) with a sample rate of 8192 Hz. Recordings were then analysed using Matlab 2020 (1994-2021 The MathWorks, Inc.) to compute the fast Fourier transform (FFT) with window length of 1024 samples, frequency resolution of 8 Hz, 66.7% overlap, and Hann window. The spectra of the recorded signals were extracted, visualized, and compared. The signal (the SIS) was transmitted at two different intensities (ratio 1:3, SIS15 and SIS50, respectively) to plants of sunflower and olive. SIS15 was tested on four sunflower plants (H), with a measurement point on two of the apical leaves and a measurement point on the stem, the recordings duration was 10 s for each point. SIS50 was tested on four sunflower plants (H) and six olive plants (O), with a measurement point on two of the apical leaves and a measurement point on the stem, the recordings duration was 10 s for each point.

3. Results

3.1. Vibration stimuli effect on *P. spumarius* probing and feeding behaviour

Test on sunflower. The greatest number of non-probing individuals during the 3h recordings was observed for SIS50, in that 31.25% of the females and 28.57% of the males performed activities other than probing, as walking and resting (Table 5.1). All these insects were alive at the end of the EPG recordings. The number of either probing males or probing females was statistically different between groups (males: G=7.93, df=2, p-value=0.019; females: G=8.59, df=2, p-value=0.014). In particular, the post-hoc test showed that the treatment with the SIS50 significantly reduced the number of probing individuals compared to either the SIS15 (which was statistically different only for females) or the control (Table 5.1).

Table 5.1. *Philaenus spumarius* tested by EPG (Electrical Penetration Graph) on sunflower. Different superscript letters indicate statistically significant difference between groups (Ryan multiple comparisons for proportions).

Female				
Treatment	Tested	Probing individuals	Non-probing individuals	Non-probing (%)
Control ^{a}	17	17	0	0
SIS15 ^{a}	15	14	1	6.66
SIS50 ^{b}	16	11	5	31.25
Male				
Treatment	Tested	Probing individuals	Non-probing individuals	Non-probing (%)
Control ^{a}	18	17	1	5.55
SIS15 ^{b}	19	19	0	0
SIS50 ^{b}	14	10	4	28.57

No significant differences were observed between treatments and control in the total time spent with stylets inserted into the plant tissues (probing time); however, probing in males on plants treated with SIS50 (91.15 min) was considerably shorter than in control males (158.43 min) in terms of median values. Considering the probes, SIS50 significantly affected the number of male probes comprising a sustained xylem ingestion (duration of the Xi event longer than 5 min) (t = -2.259, p = 0.026), which resulted reduced compared control males (t = 3.241, p = 0.021).

Regardless of sex, SIS15 and SIS50 had a significant impact on the total duration of xylem ingestion (Xi WDI) (SIS15: t = -2.932, p = 0.004; SIS50: t = -3.513, p < 0.001). Specifically, spittlebugs spent significantly less time ingesting xylem sap from plants treated with either the SIS15 (t = 2.932, p = 0.012) or the SIS50 (t = 3.513, p = 0.002) compared to control, with no differences between the signals (Figure 5.3). However, in terms of median values, the shortest xylem ingestion was observed in the group treated with the SIS50 (Control = 92.365 min; SIS15 = 51.110 min; SIS50 = 30.080 min), with a reduction of Xi of 67.43%. Similarly, we observed a sex-independent effect of SIS15 (t = -4.687, p < 0.001) and SIS50 (t = -3.686, p < 0.001) on the duration of non-pathway interruptions (npN WDI), which resulted significantly shorter in the treated groups than in the control (SIS15: t = 4.687, p < 0.001; SIS50: t = 3.687, p = 0.001). Both SIS15 and SIS50 had a significant effect on the trend of xylem ingestion duration (Xi WDI) during the 3h recording. Xylem ingestion was significantly shorter on plants treated with SIS50 starting from the first hour, whilst in either control and SIS15, it progressively decreased during the second and third hour (Table 5.2; Figure 5.4). Xylem ingestion and non-pathway interruption durations were reduced on plants treated with either the SIS15 (Xi: t = -3.021, p = 0.003; npN: t = -4.752, p < 0.001) or the SIS50 (Xi: t = -3.677, p < 0.001; npN: t = -3.466, p =< 0.001). The two events were shorter than control in both SIS15 (Xi: t=3.021, p=0.009; npN: t = -4.75, p < 0.001) and SIS50 (Xi: t = 3.678, p = 0.001; npN: t = 3.467, p = 0.002). Considering xylem ingestion, the lowest median value was observed in SIS50, with a reduction of ca. 37% compared to spittlebugs feeding on control plants (Figure 5.5). The vibrational stimuli also impacted the number of xylem ingestions (Xi NWEI; Figure 5.6) (SIS15: t = -2.886, p = 0.005; SIS50: t = -3.491, p < 0.001) and of the non-pathway interruption events (npN NWEI) (SIS15: t = -4.710, p<0.001; SIS50: t = -3.235, p = 0.001). Spittlebugs on plants treated with the SIS15 and SIS50 performed fewer xylem ingestions (SIS15: t = 2.887, p = 0.013; SIS50: t = 3.491, p = 0.002) and non-pathway interruptions (SIS15: t = 4.710, p < 0.001; SIS50: t = 3.235, p = 0.005) compared to control, with no significant difference between treatments. No statistically significant differences were observed in the median duration of single waveforms events (WDEI).

Time required by the insect from the beginning of the recording to perform the first xylem contact (np to first Xc) was significantly longer on plants treated with SIS50 than on control plants (t = -2.469, p = 0.041). Both SIS15 (t = 2.806, p = 0.006) and SIS50 (t = 3.161, p = 0.002) affected the time required for the first xylem ingestion (np to first Xi), which resulted significantly longer for the treatments than for the control (SIS15: t = -2.807, p = 0.017; SIS50: t = -3.161, p = 0.006), with no difference between the two treatments (median values: Control = 7.55 min; SIS15 = 14 min; SIS50 = 24.40 min). The vibrational signals impacted the time required for sustained xylem ingestion to occur (np to first sustained Xi), namely a xylem ingestion event longer than 5 min (SIS15: t = 2.225, p = 0.028; SIS50: t = 3.508, p < 0.001). Even so, according to Tukey's test results, the time required to perform this behaviour was significantly longer in SIS50 (35.80 min) compared to control (11.95 min) (t = -3.508, p = 0.002). No significant differences were observed when considering the time required by the spittlebug to perform the first probe (time to first probe).

As expected, considering its low occurrence rate particularly on favorable plants, waveform Xe, putatively associated in *P. spumarius* to *X. fastidiosa* inoculation (Cornara et al. 2020), was not observed in any of the recordings performed either on olive or on sunflower.

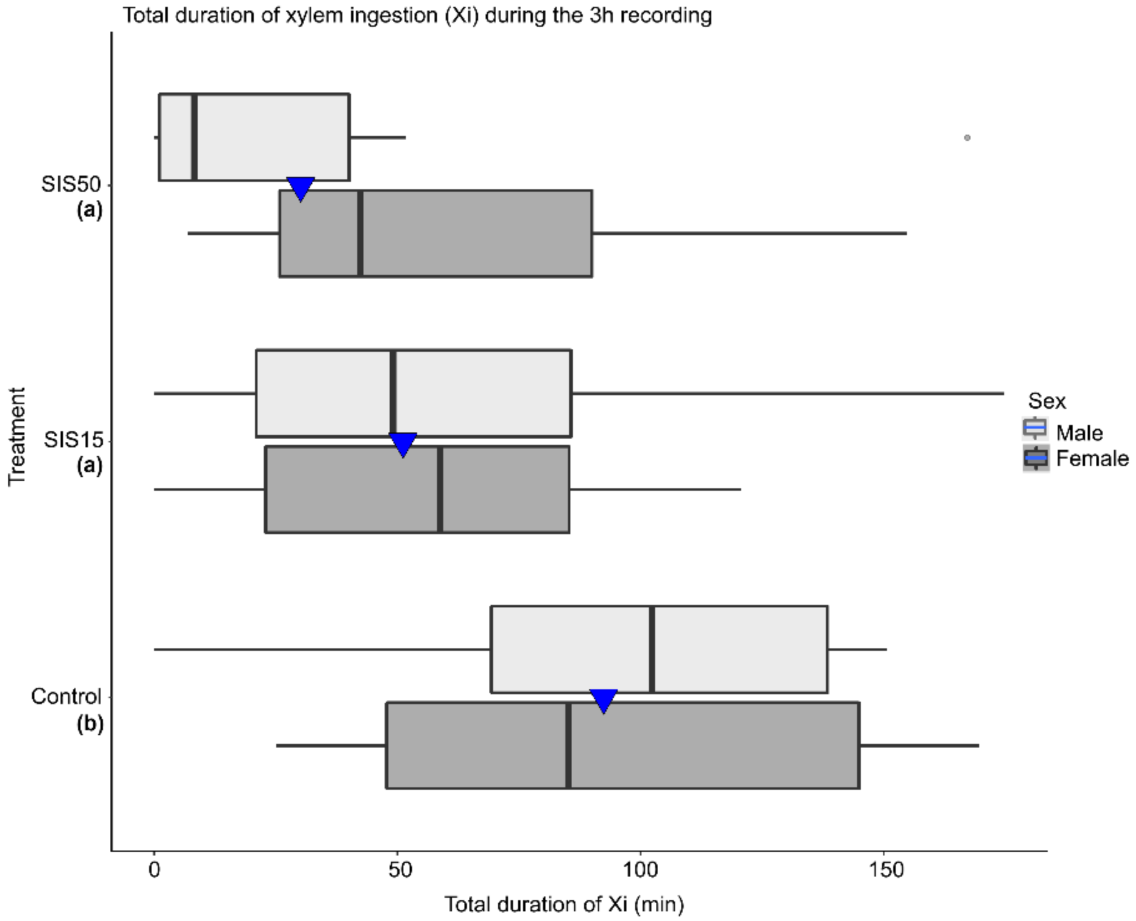

Figure 5.3. Total duration of xylem ingestion (Xi WDI) during the 3h recordings on sunflower plants treated with SIS15, SIS50 and control. The blue triangles indicate median values. Letters in bold

within brackets indicate statistically significant differences among treatments according to the results from Tukey's test.

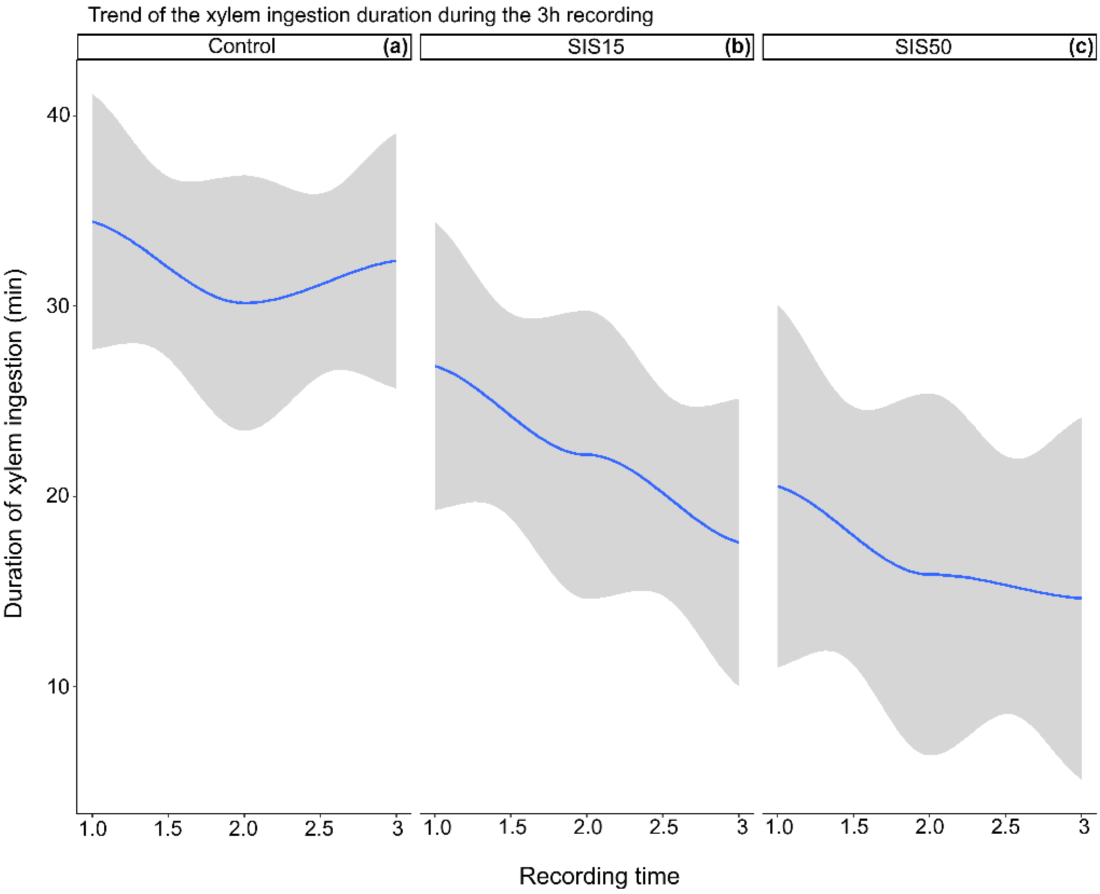

Figure 5.4. Trend of the duration of xylem ingestion (Xi WDI) during the 3h recordings on sunflower plants treated with SIS SIS15, SIS50 and control. Letters in bold within brackets indicate statistically significant differences among treatments according to the results from Tukey's test.

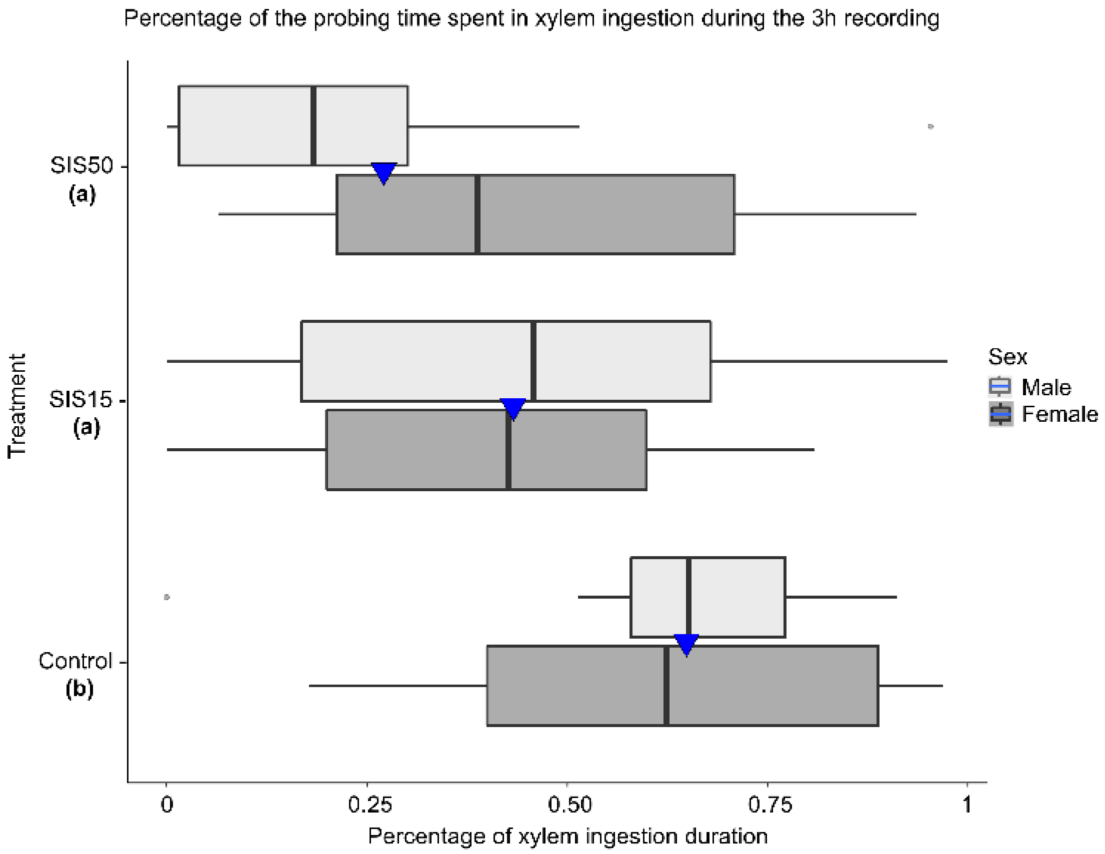

Figure 5.5. Total duration of xylem ingestion (Xi) expressed as percentage of the probing time (pWDI Xi) during the 3h recordings on sunflower plants treated with SIS SIS15, SIS50 and control. The blue dots indicate median values. Letters in bold within brackets indicate statistically significant differences among treatments according to the results from Tukey's test.

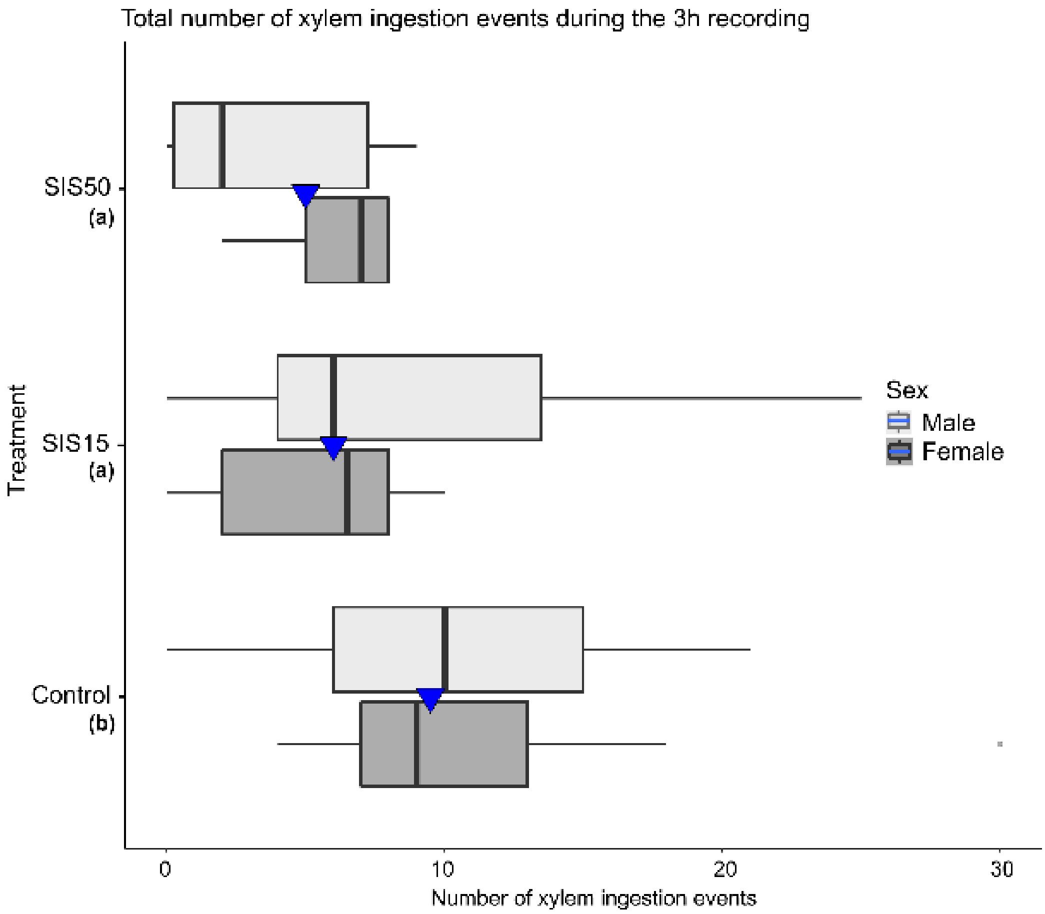

Figure 5.6. Total number of xylem ingestion events (Xi NWEI) during the 3h recordings on sunflower plants treated with SIS SIS15, SIS50 and control. The blue triangles indicate median values. Letters in bold within brackets indicate statistically significant differences among treatments according to the results from Tukey's test.

Table 5.2. Glmer model (A) and Tukey's test (B) results for trend of xylem ingestion duration (Xi WDI) during the 3h EPG recording (confidence level: 0.95).

A)

	estimate	SE	z value	*p* (>|z|)
(Intercept)	3.496	0.070	49.611	< 0.001
SIS15	0.007	0.074	0.093	0.926
SIS50	-0.343	0.095	-3.625	< 0.001
Time	-0.030	0.022	-1.374	0.169
Sex	0.019	0.027	0.722	0.470
SIS15: Time	-0.183	0.036	-5.112	< 0.001
SIS50: Time	-0.142	0.046	-3.129	0.002

B)

Contrast	estimate	SE	z ratio	*p*
Control,2 - SIS15,2	0.359	0.030	11904	< 0.001
Control,2 - SIS50,2	0.628	0.038	16678	< 0.001
SIS15,2 - SIS50,2	0.268	0.041	6625	< 0.001

“Start & stop” test. Within a short time after its transmission (median: 4 min), the SIS50 elicited the withdrawal of the stylets in 14 out of 26 males tested. Once the stimulus stopped, four of the males that withdrawn the stylets ceased their feeding activity and jumped off the plant, while the others restarted probing within ca. 3 min (median value). The other twelve tested males continued xylem ingestion during and after the treatment, with no evident difference between signal on and off.

Test on olive. In contrast with results obtained on sunflower, no statistically significant differences in probing and feeding behaviours of *P. spumarius* on olive plants between control and treatment (SIS50) were observed (data not shown).

3.2. Signal transmission on host plants

The intensity values of the SIS (SIS15 and SIS50) were lower when recorded from the leaf than from the stem of the sunflower (Figure 5.7). The intensity values of the SIS reached on sunflower a 10-fold increase when played at higher intensities (SIS50) compared to SIS15 (Figure 5.7). On the other hand, the intensity of the SIS50 did not differ from stem to leaf on olive plants (Figure 5.8). The frequency spectrum of the SISs (Figure 5.1) was rather conserved in all its components when transmitted to sunflower, as the main intensity peaks were concentrated on the original frequency bands of the signal (Figure 5.7). However, the recorded spectrum differed from the original signal in that most the signal intensity was recorded at 840 Hz, while the spectrum below 400 Hz displayed a very low intensity. By comparing the intensity values of the SIS50 on sunflower and olive, a significant decrease in the signal intensity was recorded (Figure 5.9), while the spectrum of the signal (the overall shape) was rather conserved between the two host plants. The signal showed three main frequency peaks in both hosts, namely 536, 680 and 840 Hz. The mean values of the spectra referring to SIS on olive and sunflower plants, the amplitude of the peaks (velocity expressed in µm/s) and the dominant frequencies are reported in Table 5.3.

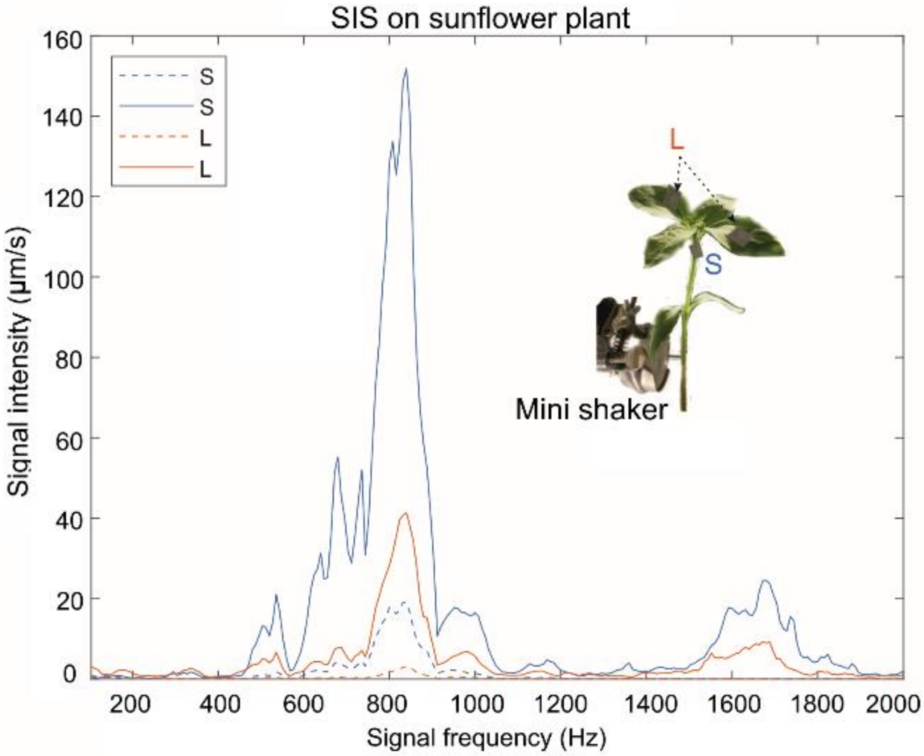

Figure 5.7. Synthetic interference signal (SIS) medium spectrum after fast Fourier transform of the signals recorded on the apical parts of four sunflower plants. Values on stem (S, blue lines) and leaves (L, orange lines) are reported, for SIS15 (dashed lines) and SIS50 (solid lines).

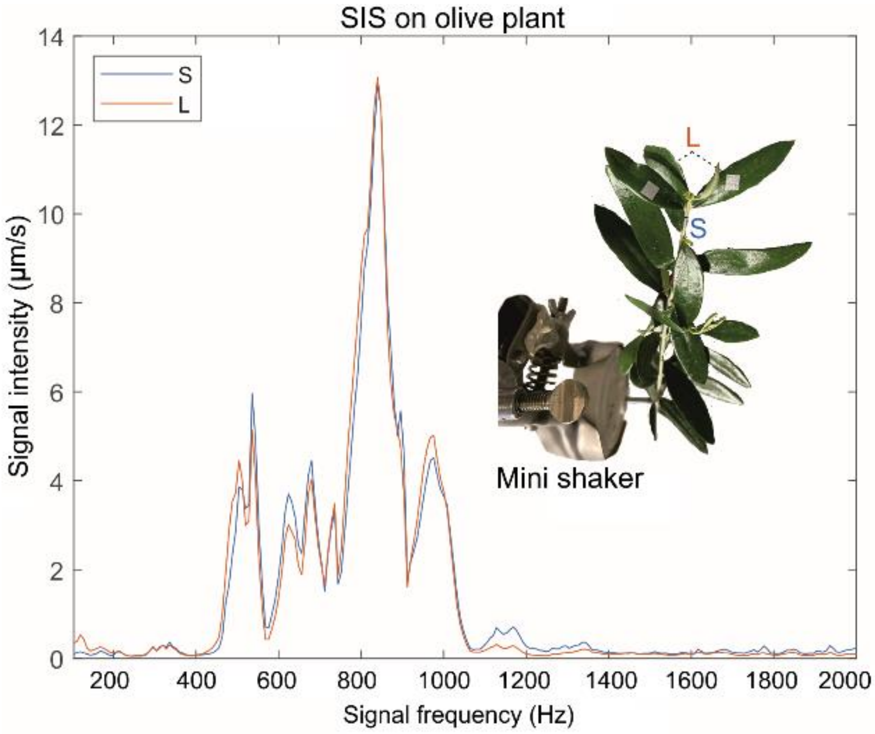

Figure 5.8. Synthetic interference signal (SIS) medium spectrum after fast Fourier transform of the signals recorded on the apical parts of six olive plants. Values on stem (S, blue line) and leaves (L, orange line) are reported for SIS50.

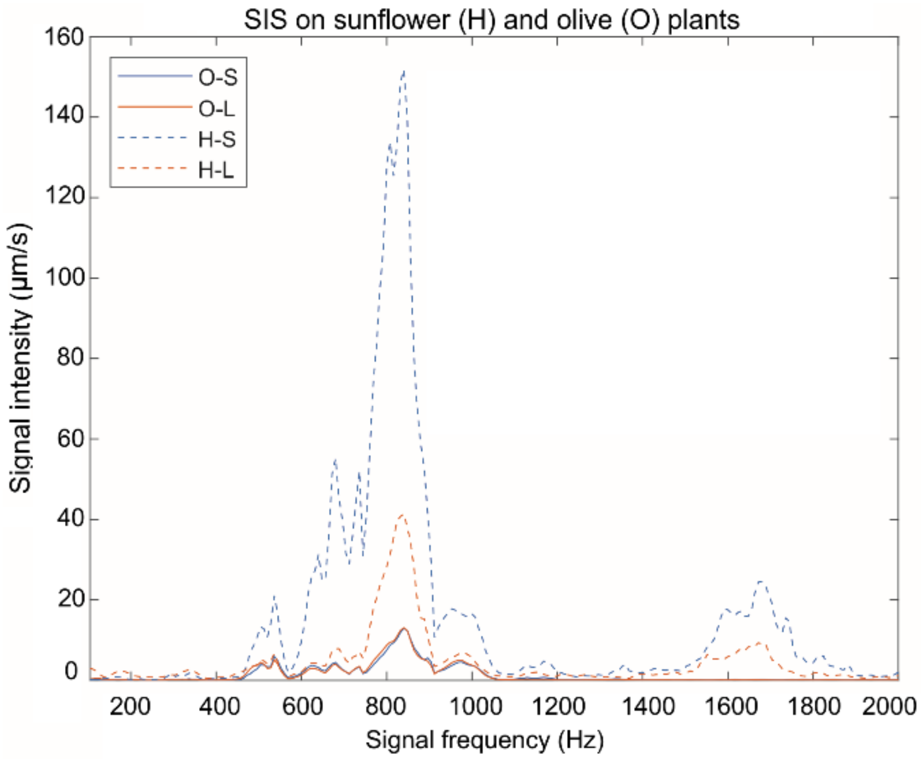

Figure 5.9. Comparison of the SIS50 (synthetic interference signal) amplitudes recorded on the apical parts of olive plants (O) and sunflower plants (H). Values on stem (S) and leaves (L) are reported.

Table 5.3. Means ± standard deviations (SD) of the highest recorded intensities (µm/s) occurring in three frequency bands of the spectrum of synthetic interference signal (SIS), which was transmitted to either sunflower or olive plants.

			Signal transmitted: SIS		
			Sunflower		Olive
Freq. band (Hz)	Rec. point	Statistics	SIS15	SIS50	SIS50
536	Stem	Mean ± SD	1.80 ± 0.73	21.03 ± 8.42	5.97 ± 6.46
	Leaves	Mean ± SD	0.41 ± 0.40	6.66 ± 4.53	5.14 ± 6.77
680	Stem	Mean ± SD	4.21 ± 3.44	55.22 ± 41.77	4.45 ± 4.66
	Leaves	Mean ± SD	0.46 ± 0.31	7.80 ± 4.76	4.03 ± 5.55
840	Stem	Mean ± SD	19.02 ± 14.85	151.88 ± 102.68	12.90 ± 11.94
	Leaves	Mean ± SD	3.13 ± 3.35	41.36 ± 34.40	13.07 ± 14.66

4. Discussion

In this work, we demonstrated that the transmission over a suitable host plant of a stimulus (SIS) designed based on a vibrational signal used by spittlebugs for intra-specific communication played at high intensity (SIS50) could disrupt relevant feeding behaviours of *P. spumarius.*

The stimulus impeded probing in ca. 30% of the insects tested, causing ceasing of probing activities in around 50% of the tested males upon signal onset. Feeding was significantly impaired in probing individuals, with a ca. 67% reduction of the time spent by the individuals in xylem ingestion over the 3h EPG recordings, 37% when just considering the time spent with stylets inserted into the host tissues (probing time). The signal also reduced the number of ingestions performed by the insects, and tripled the time needed for xylem ingestion to occur. This interference is likely the outcome of the interaction between diverse factors. For instance, being perceived as a stressing input, the signal could have a direct effect on the spittlebug that ends up diverging from its “normal” behaviour. Vibrations could also make the plant an unsuitable substrate, considering that the duration of xylem ingestion is a clear indicator of host plant suitability, with short duration indicating an unsuitable/barely suitable substrate (Sandanayaka et al. 2013; Markheiser et al. 2019). As decreased population densities of a vector may result in high crop yields, a similar effect is expected by reducing its feeding activity (Madden et al. 2000). Vector density, time spent on the host plant, and transmission efficiency accordingly concur in determining *X. fastidiosa* transmission (Purcell 1982; Daugherty and Almeida 2009). In the Apulian scenario, the relative low spittlebug efficiency in transmitting *X. fastidiosa* to the host plant is compensated by the high number of individuals residing on olive plants for one to two months from sprouting to summer drought (Cornara et al. 2016, 2017a; Bodino et al. 2020). A strategy aimed at reducing vector load and residency time on olive plants could accordingly lead to a lower transmission probability and pathogen spread. An effective tool should act as repellent by rapidly hampering vector probing and feeding behaviour, within the first minutes of the insect/plant interaction to prevent *X. fastidiosa* acquisition and transmission (Almeida et al. 2005; Cornara et al. 2020). Bacterial cells inoculation into the host plant has been recently proposed to be associated to an occasional vector behaviour, which is performed few minutes after the insertion of the stylets into the host tissues, possibly when the xylem chemical of physiological conditions fails to stimulate sap ingestion (Cornara et al. 2020). Therefore, the higher the number of probes and the greater number of vectors probing result in high chances of bacterial inoculation (Daugherty and Almeida 2009). On the other hand, bacterium acquisition is likely associated to xylem sap ingestion from an infected vessel and to the number of vessels probed by the vector giving the heterogeneous distribution of *X. fastidiosa* within the host plant (Almeida 2016b). Tackling transmission is therefore a matter of reducing the chances for a vector to acquire *X. fastidiosa* with the subsequent pathogen spill over. Fewer vector/plant contacts caused by reduced suitability of the host plant and/or reduced permanence of the vector on the substrate, would translate in fewer and shorter xylem contacts, thus in possibly reduced transmission probabilities. A candidate signal for vibrational-mediated vector control should explain its repellence action from the very beginning of the insect/plant interaction, given that bacterium inoculation was proposed to occur within the first minutes from stylets insertion into plant tissues (Cornara et al. 2020). Spittlebugs on SIS50-treated plants showed reduced ingestion of xylem sap from the beginning of the recording, additionally sharply decreasing over time, suggesting a fast repellent action that could be exploited to reduce spittlebugs population on the host plant, thus possibly reduced pathogen transmission chances. However, transmission trials with *X. fastidiosa* on plants treated with SIS50 are urgently needed to confirm our hypothesis.

The SIS15 impacted *P. spumarius* feeding behaviour, although to a minor extent compared to SIS50. Greater disruption yielded with the latter signal is likely a non-linear response to the ten-fold increase in the recorded intensity. In the leafhopper *Scaphoideus titanus,* the inhibition of a crucial behaviour such as male calling was achieved by using a noise transmitted at relatively high amplitudes. Although the noise reduced the leafhopper searching behaviour when perceived by the male at intensities above 2.5 µm/s, the complete disruption of the male-female communication occurred when the noise intensity exceeded 15 µm/s (Polajnar et al. 2016). Considering that *S. titanus* signals reached intensities of 50 µm/s when recorded from the leaf where the insect is placed, mating disruption was achieved when the noise intensity was 1/3 the intensity of the natural emitted signal. These outcomes suggest that a disturbance stimulus can be efficient even if the signal reaches the insects at very low intensities (Eriksson et al. 2011). In our study, the main SIS50 frequency peaks could reach *P. spumarius* at intensities ranging from a minimum of 6 µm/s to a maximum of 150 µm/s on sunflower, thus resulting in a strong behavioural response, i.e., the immediate alteration of the feeding activity. The inefficacy of SIS50 in affecting the spittlebug probing and feeding behaviour on olive is likely related to the dramatic decrease in signal intensity on the woody plant compared to sunflower particularly in correspondence of the three main frequency peaks recorded in both hosts, namely 536, 680 and 840 Hz. However, the lack of behavioural alteration on “vibrationally-treated” olives could not be ascribed just to signal intensity in correspondence of these three peaks, given the values of this parameter are rather similar between SIS15 on sunflower and SIS50 on olive. Low frequency components may also have played a role, given that small insects such as *S. titanus* perceive and respond to low frequencies (i.e., 220–250 Hz) when intensity values range between 1 and 0.1 µm/s (Eriksson et al. 2011, 2012). Accordingly, of particular interest is the spectrum of the SIS below 400 Hz. In fact, the fundamental frequency of *P. spumarius* vibrational signals ranges between 150 and 200 Hz and insect mechanoreceptors such as the femoral-chordotonal organ are responsive to low and medium frequency vibrations (200-900 Hz) in an intensity-dependent fashion (Stein and Sauer 1999). In olive, the spectrum below 400 Hz is drastically reduced, whilst it was conserved on sunflower although displaying a reduced intensity compared to the original signal (Figure 1, Figure 7). Lowest velocity threshold values perceived by insects are between 1 and 10 µm/s, which corresponds to the intensities measured in the frequency components below 400 Hz when the SIS was played on sunflower at both intensities. In the case of the SIS15, the long exposure to low-frequency components likely resulted in an accumulation of stress for the insect and in a progressive reduction of the feeding activity, while no behavioural effects were observed in olive due to the absence of these components and the overall lower intensity values. By increasing the intensity of the frequency peaks within the 0-400 Hz range it would possibly obtain a greater impact in terms of spittlebugs feeding disruption. On the other hand, the SIS frequency components recorded on the tested plants likely elicited the feeding impairment on sunflower because it reached the insect at high intensities, although further research is needed to characterize the impact of each of the signal features on spittlebugs behaviour. Lastly, considering that plants are frequency and intensity filters characterised by different resonance proprieties (Michelsen et al. 1982; Polajnar et al. 2012; Mazzoni et al. 2014), studies should be addressed at effectively transmitting the candidate signal to olive plants, maximizing the feeding disruption effect.

Beside the signal features and its behavioural role, the phenology and life cycle of the insect must be considered while designing “behavioural” control methods. In *P. spumarius*, the SIS50 affected the feeding behaviour in August-September (when females in nature emit mating signals), whereas rejection signals are mainly produced in spring (when females are sexually immature) (Avosani et al. 2020). The SIS50 had an apparently, although non-statistically significant, more marked effect on males than on females, being males responsive to female mating signals (Avosani et al. 2020), which could have been masked by SIS in September. If temporal and spectral features of SIS50 could both mask the female signals and cause distress, they could lead *P. spumarius* males and females to find a more suitable host for feeding and mating. Different levels of disturbance (i.e., soil tilling and vibrational stimuli) could accordingly affect adult populations by eliciting mass movements to other hosts and influence the oviposition suitability of certain sites, thus impacting the following populations (Bodino et al. 2019). A strategy aimed at repelling the spittlebug from olive plants is very promising, as this insect is highly polyphagous and can thrive to hosts alternative to olive (Weaver & King 1954; Bodino et al. 2019). In this regard, to estimate the efficiency of a similar strategy, it is crucial to assess the landscape composition of olive groves, considering the surrounding habitats, which can provide alternative hosts for spittlebug adults. Beside the landscape composition, other factors such as the phenology and mating status could influence the efficacy of the vibrational disturbance. Further research should therefore analyse the effect of the SIS, together with other species-specific signals in the vast repertoire of *P. spumarius* communication, throughout the season and on mated and unmated individuals, with a particular emphasis on spring and summer period, when *X. fastidiosa* transmission to olive occurs (Cornara et al. 2017).

To conclude, this work demonstrates for the first time that the playback of an *ad-hoc* designed signal based on species-specific vibrations can be exploited to impair probing and feeding behaviours of *P. spumarius*, the main European vector of *X. fastidiosa*. This strategy is potentially applicable to all those vectors of plant pathogens using vibrations for short-range communication. Although our results raise numerous further experimental questions, they also could pave the way for sustainable strategies to cohabit with the fastidious bacterium, by mitigating the ecosystem impact either of the pathogen itself or of the suggested or currently applied integrated pest management approaches.

References

Alexander RD (1957) Sound production and associated behavior in insects. The Ohio Journal of Science, 57(2):101–113

Ali BM, van der Werf W, Oude Lansink A (2021) Assessment of the environmental impacts of *Xylella fastidiosa* subsp. *pauca* in Puglia. Crop Prot 142:105519. https://doi.org/10.1016/j.cropro.2020.105519

Almeida RPP (2016a) Can Apulia's olive trees be saved? Science 353(6297):346–348. https://doi.org/10.1126/science.aaf9710

Almeida RPP (2016b) Chapter 12: *Xylella fastidiosa* Vector Transmission Biology. Vector-Mediated Transm Plant Pathog 165–173. https://doi.org/10.1094/9780890545355.012

Almeida RPP, Blua MJ, Lopes JRS, Purcell AH (2005) Vector transmission of *Xylella fastidiosa*: Applying fundamental knowledge to generate disease management strategies. Ann Entomol Soc Am 98:775–786. https://doi.org/10.1603/0013-8746(2005)098[0775:VTOXFA]2.0.CO;2

Avosani S, Daher E, Franceschi P, et al (2020) Vibrational communication and mating behavior of the meadow spittlebug *Philaenus spumarius*. Entomol Gen 40:307–321. https://doi.org/10.1127/entomologia/2020/0983

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-effects models using lme4. arXiv Prepr arXiv14065823

Bjornstad ON (2013) ncf: Spatial nonparametric covariance functions. 2016. R Packag version 1

Bodino N, Cavalieri V, Dongiovanni C, et al (2019) Phenology, seasonal abundance and stage-structure of spittlebug (Hemiptera: Aphrophoridae) populations in olive groves in Italy.

Scientific Reports, 9(1), 1–17. https://doi.org/10.1038/s41598-019-54279-8

Bodino N, Cavalieri V, Dongiovanni C, et al (2020) Spittlebugs of mediterranean olive groves: Host-plant exploitation throughout the year. Insects 11:. https://doi.org/10.3390/insects11020130

Brühl CA, Zaller JG (2019) Biodiversity decline as a consequence of an inappropriate environmental risk assessment of pesticides. Front Environ Sci 7:2013–2016. https://doi.org/10.3389/fenvs.2019.00177

Caorsi VZ, Both C, Cechin S, et al (2017) Effects of traffic noise on the calling behavior of two Neotropical hylid frogs. PLoS One 12:1–14. https://doi.org/10.1371/journal.pone.0183342

Chávez-Dulanto PN, Thiry AAA, Glorio-Paulet P, et al (2020) Increasing the impact of science and technology to provide more people with healthier and safer food. Food Energy Secur 1–31. https://doi.org/10.1002/fes3.259

Civitello DJ, Cohen J, Fatima H, et al (2015) Biodiversity inhibits parasites: Broad evidence for the dilution effect. Proc Natl Acad Sci U S A 112:8667–8671. https://doi.org/10.1073/pnas.1506279112

Cornara D, Cavalieri V, Dongiovanni C, et al (2017a) Transmission of *Xylella fastidiosa* by naturally infected *Philaenus spumarius* (Hemiptera, Aphrophoridae) to different host plants. J Appl Entomol 141:80–87.

Cornara D, Garzo E, Morente M, Moreno A, Alba-Tercedor J, & Fereres A (2018) EPG combined with micro-CT and video recording reveals new insights on the feeding behavior of *Philaenus spumarius.* PLoS One, 13(7). https://doi.org/10.1371/journal.pone.0199154

Cornara D, Marra M, Morente M, et al (2020) Feeding behavior in relation to spittlebug transmission of *Xylella fastidiosa*. J Pest Sci (2004) 93:1197–1213. https://doi.org/10.1007/s10340-020-01236-4

Cornara D, Morente M, Markheiser A, et al (2019) An overview on the worldwide vectors of *Xylella fastidiosa*. Entomol Gen 39:157–181. https://doi.org/10.1127/entomologia/2019/0811

Cornara D, Saponari M, Zeilinger AR, et al (2017b) Spittlebugs as vectors of *Xylella fastidiosa* in olive orchards in Italy. J Pest Sci (2004) 90:521–530. https://doi.org/10.1007/s10340-016-0793-0

Cornara D, Sicard A, Zeilinger AR, et al (2016) Transmission of *Xylella fastidiosa* to grapevine by the meadow spittlebug. Phytopathology 106:1285–1290. https://doi.org/10.1094/PHYTO-05-16-0202-R

Daugherty MP, Almeida RPP (2009) Estimating *Xylella fastidiosa* transmission parameters: Decoupling sharpshooter number and feeding period. Entomol Exp Appl 132:84–92. https://doi.org/10.1111/j.1570-7458.2009.00868.x

Daugherty MP, O'Neill S, Byrne F, Zeilinger A (2015) Is vector control sufficient to limit pathogen spread in vineyards? Environ Entomol 44:789–797. https://doi.org/10.1093/ee/nvv046

Derlink M, Pavlovčič P, Stewart AJA, Virant-Doberlet M (2014) Mate recognition in duetting species: The role of male and female vibrational signals. Anim Behav 90:181–193. https://doi.org/10.1016/j.anbehav.2014.01.023

Eriksson A, Anfora G, Lucchi A, et al (2011) Inter-plant vibrational communication in a leafhopper insect. PLoS One 6(5), e19692. https://doi.org/10.1371/journal.pone.0019692

Eriksson A, Anfora G, Lucchi A, et al (2012) Exploitation of insect vibrational signals reveals a new method of pest management. PLoS One 7:1–5. https://doi.org/10.1371/journal.pone.0032954

Fereres A, Moreno A (2009) Behavioural aspects influencing plant virus transmission by homopteran insects. Virus Res 141:158–168. https://doi.org/10.1016/j.virusres.2008.10.020

Fox J, Weisberg S (2019) An R companion to applied regression (Third). Thousand Oaks CA Sage

Gordon SD, Krugner R (2019) Mating Disruption by Vibrational Signals: Applications for Management of the Glassy-Winged Sharpshooter. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 355–373

Hill PSM, Wessel A (2016) Biotremology. Current Biology, 26(5):187–191, ISSN 0960-9822, https://doi.org/10.1016/j.cub.2016.01.054

Kairis O, Karavitis C, Kounalaki A, et al (2013) The effect of land management practices on soil erosion and land desertification in an olive grove. Soil Use Manag 29:597–606. https://doi.org/10.1111/sum.12074

Karamaouna F, Kati V, Volakakis N, et al (2019) Ground cover management with mixtures of flowering plants to enhance insect pollinators and natural enemies of pests in olive groves. Agric Ecosyst Environ 274:76–89. https://doi.org/10.1016/j.agee.2019.01.004

Lakes-Harlan R, Strauß J (2014) Functional morphology and evolutionary diversity of vibration receptors in insects. In: Studying vibrational communication. Springer, pp 277–302

Lazzari CR, Manrique G, Schilman PE (2005) Vibratory communication in Triatominae (Heteroptera). In Insect Sounds and Communication: Physiology, Behaviour, Ecology, and Evolution, (pp. 297-304). Taylor & Francis.

Madden LV, Jeger MJ, Van Den Bosch F (2000) A theoretical assessment of the effects of vector-virus transmission mechanism on plant virus disease epidemics. Phytopathology 90:576–594. https://doi.org/10.1094/PHYTO.2000.90.6.576

Mankin RW (2019) Vibrational Trapping and Interference with Mating of *Diaphorina citri*. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 399–413

Markheiser A, Cornara D, Fereres A, Maixner M (2019) Analysis of vector behavior as a tool to predict *Xylella fastidiosa* patterns of spread. Entomol Gen XX: https://doi.org/10.1127/entomologia/2019/0841

Mazzoni V, Eriksson A, Anfora G, et al (2014) Active space and the role of amplitude in plant-borne vibrational communication. In: Studying vibrational communication. Springer, pp 125–145

Mazzoni V, Lucchi A, Čokl A, et al (2009) Disruption of the reproductive behaviour of *Scaphoideus titanus* by playback of vibrational signals. Entomol Exp Appl 133:174–185. https://doi.org/10.1111/j.1570-7458.2009.00911.x

Mazzoni V, Nieri R, Eriksson A, et al (2019) Mating Disruption by Vibrational Signals: State of the Field and Perspectives. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 331–354

Mesmin X, Chartois M, Genson G, et al (2020) *Ooctonus vulgatus* (Hymenoptera, Mymaridae), a potential biocontrol agent to reduce populations of *Philaenus spumarius* (Hemiptera, Aphrophoridae) the main vector of *Xylella fastidiosa* in Europe. PeerJ 2020:e8591. https://doi.org/10.7717/peerj.8591

Michelsen A, Fink F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. Behav Ecol Sociobiol 11:269–281

Mokrane S, Cavallo G, Tortorici F, et al (2020) Behavioral effects induced by organic insecticides can be exploited for a sustainable control of the Orange Spiny Whitefly *Aleurocanthus spiniferus*. Sci Rep 10:1–12

Molinatto G, Demichelis S, Bodino N, et al (2020) Biology and prevalence in northern italy of *Verrallia aucta* (Diptera, Pipunculidae), a parasitoid of *Philaenus spumarius* (Hemiptera, Aphrophoridae), the main vector of *Xylella fastidiosa* in Europe. Insects 11:1–16. https://doi.org/10.3390/insects11090607

Morente M, Cornara D, Moreno A, Fereres A (2018) Continuous indoor rearing of *Philaenus spumarius*, the main European vector of *Xylella fastidiosa*. J Appl Entomol 142:901–904. https://doi.org/10.1111/jen.12553

Pinheiro J, Bates D, DebRoy S S (2016) Linear and Nonlinear Mixed Effects Models_. R package version 3.1-127. J. Apic. Res.

Polajnar J, Eriksson A, Lucchi A, et al (2015) Manipulating behaviour with substrate-borne vibrations - Potential for insect pest control. Pest Manag Sci 71:15–23. https://doi.org/10.1002/ps.3848

Polajnar J, Eriksson A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. J Pest Sci (2004) 89:909–921. https://doi.org/10.1007/s10340-015-0726-3

Polajnar J, Maistrello L, Ibrahim A, Mazzoni V (2019) Can Vibrational Playback Improve Control of an Invasive Stink Bug? In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 375–398

Polajnar J, Svenšek D, Čokl A (2012) Resonance in herbaceous plant stems as a factor in vibrational communication of pentatomid bugs (Heteroptera: Pentatomidae). J R Soc Interface 9:1898–1907. https://doi.org/10.1098/rsif.2011.0770

Purcell AH (1982) Insect vector relationships with procaryotic plant pathogens. Annu Rev Phytopathol 20:397–417. https://doi.org/10.1146/annurev.py.20.090182.002145

Purcell AH (1980) Environmental therapy for Pierce's disease of grapevines. Plant Dis 64:388–390. https://doi.org/10.1094/pd-64-388

Ryan TH (1960) Significance tests for multiple comparison of proportions, variances, and other statistics. Psychological Bulletin 57:318–328

Samarra FIP, Klappert K, Brumm H, Miller PJO (2009) Background noise constrains communication: Acoustic masking of courtship song in the fruit fly *Drosophila montana*. Behaviour 146:1635–1648. https://doi.org/10.1163/156853909X463713

Sánchez-Bayo F, Wyckhuys KAG (2019) Worldwide decline of the entomofauna: A review of its drivers. Biol Conserv 232:8–27. https://doi.org/10.1016/j.biocon.2019.01.020

Sandanayaka WRM, Jia Y, Charles JG (2013) EPG technique as a tool to reveal host plant acceptance by xylem sap-feeding insects. J Appl Entomol 137:519–529. https://doi.org/10.1111/jen.12025

Saponari M, Giampetruzzi A, Loconsole G, et al (2019) *Xylella fastidiosa* in olive in apulia: Where we stand. Phytopathology 109:175–186. https://doi.org/10.1094/PHYTO-08-18-0319-FI

Simberloff D, Martin JL, Genovesi P, et al (2013) Impacts of biological invasions: What's what and the way forward. Trends Ecol Evol 28:58–66. https://doi.org/10.1016/j.tree.2012.07.013

Stein W, Sauer AE (1999) Physiology of vibration-sensitive afferents in the femoral chordotonal organ of the stick insect. J Comp Physiol - A Sensory, Neural, Behav Physiol 184:253–263. https://doi.org/10.1007/s003590050323

Takanashi T, Uechi N, Tatsuta H (2019) Vibrations in hemipteran and coleopteran insects: behaviors and application in pest management. Appl Entomol Zool 54:21–29. https://doi.org/10.1007/s13355-018-00603-z

Valero-Mora PM (2010) ggplot2: Elegant Graphics for Data Analysis. Springer

Virant-Doberlet M, Cokl A (2004) Vibrational communication in insects. Neotrop Entomol 33:121–134. https://doi.org/10.1590/s1519-566x2004000200001

Virant-Doberlet M, Kuhelj A, Polajnar J, Šturm R (2019) Predator-prey interactions and eavesdropping in vibrational communication networks. Front Ecol Evol 7:1–15. https://doi.org/10.3389/fevo.2019.00203

Vos S, Camilleri M, Diakaki M, et al (2019) Pest survey card on *Xylella fastidiosa*. EFSA Support Publ 16:1667E. https://doi.org/10.2903/sp.efsa.2019.en-1667

Weaver CR, King DR (1954) Meadow spittlebug, *Philaenus leucophthalmus* (L.). Research Bulletin 741

Wickham H (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York

Witsack W (1973) Experimentell-okologische Untersuchungen uber Dormanz-Formen von Zikaden (Homoptera, Auchenorrhyncha). 2. zur Ovarial-Parapause und obligatorischen Embryonal-Diapause von *Philaenus spumarius* (L.) (Aphrophoridae). Zool Jahrbücher Abteilung für Syst Okol und Geogr der Tiere 100:517–562

Vibrational communication and evidence for vibrational behavioural manipulation of the tomato potato psyllid, *Bactericera cockerelli*

Sabina Avosani, Thomas E.S. Sullivan, Marco Ciolli, Valerio Mazzoni, and David Maxwell Suckling

Entomologia Generalis, **2020**, 40(4), 351-363

DOI: 10.1127/entomologia/2020/0984

Abstract

Vibrational pest management techniques involve the manipulation of the mating behaviour of the target species. Development of such techniques require a deep understanding of the pair formation process. Two types of bioassays were conducted to determine whether a vibrational disruptive approach could be used for *Bactericera cockerelli.* First, we conducted trials with either single individuals or pairs of a male and a female to ascertain whether *B. cockerelli* uses vibrational signals to achieve mating. Second, playback trials were conducted using two different female signals to manipulate the behaviour of males. Signals emitted during the trials were recorded using a laser vibrometer from the surface of a leaf where either single individuals or pairs of *B. cockerelli* were released. We described the communication phases that led to mating and the associated male and female signals. Pair formation started with a male call followed by a female response, which established a vibrational duet. During the female reply, the male searched for the female without emitting vibrational signals. Mating could be achieved if the male could maintain the female replies and reach her. In the playback trials, two different female playbacks attracted males to the signal source. Furthermore, males stimulated with female playbacks spent more time near the source of the signal than insects in the silent control group. We concluded that the *B. cockerelli* mating behaviour relies on vibrational signals and is potentially susceptible to vibrational manipulation, thus a future vibrational management technique could be developed to monitor or control this species.

Keywords: vibrational pest control; behaviour; tomato potato psyllid, (Hemiptera: Triozidae); pair formation

1. Introduction

Innovative pest management strategies can involve manipulation of the target organism's sexual behaviour using specific external stimuli, to reduce the use of pesticides in agriculture (Polajnar et al. 2015). To control the population of an insect pest, its' behaviour can be altered either to attract/repel it with a lure-and-kill/push-and-pull tactic or to disrupt mating (Foster and Harris 1997). However, the prerequisite for setting an efficient control method is to identify and characterise the key signals used to achieve mating by the target species (Pedigo and Rice 2014; Pertot et al. 2017). Therefore, it is necessary to assess which sensory modalities control relevant behaviours (Mazzoni et al. 2017). During communication, emitted and received signals convey information about identity, physiological condition and position of the sender, which can elicit specific responses in the receiver (Endler 1992).

Exchange of substrate-borne vibrations is a widespread communication modality among insects and is of crucial importance for mating (Čokl and Virant-Doberlet 2003; Virant-Doberlet and Cokl 2004; Cocroft and Rodríguez 2005; Virant-Doberlet and Zezlina 2007). In this regard, many hemipteran species that use vibrations to communicate are agricultural pests (Kanmiya 2006; Mazzoni et al. 2009; Mazzoni et al. 2017; Derlink et al. 2018). It has been recently suggested that the behaviour of these insects can be manipulated by means of mechanical stimuli that interfere with the vibrational communication (Polajnar et al. 2016). For instance, specific disruptive vibrational signals transmitted through plants decreased the mating success of the grapevine leafhopper, *Scaphoideus titanus*, in both laboratory and open field trials (Eriksson et al., 2012, Polajnar et al., 2015). A similar approach was proposed for *Homalodisca vitripennis* (Gordon et al. 2017; Nieri et al. 2017; Krugner and Gordon 2018) and *Empoasca vitis* (Nieri and Mazzoni 2018), for which specific playbacks were designed to interfere with intraspecific communication and thus to disrupt the pair formation process. Besides mating disruption, another vibrational approach for pest control involves mechanical stimuli to attract insects into traps, as suggested for the brown marmorated stink bug, *Halyomorpha halys.* In this case, the existing pheromone trap is under implementation with a specific vibratory stimulus that brings males into the device (Mazzoni et al. 2017). Hence, the playback of species-specific vibrations in an integrated pest management approach could be expected to be applied for other pest insects whose communication relies on vibrational signals.

According to this, many species among Psylloidea are important agricultural pest (Munyaneza 2010) and use vibrational signals to achieve mating (Percy et al. 2006), as reported for the Asian citrus psyllid *Diaphorina citri* (Wenninger et al. 2009; Hall et al. 2013). In this and other psyllid species, the exchange of signals between the male and female allows the male to reach the replying partner on the leaf (Percy et al. 2006; Wenninger et al. 2009; Eben et al. 2015). With this knowledge, a trapping strategy has been developed and tested with *D. citri*, using a system that detected the male signals and transmitted an attractive vibrational stimulus as response (Mankin et al. 2013). For these reasons, it would be worthy to assess whether similar vibrational control techniques could be designed and applied against other psyllid pests.

The tomato potato psyllid, *Bactericera cockerelli* (Šulc) (Hemiptera: Triozidae) became one of the major economic pests in New Zealand after its introduction from North America (Liu and Trumble 2007; Teulon et al. 2009). *Bactericera cockerelli* can transmit a bacterium, *Candidatus* Liberibacter solanacearum, which is the causal agent of 'zebra chip' disease in potato plants (Hansen et al. 2008; Liefting et al. 2009). Characterisation of the signals involved in the sexual communication of this pest could lead to the development of control practices based on behavioural manipulation, providing an alternative to insecticides, which represent the current management approach (Goolsby et al. 2007; Berry et al. 2009). Chemical signals are involved in the sexual communication of some species within the *Cacopsylla* genus (Soroker et al. 2004; Horton and Landolt 2007) and there is evidence that *B. cockerelli* males move towards volatile chemical compounds emitted by conspecifics (Guédot et al. 2010). However, the role of vibrational signals in the mating behaviour and the attractiveness of these stimuli remain unclear. Some psyllids use vibrational signals to identify and eventually localise a potential mate, and in some taxa, pair formation relies on a tightly synchronised duet (Tishechkin 2005; Percy et al. 2006; Wenninger et al. 2009). It has been recently reported that *B. cockerelli* males emit substrate-borne signals and that the playback of synthetic male signals could elicit behavioural responses from other conspecifics (Sullivan et al., submitted for publication). Nevertheless, neither the pair formation process nor the male and female signals have been described yet. Hence, we carried out behavioural assays aimed to provide a detailed characterisation of the inter-sexual communication and the associated vibrational signals. Moreover, we conducted playback trials using pre-recorded signals of *B. cockerelli* female to evaluate whether the male behaviour is vulnerable to manipulation.

2. Materials and methods

2.1. Insect rearing

A colony of *B. cockerelli* was established under controlled conditions (23 ± 2 °C, 60% ± 5% RH, 16:8 L:D) in a climate chamber at Plant and Food Research in Lincoln (Canterbury, New Zealand). Insects were maintained on capsicum (*Capsicum annuum*) inside rearing cages (Bugdorm-6620, MegaView Science Co., Ltd., Taiwan; 60 cm× 60 cm×120 cm). Nymphs were collected from the colony and reared to the adult stage in a separate cage, which had same size than the previous ones. Recently molted virgin adult males and females were used for experiments after melanisation (2-4 days).

2.2. Recording vibrational signals

Recordings were conducted at room temperature (25 ± 2 °C) on a vibrational dampening steel plate (1.2mx1.2mx10 mm; Slade Engineering, Christchurch, New Zealand) that was placed on an array of nine insulating rubber pads (diameter 6.35 cm; Sorbothane Hemisphere Rubber Bumper, IsolateIT, Burlington, USA) over a table. Vibrational signals and behaviours were recorded from a detached capsicum leaf with the stem placed in a plastic vial (100 ml) filled with water. To detect the vibrational signals, a laser vibrometer (Polytec model PDV 100, Waldbrann, Germany) was set on the steel plate and pointed onto a piece (0.5x0.5 cm) of reflective tape glued to the adaxial surface of a capsicum leaf (approximate surface 12x8 cm). To prevent insects from escaping, the leaf was contained in a plastic cage (20 cm^2) with a circular opening on the top for the laser beam (diameter 6 cm). The cage was set above a Plexiglas cube (30 cm^3), which was placed on the steel plate. In this way, the laser and the leaf were at the optimal stand-off distance that ensures a better signal-to-noise ratio. Four insulating rubber pads were placed under the cube to reduce background vibrational noise. Recorded signals were digitized and stored via a sound card (M-Audio Fast Track Pro, M-Audio, United Kingdom) at a 48 kHz sample rate and 24-bit resolution on a laptop computer (HP, EliteBook 8460 p) using Raven Pro 1.5 (The Cornell Lab of Ornithology, Ithaca, NY, USA). To associate the behaviour and respective emission of vibrational signals, insect movements were continuously monitored using two cameras (Logitech Quickcam Pro 9000 webcam, Logitech

International S.A., Lausanne, Switzerland, and Microsoft® LifeCam HD-5000, Microsoft Corporation; U.S.A.), focused on the adaxial and abaxial sides of the leaf, respectively. The HandyAvi (Version 4.3C © Anderson's AZcendant Private Ltd, Singapore) time lapse software was used to capture psyllid signalling behaviour at 1 frame/s.

2.2.1. Test 1: Vibrational signals associated with pair formation in *B. cockerelli*

To characterise the vibrational signals associated with pair formation in *B. cockerelli*, behavioural trials were conducted during November and December 2018 between 08:00 and 17:00h using either individuals or male-female pairs. In the individual trials, a male (n = 56) or a female (n = 37) was placed on the leaf and its vibrational signalling behaviour was recorded for 15 minutes. In the trial with pairs (n = 62), a male and a female were released in a random order on opposite sides of the leaf. The behaviour was recorded until copulation occurred or for 15 minutes in absence of vibrational signals. If a duet was established within the 15-min period, the trial was extended for another 30 min to provide a reasonable amount of time to the searching individual to find the partner on the leaf. To describe the psyllids' signalling activity, when applicable, we measured: (1) the calling latency, i.e., the time from the beginning of the trial to the emission of the first vibrational signal; (2) the number of signals emitted during the trial; (3) the latency to first duet, as the first reply to a signal regardless of gender; (4) the female response latency, as the delay of the female signal from the onset of the male signal; (5) the searching time, i.e., the time between the onset of the male search and the copulation; (6) and the replying rate of female, as the rate between the total number of female replies and male calls during the trial. We also measured (7) the mating success as the number of copulation events after the establishment of the male-female duet. To identify possible differences both between and within each phrase (see definitions below) of the Male Calling Signal (MCS, see results below), we compared the temporal and spectral parameters of the pulses at the beginning and at the end of each phrase with the Friedman test (nonparametric repeated measures ANOVA) with replications followed by pairwise multiple comparisons (Siegel and Castellan Jr 1988). We selected six non-consecutive pulses for each phrase within a signal. Three pulses were sampled at the beginning and three pulses were sampled at the end of each phrase, always including the first and last pulse. A Wilcoxon signed rank test was performed to detect possible differences in the spectral or temporal parameters between the MCS and the Female Response Signal (FRS, see results below). Specifically, the first 50 and the last 50 pulses were sampled from both the MCS and the FRS of the male (n = 22) and the female (n = 22), respectively, and then compared. The criterion of 50 pulses provided an appropriate description of the first and last sections of the MCS and FCS, since these are very long signals composed of brief pulses with a high repetition time (see results below). The durations of MCS and FRS were compared using a non-parametric Wilcoxon test with a continuity correction (Steel 1959). Statistical analyses were conducted using KyPlot version 2.0 beta 15 (1997–2001 Koichi Yoshioka) and Past 3 (Hammer and Harper Øyvind Hammer, Natural History Museum, and University of Oslo). Vibrational signals were identified and named according to their structure and behavioural context (see definition below). The spectral analysis was performed with Raven Pro 1.5 (The Cornell Lab of Ornithology, Ithaca, NY, USA) after a Fast Fourier Transform (FFT) type Hann, window length of 256 samples and 89.9% of overlap. The following parameters were measured when applicable for each signal, phrase, and pulse: duration, pulse repetition time (PRT, time from the onset of two consecutive pulses) and dominant frequency (Df). Results are presented as means with standard deviation (SD), range (min. and max.), together with the number of signals analysed for each individual (N) and number of insects (n) from which signals were obtained.

2.2.2. Test 2: Signal playback tests

The aim of this test was to assess the effect of vibrational stimuli on males. The tested stimuli consisted of two playbacks of natural female signals, which were previously recorded from a capsicum leaf. Two female playbacks were made using the audio software Adobe Audition 3.0 (Adobe Systems, Inc., San Jose, CA, USA). Specifically, female playback 1 (FPB1) consisted of two female signals. The first signal was obtained from a Female Attraction Signal (FAS, see results below) (duration: 23.72 s) and was composed of 37 pulse trains of increasing duration (range 0.08 to 3.56 s). The second signal was obtained from a Female Response Signal (FRS, see results below); it was shorter (21.53 s) than the FAS and consisted of five long pulse trains (range 1.51 to 8.02 s). Between the two signals, there was a period of silence of 25.05 s. The female playback 2 (FPB2) was similar to FPB1, except for a longer second signal (38.55 s, with the last pulse train of 24.97 s instead of 8.02 s), whose duration was more similar to the mean duration of the natural FRS (see results below) compared to the duration of the second signal of the female playback 1. For both playbacks, after the second signal, there were 25 seconds of silence. The PRT and the frequency ranges within the signals were not modified, while the relative amplitude of the playbacks measured from the plant was adjusted to not exceed the insect natural amplitude. Playbacks were transmitted into the substrate using an electromagnetic mini shaker (Direct Drive Linear Motor, model DDLM-038-051-01, MotiCont, Los Angeles, California, USA) in direct contact with the adaxial side of the capsicum leaf (approximate dimensions of the leaf, considering both lamina and petiole : 18x10 cm). A sharpened steel rod was screwed at the top of the device, which was physically separated from the anti-vibration table with a clamp standing on a nearby table. A laser vibrometer was pointed on the adaxial side of the leaf to ensure that the playback was being transmitted and to record the vibrational signals emitted by the insects during the trials.

Test 2a: Female playback 1 (FPB1). A male (n = 19) was placed on the leaf, at least 15 cm away from the tip of the mini shaker. After 1 minute of a silent acclimation period, the FPB1 was transmitted as a loop into the substrate for 10 minutes. The control group consisted of males (n = 19) left in silence for 12 minutes.

Test 2b: Female playback 2 (FPB2). A male (n = 19) was placed on the leaf, at least 15 cm away from the tip of the mini shaker. After 12 minutes of silence, the FBP2 was transmitted as a loop into the substrate for 10 minutes. In this way, the males left in silence for 12 minutes provided the control group of the trial with the FPB1. As control for the FPB2 test, a trial (n = 19) with identical setup but in absence of playback (22 minutes of silence) was performed.

For both tests, if the playback did not elicit the emission of any vibrational signal by the insect, the recording was stopped at one minute after the looped playbacks. However, if the male responded to the stimulus, the trial was extended by 30 minutes to determine whether the male reached the mini shaker. In the playback tests (the ‘treatments’), we counted the number of (1) signalling males, (2) searching males, (3) males that reached the stimulation point (see definitions below), (4) males that touched or walked onto the mini shaker, and (5) the searching time. As a measure of the signal attractiveness to males, we counted (6) the number of males that remained close to the signal source (stimulation point) for a minimum of 1 min from the moment they reached it. We also counted the (7) number of males that responded to the playback. In Test 2a and 2b, a G test in contingency tables (2x2), Williams-corrected was used to evaluate the effect of the playback on males by comparing treatments and silent controls for (1), (3), (4), and (6). Since we defined the ‘searching males’ as individuals that walked when stimulated with playback and the ‘duetting males’ as individuals that responded to the playback (see definitions below), we did not perform any statistics on (2), (5), and (7) between treatments and silent controls. The G test in contingency tables (2x2), Williams-corrected, was used to compare (2), (3), (4), (6), and (7) between the playback treatments of the Test 2a and 2b. Since the acclimation time differed between the treatments of Test 2a and 2b, we did not compare (1). A Kruskal–Wallis test followed by Mann–Whitney pairwise, Bonferroni-adjusted, was used to compare (5) between treatments.

2.3. Definitions

Calling signal. Signal emitted spontaneously to trigger a reply from the opposite sex (Booij 1982; Tishechkin 2003).

Pulse. Physically unitary or homogeneous sound, composed of a brief succession of sine waves (Broughton 1963, Alexander 1967).

Pulse train. Succession of repetitive and temporally well-distinct pulses (e.g., Mazzoni et al. 2009).

Phrase. Sequence of regularly repeated pulse trains with distinctive time and amplitude pattern.

PRT. Pulse repetition time, time from the onset of two consecutive pulses within a pulse train.

MCS. Male Calling Signal of *B. cockerelli.*

FRS. Female Response Signal of *B. cockerelli.*

FAS. Female Attraction Signal of *B. cockerelli.*

Signalling males. Individuals that emitted, spontaneously or after the playback, at least one vibrational signal during the trial.

Searching males. Individuals that responded to playback by emission of vibrational signals and walked upon stimulation by playback.

Duetting males. Individuals that established a duet with the female playback.

Searching time. Period from the onset of the search until the arrival of the male to the stimulation point or until the end of trial.

Stimulation point. Area (Ø 1 cm) around the point of the leaf in direct contact with the mini shaker considering both sides of the leaf.

3. Results

Emission of vibrational signals by *B. cockerelli* males and females was always accompanied by rapid wing movements in a vertical plane and dorso-ventral movements of the abdomen.

3.1. Vibrational signals associated with pair formation in *B. cockerelli*

Single males. Among males tested alone, 50% (n = 56) emitted at least one signal after being released on the leaf, and 24 of these males were analysed. Signalling latency varied considerably amongst individuals (Table 6.1), while the duration of the signal was relatively constant (Table 6.1). The number of signals emitted in the given time of the trial varied amongst males (Table 6.1) with the highest and lowest number of recorded signals from a male being 14 and 1, respectively. The Male Calling Signal (MCS) consisted of a series of pulse trains (defined as ‘phrases’) composed of a rapid succession of broad-band pulses (Table 6.1, Figure 6.1 A, B, C and D). The male phrases were grouped in function of their spectral and temporal parameters, which clearly changed as the MCS proceeded. In particular, the first emitted phrase (‘phrases 1’) was followed by a series of pulse trains (‘phrases 2’) (Figure 6.1B), whose first and last pulses had a lower relative amplitude compared to the first (Friedman Statistic; $X^2 = 6.5$, df = 1, p = 0.02) and last pulses (Friedman Statistic; $X^2 = 18.2$, df

= 1, $p < 0.001$) of the phrase 1. The final pulses significantly differed between phrases 1 and 2 also in the PRT (Friedman Statistic; $X^2 = 3.9$, df = 1, $p = 0.04$), while the PRT of the first pulses did not differ between phrases (Friedman Statistic; $X^2 = 2.2$, df = 1, $p = 0.1$). Phrase 1 was emitted once, while the number of phrase 2 within a signal varied among individuals (30 ± 9; n = 22; N = 2), nevertheless the durations of phrases 1 and 2 were similar (Friedman Statistic; $X^2 = 64.9$, df = 3, $p = 1$). After the sequence of phrase 2, the MCS finished with a series of longer (Friedman Statistic; $X^2 = 64.9$, df = 3, $p < 0.001$) pulse trains (named 'phrase 3') (Figure 6.1C). The first pulses of phrase 3 had higher relative amplitude when compared to the first pulses of phrases 2 (Friedman Statistic; $X^2 = 18.2$, df = 1, $p < 0.001$). Similarly, the relative amplitude of the final pulses of phrase 3 was higher than the amplitude of the last pulses of phrases 2 (Friedman Statistic; $X^2 = 14.7$, df = 1, $p < 0.001$). In addition, the final pulses of phrase 3 had significantly higher PRT (Friedman Statistic; $X^2 = 8$, df = 1, $p < 0.001$) than the final pulses of phrase 2. The duration of phrase 3 was highly variable, ranging from a minimum of 0.62 s to a maximum of 16.7 s (5.34 ± 4.86 s, n = 22; N = 2). Phrases 1 and 3 differed in their duration (Friedman Statistic; $X^2 = 64.9$, df = 3, $p < 0.001$) and in the PRT of the final pulses (Friedman Statistic; $X^2 = 28.5$, df = 3, $p < 0.001$). Moreover, the internal structure differed within each phrase, since both the duration and the PRT between the first and last pulses were significantly different (Table 6.2).

Females. None of the females tested individually (n = 37) emitted spontaneous calls in the given time.

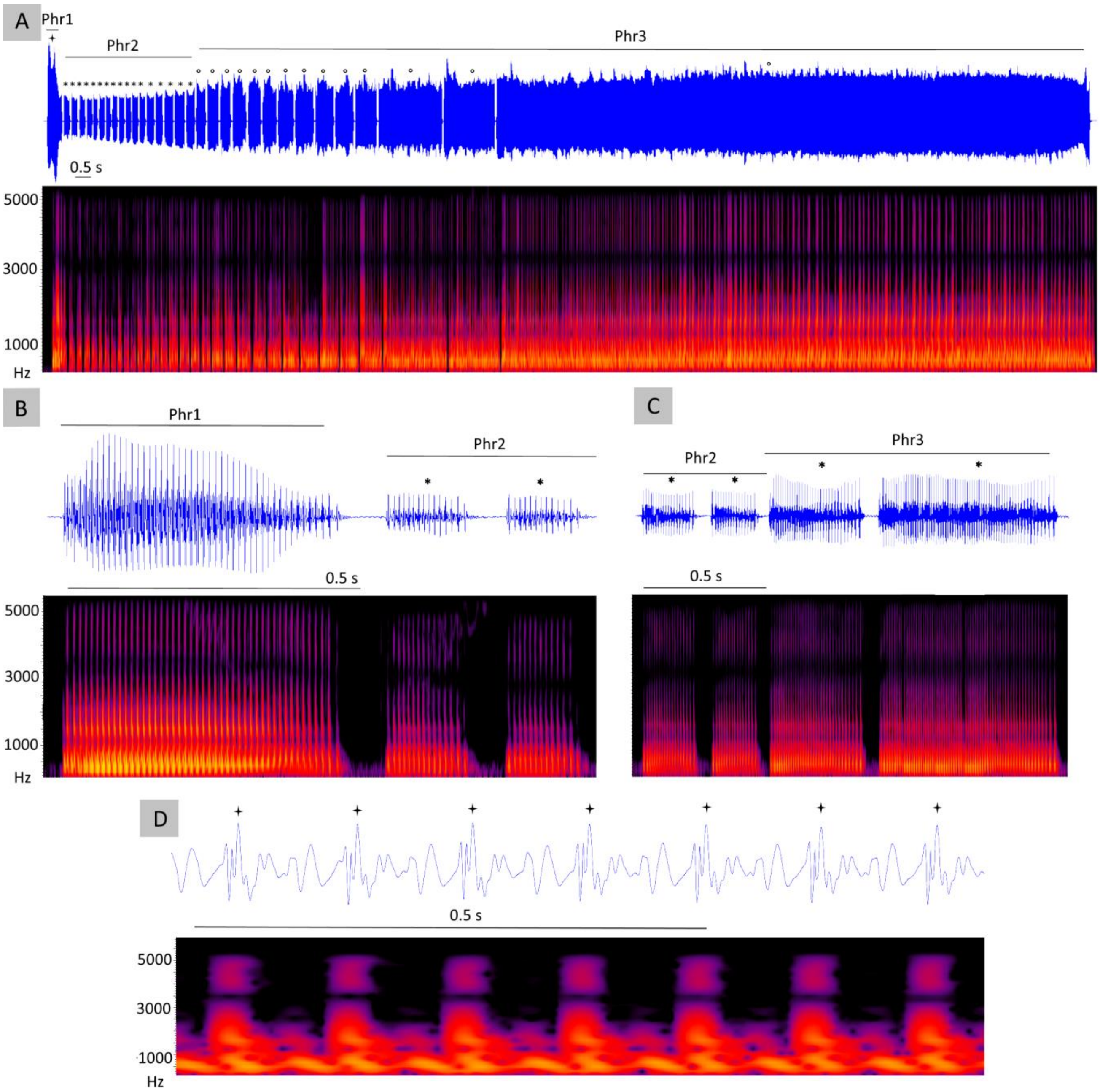

Figure 6.1. Oscillogram (above) and spectrogram (below) of the Male Calling Signal (MCS) of *Bactericera cockerelli*. A) Entire MCS. B) Detail of the first section of a MCS. C) Detail of the last section of a MCS. D) Detail of the pulses emitted within a phrase of a MCS. Phr1: phrase 1, Phr2: series of phrase 2, Phr3: series of phrase 3. Asterisks indicate each phrase emitted within the MCS, diamonds indicate each pulse emitted within a phrase.

Table 6.1. Temporal and spectral parameters of the Male Calling Signal (MCS) of *B. cockerelli.*

	Mean ± SD	Min	Max	n	N
Signalling latency (s)	292.9 ± 224.4	0	877.6	24	1
Duration of MCS (s)	24.4 ± 4.3	17.5	33.4	24	2
Number of MCS	4.7 ± 3.2	1	14	24	1
Pulse Repetition Time (s)	0.0096 ± 0.0012	0.0078	0.0118	24	48
Phr1 duration (s)	0.29 ± 0.09	0.12	0.47	24	2

Phr2 duration (s)	0.28 ± 0.15	0.12	0.61	24	2
Phr3 duration (s)	5.35 ± 4.87	0.63	16.74	24	2
Df (Hz) of first pulses	485.6 ± 243.6	31.3	1000	22	24
Df (Hz) of last pulses	548.5 ± 257.6	187.5	1125	22	24

n: number of analysed individuals, N: number of analysed signals per individual, MCS: Male Calling Song, Phr1: phrase 1, Phr2: phrase 2, Phr3: phrase 3. Df: dominant frequency of either the first or the last pulses within the phrases

Table 6.2. Differences in the temporal parameters between the beginning and the end of each phrase of the Male Calling Signal. Friedman test (nonparametric repeated measures ANOVA) with replications followed by pairwise multiple comparisons.

Phrase	Parameter	Chi-Square	*p*	n	N
Phr1	Duration (s)	17.9	<0.001	22	12
Phr2	Duration (s)	21.7	<0.001	22	12
Phr3	Duration (s)	17.7	<0.001	22	12
Phr1	Pulse Repetition Time (s)	72.9	<0.001	22	12
Phr2	Pulse Repetition Time (s)	104.8	<0.001	22	12
Phr3	Pulse Repetition Time (s)	120.1	<0.001	22	12

n: number of analysed individuals, N: number of analysed signals per individual, MCS: Male Calling Signal, Phr1: phrase 1, Phr2: phrase 2, Phr3: phrase 3.

Pairs. Pair formation in *B. cockerelli* could be divided in three phases: (i) identification, (ii) localisation, and (iii) copulation. Vibrational signals were emitted during the first two phases, while during copulation both male and female were silent. Identification started with the emission of a MCS (male signalling rate = 51.6%, n = 62) that could elicit the onset of a vibrational duet (n = 25, Table 6.3, Figure 6.2A and 6.2B) with a receptive female (female response rate = 78%) that emitted the Female Response Signal (FRS; Table 6.4, Figure 6.2A). The FRS consisted of a long series of pulse trains (or 'phrases'), with the number ranging from 2 to 37 (Table 6.4) and whose duration was very variable (Table 6.4). The female phrases were not grouped since there were neither a clear temporal nor spectral pattern throughout its emission, as observed in the MCS. Nonetheless, there were significant differences in the spectral and temporal features within the female phrases, in particular in terms of dominant frequency (Friedman Statistic; X^2 = 6.76 =, df = 1, p < 0.001), amplitude (Friedman Statistic; X^2 = 6.76, df =1, p < 0.001), duration (Friedman Statistic; X^2 = 21.16, df = 1, p < 0.001), and PRT (Friedman Statistic; X^2 =, df =1, p < 0.001) between the first and final pulses of each pulse trains. Spectral and temporal parameters were significantly different between the MCS and the FRS; in particular, they differed both in the duration of both the first (Friedman Statistic; X^2 = 14.73, df = 1, p < 0.001) and the last (Friedman Statistic; X^2 = 21.73, df = 1, p < 0.001) pulses within the phrases and in the frequency composition of the first (Friedman Statistic; X^2 = 6.4016, df = 1, p < 0.001) and the last (Friedman Statistic; X^2 = 2.80, df = 1, p = 0.02) pulses within the phrases. The durations of MCS and FRS were significantly different from each other (Wilcoxon's test; z = 4.6, p < 0.001). The female response always overlapped the final part of the MCS and continued after its end, while further MCSs were emitted when the female ceased to reply. The male searching behaviour occurred during the long female response (localisation phase). Mating was achieved if the male was capable to maintain the duet until he reached the female, which remained stationary on the leaf (mating success of duetting males = 76%, n = 25). Interestingly, 12.5% of the duets (n = 25) were started by the female with emission of a Female Attraction Signal (FAS), which consisted of a series of pulse trains (Table 6.5, Figure 6.3A and 6.3B). The FAS could elicit the emission of the MCS, and thus the onset of a duet and the male search. In one case, the male started to search after the emission of the FAS, and silently reached the signalling female and mated with her. Similarly, 9.7% of the tested males (n = 62) reached the female without emitting any vibrational signal, while 38.7% of males established and maintained a duet until successfully localisation of the mate. Two females (out of 25) ceased to reply after having established a duet even when the male continued to emit MCSs, while three males (out of 25) did not find the female in the given time of the trial. Vibrational signals were not emitted during copulation.

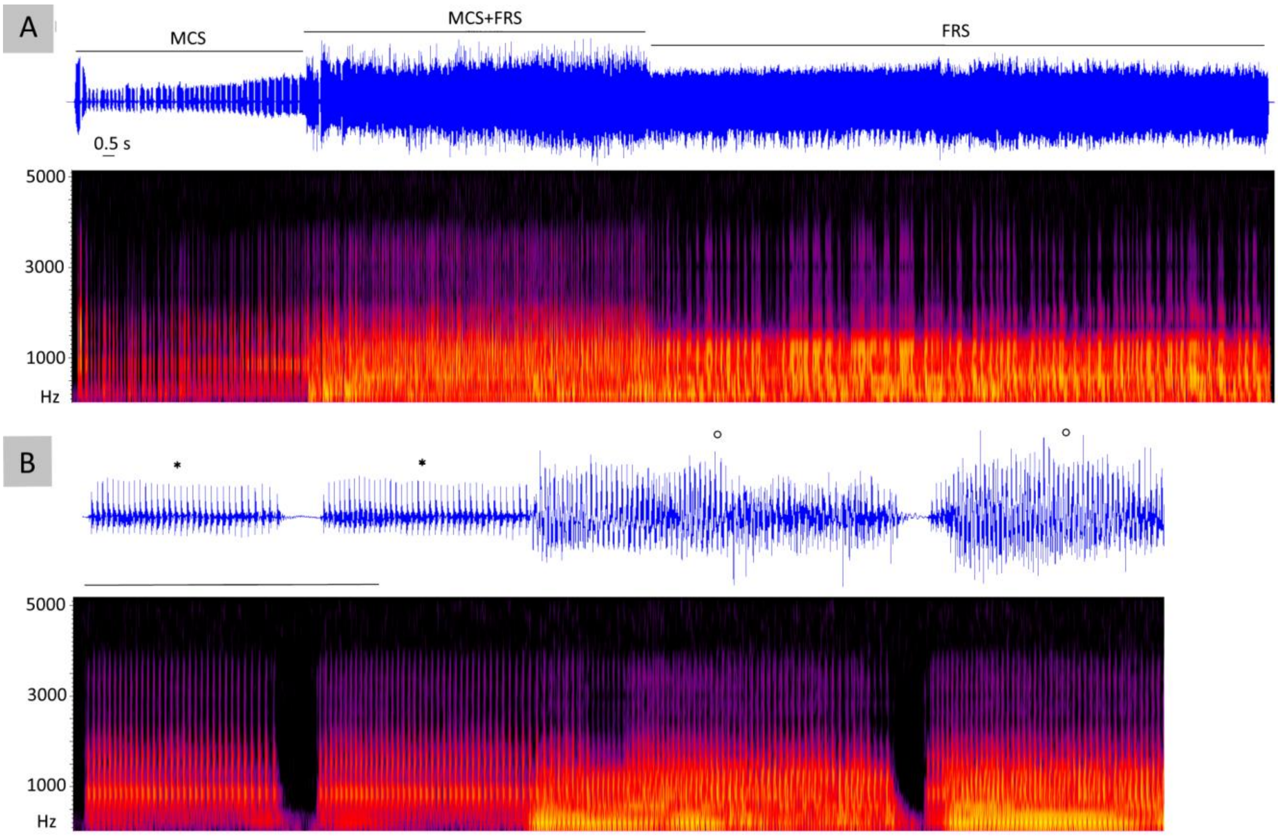

Figure 6.2. Oscillogram (above) and spectrogram (below) of the male-female duet in *Bactericera cockerelli*. A) Entire duet, consisting of a MCS and a FRS. B) Detail of a duet, showing the overlap between the MCS and the FRS. Asterisks indicate the pulse trains within the MCS, empty circles indicate the overlap between the male and female pulse trains. MCS: Male Calling Signal, FRS: Female Response Signal.

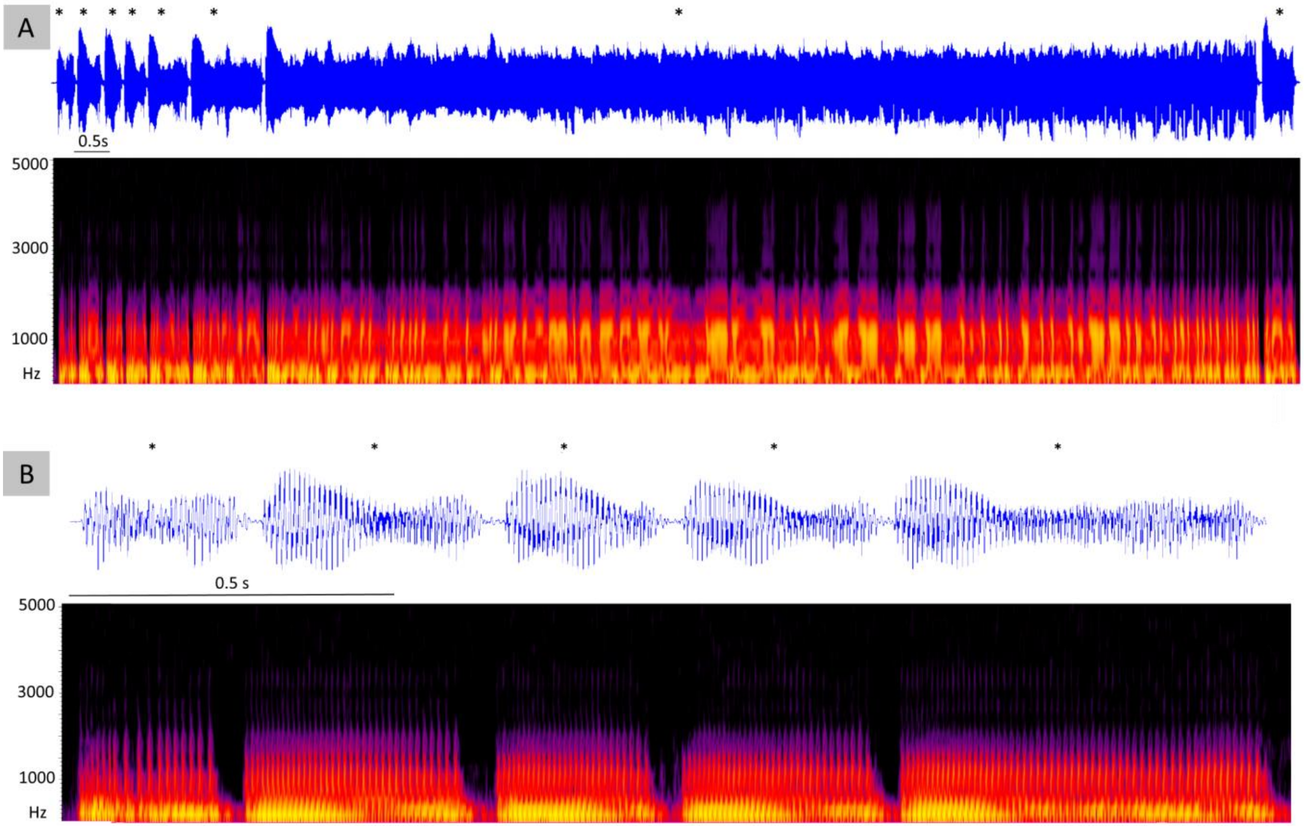

Figure 6.3. Oscillogram (above) and spectrogram (below) of a Female Attraction Signal (FAS) of *Bactericera cockerelli*. A) Entire FAS. B) Detail of the first section of a FAS. Asterisks indicate each phrase emitted within the signal.

Table 6.3. Features of the male-female duet in *B. cockerelli.*

	Mean ± SD	Min	Max	n	N
Latency from first duet (s)	392.7 ± 272.7	30.07	823.9	25	1
Female Reply Latency (s)	12.3 ± 7.1	5.2	41.8	25	1
Searching time (s)	404.5 ± 436.5	62.1	1461.6	20	1
Female Replying Rate	0.9 ± 0.1	0.6	1	25	1

Female Reply Latency: delay of the female signal from the onset of the Male Calling Signal, searching time: time between the onset of the male search and the copulation, Female Replying Rate: rate between the total number of female replies and male signals during the trial, n: number of analysed individuals, N: number of analysed signals per individual

Table 6.4. Features of the Female Response Signal (FRS) of *B. cockerelli.*

	Mean ± SD	Min	Max	n	N
Duration of FRS (s)	42.8 ± 19.4	10.5	100.6	25	2
Number of FRS	15.3 ± 9.9	2	41	25	1
Phrases duration (s)	5.64 ± 5.04	0.60	24.04	25	2
Df (Hz) of first pulses	694.1 ± 293.8	303.8	1488.8	25	50
Df (Hz) of last pulses	749.3 ± 299.3	240	1458.8	25	50

n: number of analysed individuals, N: number of analysed signals per individual, Df: dominant frequency of either the first or the last pulses within the phrases

Table 6.5. Temporal and spectral parameters of the Female Attraction Signal (FAS) of *B. cockerelli.*

	Mean ± SD	Min	Max	n	N
Duration of FAS (s)	29.2 ± 3.8	24.3	33.4	4	1
Number of phrases	29 ± 14.7	7	38	4	1
Phrases duration (s)	0.66 ± 0.56	0.31	1.48	4	14
Df (Hz) of first pulses	336.6 ± 225.7	183.8	667.5	4	50
Df (Hz) of last pulses	443.4 ± 248.6	187.5	746.3	4	50

n: number of analysed individuals, N: number of analysed signals per individual, Df: dominant frequency of either the first or the last pulses within the phrases

3.2. Signal playback trials

In Test 2a (Table 6.6), the number of males that emitted at least one MCS (1) and touched the mini shaker (4) did not differ between treatment with the playback and silent control. In contrast, the number of males that reached the signal source (stimulation point, (3)) and spent at least 1 minute nearby (6) during playback was significantly higher in the treatment with the playback than in the silent control. In Test 2b (Table 6.7), the number of signalling males (1) and the number of individuals that reached the stimulation point (3), touched the mini shaker (4) and spent time on the stimulation point (6) when the mini shaker was on was significantly higher in the group treated with the playback than in the silent control group. When comparing insect responses to the playbacks used in Test 2a and 2b, there were no significant difference in parameters (1), (2), (3), (4), (5), and (6) between the playback treatments (Table 6.8). The searching time (5) also did not differ between the playback treatments (Kruskal-Wallis Statistic, X^2 = 1.48, df = 1, p > 0.05). However, the number of duetting males (7) was significantly higher when males were treated with the FPB2 (Test 2b) than when they were treated with the FPB1 (Test 2a) (Table 6.8).

Table 6.6. Results of the G test (G and p are given) in a contingency table (2x2) of Test 1 for treatment (playback stimulation, FPB1+) and control (FPB1-) trials.

	FPB1+	FPB1-	G	*p*
n	19	19		
(1) signalling males	10	8	0.4	NS (>0.05)
(3) males that reached stimulation point	6	1	4.8	<0.001
(4) males that touched the mini shaker	3	1	1.2	NS (>0.05)
(6) close to stimulation point for more than 1 min	6	0	5	<0.001

n: number of tested males

Table 6.7. Results of G test (G and p) in a contingency table (2x2) of Test 2 for treatment (playback stimulation, FPB2+) and control (FPB2-) trials.

	FPB2+	FPB2-	G	*p*
n	19	19		
(1) signalling males	15	15	11.1	<0.001
(3) males that reached stimulation point	10	2	8.3	<0.001
(4) males that touched mini shaker	6	0	5	<0.05
(6) close to stimulation point for more than 1 min	10	0	11	<0.001

n: number of tested males

Table 6.8. Results of G test (G and p) in a contingency table (2x2) between treatment 1 (playback1, FPB1+) and treatment 2 (playback 2, FPB2+).

	FPB1+	FPB2+	G	*p*
n	19	19		
(2) searching males	9	11	0.4	NS (>0.05)
(3) males that reached stimulation point	6	10	1.7	NS (>0.05)
(4) males that touched mini shaker	3	6	1.3	NS (>0.05)
(6) close to SP for more than 1 min	6	10	1	NS (>0.05)
(7) duetting males	8	15	5.6	<0.05

n: number of tested males

4. Discussion

In this study, we described the mating behaviour and the associated vibrational signals of the tomato potato psyllid, *B. cockerelli*. We provide evidence that the sexual behaviour of males is vulnerable to manipulation, thus the development of a future vibrational pest control tool is worthy of further exploration.

B. cockerelli shares several similarities with other psyllid species in terms of mating behaviour and emission of vibrational signals. For instance, the movement of the wings in a vertical plane during signalling suggest that vibrations are produced by stridulation, as previously proposed for *B. cockerelli* (Sullivan et al., submitted for publication) and for many Psylloidea, where the forewing act as a 'plectrum' that rubs against structures on the thorax (the 'pars stridens') (Taylor 1985; Tishechkin 2005; Eben et al. 2014; Wood et al. 2016; Liao et al. 2019). Also, the pair formation process resembled the general scheme described for other psyllids in which a male and a female established a vibrational duet (Tishechkin 2005; Percy et al. 2006). Similar to these species, the *B. cockerelli* male was the first to emit a calling signal, while the female did not produce spontaneous signals in absence of a potential partner and only replied to the male. However, during copulation, neither the male nor the female produced vibrations, possibly because there was no need to maintain the female motivation and/or due to difficulties in using the wings to stridulate while mating. As reported for many Australian Psylloidea species (Percy et al. 2006), *B. cockerelli* male and female signals were structured in phrases and differed in duration and dominant frequency of the pulses. Moreover, the female reply occurred before the end of the male signal, similar to some *Schedotrioza* spp. and *Trioza* spp. (Percy et al. 2006), and to other hemipteran insects such as *Aphrodes makarovi* (Cicadellidae) (De Groot et al. 2012). De Groot and colleagues (2012) suggested that the partial overlap between male and female signals could be advantageous for the female, if this could reduce the risk of localisation by eavesdropping predators, which could perceive the signal arriving from two different sources. As for *A. makarovi* (De Groot et al. 2012), the long and complex male call in *B. cockerelli* could also ensure species recognition, thus the female reply would occur when the male signal reaches a critical duration and before its end. In this regard, the female replying latency was quite variable, ranging from a minimum of 5.2 to a maximum of 41.8 seconds from the beginning of the MCS. The time between the onset of the male calling signal and the onset of the female reply could be used by the female to evaluate the quality of the potential partner. In this way, the female could assess the MCS before emitting her signal and choose whether to reply and elicit the male search. In fact, some *B. cockerelli* females (20%) did not respond to the male calling signal in our trials. Nonetheless, after the establishment of a duet, mating was successfully achieved by most males (90% of duetting pairs mated), suggesting that female choice likely occurred together with the identification, during the first phase of pair formation. Mating success of psyllids males is usually high (Eben et al. 2014; Liao and Yang 2015; Liao et al. 2016), since females are ready to mate soon after eclosion as adults (Burts and Fischer 1967) and promptly reply to male vibrations (Percy et al. 2006). Nevertheless, psyllid mating behaviour could depend on additional signals (e.g., specific odours), which could elicit the female motivation and increase the ability of males to reach a mate. Psyllid species such as *D. citri* and *B. cockerelli* bear complex sensory arrays on the antennae, which permit chemical detection, most likely used to locate host plants and mates (Arras et al. 2012). Although vibrational signals seem to be the primary sensory modality in *B. cockerelli* for mate finding, few males (10%, n = 62) successfully mated with the female without the emission of any signal. The small size of our experimental substrate (the leaf) could have been advantageous for the male and thus he could have reached the female by chance. Nevertheless, we cannot exclude the possible role of chemical or visual stimuli, which could be used by the male to perceive the presence of a female. Accordingly, these stimuli could allow the male to delay or even prevent the emission of a calling signal, if he can use them either to orient closer to or reach the female. In this way, the male could decrease both the mating success of eavesdropping rivals and probability of being attacked by a predator (Wenninger et al. 2009).

The role of semiochemicals in psyllids has been reported for several species (Soroker et al. 2004; Horton and Landolt 2007; Horton et al. 2008; Wenninger et al. 2009) including *B. cockerelli* (Guédot et al. 2010). In this regard, *B. cockerelli* males were weakly attracted to conspecifics' odorants and, in a two-choice test, they moved preferentially towards live males or females, rather than towards the respective insect extracts alone. Females, on the other hand, were not attracted by conspecifics' odorants, while in the two-choice test they showed neither preference nor avoidance (Guédot et al. 2010). However, this study did not take into account the potential role of vibrational signals that could have potentially elicited males' search in the two-choice tests, while females did not move since mate searching is accomplished by males. At any rate, it would be worthy of investigation to assess whether the combination of chemical and vibrational signals would increase the male searching activity and finally confirm the use of multiple signals for mate finding in psyllids. In this regard, experiments involving multiple signals could provide both new ethological insights about psyllids and be useful for development of integrated pest management techniques. Although female sex pheromones are involved in the mating behaviour of two species of *Cacopsylla* (Soroker et al. 2004; Horton and Landolt 2007), and *Cacopsylla pyri* uses vibrations to identify and localise potential partners (Eben et al. 2014), synergy between chemical and vibrational signals has not been yet well explored. Additional evidence of the role of chemical signals in psyllids' mating behaviour could be from the signalling activity of *B. cockerelli* female. In contrast with most Psylloidea, mating was achieved when the female emitted the first vibrational signal (16%, n = 62), eliciting the male signalling and searching activity. Since none of the females spontaneously produced a signal when individually tested, female signalling could depend on the perception of the male odour.

Alternatively, other signals could indicate the presence of a conspecific; for instance, the female could have perceived the incidental vibrations produced by the male while walking or feeding on the leaf. In fact, the signalling rate of *D. citri* males was not affected by the presence of female odours (Wenninger et al. 2009), while the playback of specific vibrational signals to the plant evoked calling signals from *D. citri* males that eventually located the source of the signals (Rohde et al. 2013), suggesting that the latter could be considered as a lure. Therefore, not only the mating behaviour of this species strongly relies on vibrations, but it is vulnerable to manipulation as well. Similarly, our female playbacks elicited the signalling activity of *B. cockerelli* males that eventually reached and remained in vicinity of the stimulation point. The number of males that established a proper duet was higher when we transmitted the female playback 2, whose second signal was as long as the natural female response, while the second signal of the female playback 1 was shorter. Since species identification usually relies on the estimation of the temporal parameters of the mating signals (Claridge 1985; Čokl and Virant-Doberlet 2003; Virant-Doberlet et al. 2014), males likely preferred the playback that most resembled a conspecific female response. In addition, a synthetic stimulus able to efficiently attract a male should necessarily occur in a specific time window to be reliable. The trapping system used for *D. citri*, for example, was improved using a device that automatically emitted the female playback after the detection of a male calling signal (Mankin et al. 2013). However, once recognition occurred, the number of *B. cockerelli* males that reached the stimulation point did not significantly differ between the group treated with the FPB1 (Test 2a) and the group treated with the FPB2 (Test 2b). Although the longer playback elicited higher male signalling activity (in terms of duetting), likely it was not more informative than the female playback 1 about direction and distance from the source. Besides species identification, the temporal features of a signal can convey relevant directional orientation. In *A. makarovi*, the success of the male in locating the female was significantly affected by the length of the female response signal (Kuhelj et al. 2015), while in the planthopper *Hyalesthes obsoletus*, the spectral features of the female call influenced the signalling and searching activity of the male, which moved towards the preferred female playback in a series of two choice tests (Mazzoni et al.; 2015). Therefore, to develop an efficient behavioural manipulation strategy for *B. cockerelli*, research should focus on the temporal and spectral parameters of the female playback, specifically determining which characteristics provide directional cues to the male and maintain his motivation to search.

To conclude, we have provided evidence that vibrational signals are crucial for mate finding in *B. cockerelli* and that the development of a future vibrational control tool against this pest may be technically feasible. Indeed, we suggest further research aimed to set more attractive stimuli by improving the tested playback signals and/or implementing them with a chemical cue.

References

Alexander RD (1967) Acoustical communication in arthropods. Annual Review of Entomology 12(1):495-526.

Arras J, Hunter W, Bextine B (2012) Comparative analysis of antennae sensory arrays in asian citrus psyllid, *Diaphorina citri*, and potato psyllid, *Bactericera cockerelli* (Hemiptera). Southwestern Entomologist 37(1):1-12, 12.

Berry NA, Walker MK, Butler RC (2009) Laboratory studies to determine the efficacy of selected insecticides on tomato/potato psyllid. New Zealand Plant Protection 62(0).

Booij CJH (1982) Biosystematics of the Muellerianella complex (Homoptera, Delphacidae), interspecific and geographic variation in acoustic behaviour. Zeitschrift für Tierpsychologie 58:31-52.

Broughton WB (1963) Method in bio-acoustic terminology. In Acoustic Behavior of Animals. (Busnel, R. G. (ed)) Elsevier Publishing Co., New York.

Burts EC, Fischer WR (1967) Mating behavior egg production and egg fertility in Pear psylla. Journal of Economic Entomology 60(5):1297-300.

Claridge MF (1985) Acoustic-signals in the Homoptera - behavior, taxonomy, and evolution. Annual Review of Entomology 30:297-317.

Cocroft RB, Rodríguez RL (2005) The behavioral ecology of insect vibrational communication. Bioscience 55:323-334.

Čokl A, Virant-Doberlet M (2003) Communication with substrate-borne signals in small plant-dwelling insects. Annual Review of Entomology 48(1):29-50.

De Groot M, Derlink M, Pavlovčič P, Prešern J, Čokl A, Virant-Doberlet M (2012) Duetting behaviour in the leafhopper *Aphrodes makarovi* (Hemiptera: Cicadellidae). Journal of Insect Behavior 25(5):419-440.

Derlink M, Abt I, Mabon R, Julian C, Virant-Doberlet M, Jacquot E (2018) Mating behavior of *Psammotettix alienus* (Hemiptera: Cicadellidae). Insect Science 25(1):148-160.

Eben A, Mühlethaler R, Gross J, Hoch H (2014) First evidence of acoustic communication in the pear psyllid *Cacopsylla pyri* L. (Hemiptera: Psyllidae). Journal of Pest Science:1-9.

Endler JA (1992) Signals, signal conditions, and the direction of evolution. American Naturalist 139:S125-S153.

Eriksson A, Anfora G, Lucchi A, Lanzo F, Virant-Doberlet M, Mazzoni V (2012) Exploitation of Insect Vibrational Signals Reveals a New Method of Pest Management. Plos One 7(3).

Foster SP, Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annual Review of Entomology 42:123-146.

Goolsby J, Adamczyk J, Bextine B, Lin D, Munyaneza J, Bester G, Lay F (2007) development of an IPM program for management of the potato psyllid to reduce incidence of zebra chip disorder in potatoes. Subtropical Plant Science 59:85-94.

Gordon SD, Sandoval N, Mazzoni V, Krugner R (2017) Mating interference of glassy-winged sharpshooters, *Homalodisca vitripennis*. Entomologia Experimentalis et Applicata 164(1):27-34.

Guédot C, Horton DR, Landolt PJ (2010) Sex attraction in *Bactericera cockerelli* (Hemiptera: Triozidae). Environmental Entomology 39(4):1302-1308.

Hall DG, Richardson ML, Ammar ED, Halbert SE (2013) Asian citrus psyllid, *Diaphorina citri*, vector of citrus huanglongbing disease. Entomologia Experimentalis et Applicata 146:(2):207-223.

Hansen AK, Trumble JT, Stouthamer R, Paine TD (2008) A new huanglongbing species, "*Candidatus* liberibacter psyllaurous," found to infect tomato and potato, is vectored by the psyllid *Bactericera cockerelli* (Sulc). Applied and Environmental Microbiology 74(18):5862-5865.

Horton DR, Guedot C, Landolt, PJ (2008) Attraction to volatiles of male summer form pear psylla from female pear psylla: effects of female age, mating status, and presence of host plant. Canadian Entomologist 140(2):184-191.

Horton DR, Landolt PJ (2007) Attraction of male pear psylla, *Cacopsylla pyricola*, to female-infested pear shoots. Entomologia Experimentalis et Applicata 123(2):177-183.

Kanmiya K (2006) Mating Behaviour and Vibratory Signals in Whiteflies (Hemiptera: Aleyrodidae). In Insect Sounds and Communication, Physiology, Behaviour, Ecology, and Evolution. (Drosopoulos, S., and Claridge, M. F. (eds)) CRC Press Boca Raton, pp. 365-379.

Krugner R, and Gordon SD, 2018 Mating disruption of *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae) by playback of vibrational signals in vineyard trellis. Pest Management Science 74: 2013-2019.

Kuhelj A, Groot MD, Blejec A, Virant-Doberlet M (2015) The effect of timing of female vibrational reply on male signalling and searching behaviour in the leafhopper *Aphrodes makarovi*. Plos One 10(10):e0139020.

Liao YC, Wu ZZ, Yang MM (2019) Vibrational behavior of psyllids (Hemiptera: Psylloidea): functional morphology and mechanisms. bioRxiv:593533.

Liao YC, Huang SS, Yang MM (2016) Substrate-Borne Signals, Specific Recognition, and Plant Effects on the Acoustics of Two Allied Species of Trioza, With the Description of a New Species (Psylloidea: Triozidae). Annals of the Entomological Society of America 109(6):906-917.

Liao YC, Yang MM (2015) Acoustic communication of three closely related psyllid species: a case study in clarifying allied species using substrate-borne signals (Hemiptera: Psyllidae: Cacopsylla). Annals of the Entomological Society of America 108(5):902-911.

Liefting LW, Sutherland PW, Ward LI, Paice KL, Weir BS, Clover GRG (2009) A new '*Candidatus* Liberibacter' species associated with diseases of solanaceous crops. Plant Disease 93(3):208-214.

Liu D, Trumble JT (2007) Comparative fitness of invasive and native populations of the potato psyllid (*Bactericera cockerelli)*. Entomologia Experimentalis et Applicata 123(1):35-42.

Mankin RW, Rohde BB, Mcneill SA, Paris TM, Zagvazdina NI, Greenfeder S (2013) *Diaphorina citri* (Hemiptera: Liviidae) responses to microcontroller-buzzer communication signals of potential use in vibration traps. Florida Entomologist 96(4):1546-1555.

Mazzoni V, Lucchi A, Cokl A, Presern J, Virant-Doberlet M (2009) Disruption of the reproductive behaviour of *Scaphoideus titanus* by playback of vibrational signals. Entomologia Experimentalis et Applicata 133(2):174-185.

Mazzoni V, Polajnar J, Baldini M, Stacconi MVR, Anfora G, Guidetti R, Maistrello L (2017) Use of substrate-borne vibrational signals to attract the Brown Marmorated Stink Bug, *Halyomorpha halys*. Journal of Pest Science 90(4):1219-1229.

Mazzoni V, Polajnar J, Virant-Doberlet M (2015) Secondary spectral components of substrate-borne vibrational signals affect male preference. Behavioural Processes 115:53-60.

Munyaneza JE (2010) Psyllids as vectors of emerging bacterial diseases of annual crops. Southwestern Entomologist 35(3):471-477.

Nieri R, Mazzoni V (2018) The reproductive strategy and the vibrational duet of the leafhopper *Empoasca vitis.* Insect Science 25(5):869-882.

Nieri R, Mazzoni V, Gordon SD, Krugner R (2017) Mating behavior and vibrational mimicry in the glassy-winged sharpshooter, *Homalodisca vitripennis*. Journal of Pest Science 90(3):887-899.

Pedigo LP, Rice ME (2014) Entomology and pest management. Waveland Press.

Percy DM, Taylor GS, Kennedy M (2006) Psyllid communication: acoustic diversity, mate recognition and phylogenetic signal. Invertebrate Systematics 20(4):431-445.

Pertot I, Caffi T, Rossi V, Mugnai L, Hoffmann C, Grando MS, Gary C, Lafond D, Duso C, Thiery D, Mazzoni V, Anfora G (2017) A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Protection 97:70-84.

Polajnar J, Eriksson A, Lucchi A, Anfora G, Virant-Doberlet M, Mazzoni V (2015) Manipulating behaviour with substrate-borne vibrations - potential for insect pest control. Pest Management Science 71(1):15-23.

Polajnar J, Eriksson A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. Journal of Pest Science 89(4):909-921.

Rohde B, Paris TM, Heatherington EM, Hall DG, Mankin RW (2013) Responses of *Diaphorina citri* (Hemiptera: Psyllidae) to Conspecific Vibrational Signals and Synthetic Mimics. Annals of the Entomological Society of America 106(3):392-399.

Siegel S, Castellan Jr NJ (1988) Nonparametric statistics for the behavioral sciences, 2nd ed. New York, NY, England, Mcgraw-Hill Book Company.

Soroker V, Talebaev S, Harari AR, Wesley SD (2004) The role of chemical cues in host and mate location in the pear psylla *Cacopsylla bidens* (Homoptera: Psyllidae). Journal of Insect Behavior 17(5):613-626.

Steel RGD (1959) A Multiple Comparison Rank Sum Test: Treatments versus Control. Biometrics 15(4):560-572.

Taylor KL (1985) A possible stridulatory organ in some Psylloidea (Homoptera). Australian Journal of Entomology 24(1):77-80.

Teulon DaJ, Workman PJ, Thomas KL, Nielsen MC (2009) *Bactericera cockerelli*: incursion, dispersal and current distribution on vegetable crops in New Zealand. New Zealand Plant Protection 62:136-144.

Tishechkin DY (2003) Vibrational communication in Cercopoidea and Fulgoroidea (Homoptera: Cicadina) with notes on classification of higher taxa. Russian Entomological Journal 12(2):127-181.

Tishechkin DY (2005) Vibratory Communication in Psylloidea (Homoptera). In Insect Sounds and Communication: Physiology, Behaviour, Ecology and Evolution. (Drosopoulos S, Claridge MF (eds)) Taylor and Francis Group, Boca Raton, New York, pp. 357–363.

Virant-Doberlet M, Cokl A (2004) Vibrational communication in insects. Neotropical Entomology 33(2):121-134.

Virant-Doberlet M, Mazzoni V, De Groot M, Polajnar J, Lucchi A, Symondson WOC, Čokl A (2014) Vibrational Communication Networks: Eavesdropping and Biotic Noise. In Studying Vibrational Communication. (Cocroft RB, Gogala M, Hill PS, Wessel A (eds)) Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 93-123.

Virant-Doberlet M, Zezlina I (2007) Vibrational communication of *Metcalfa pruinosa* (Hemiptera: Fulgoroidea: Flatidae). Annals of the Entomological Society of America 100(1):73-82.

Wenninger EJ, Hall DG, Mankin RW (2009) Vibrational communication between the sexes in *Diaphorina citri* (Hemiptera: Psyllidae). Annals of the Entomological Society of America 102(3):547-555.

Wood R, Peters R, Taylor G, Steinbauer M (2016) Characteristics of the signals of male *Anoeconeossa bundoorensis* Taylor and Burckhardt (Hemiptera: Aphalaridae) associated with female responsiveness. Journal of Insect Behavior 29(1):1-14.

Chapter 7

Can vibrational playbacks either disrupt mating or influence other relevant behaviours in *Bactericera cockerelli* (Triozidae: Hemiptera)?

Sabina Avosani, Thomas E. Sullivan, Marco Ciolli, Valerio Mazzoni, and David M. Suckling

Insects. **2020**, 11(5), 299

DOI: 10.3390/insects11050299

Abstract

Behaviours of insects can be manipulated by transmitting vibrational signals to host plants to develop pest management techniques. *Bactericera cockerelli* is an important pest and uses vibrations for mate finding. To design a future control strategy for *B. cockerelli*, three different bioassays were performed to assess whether vibrational signals could affect relevant behaviours. Either single males or pairs, were treated with a female playback in test 1 and 2, respectively. In test 3, mixed sex groups received different disturbance playbacks. The use of a female playback significantly reduced the mating success of males since they were attracted towards the source of the stimulus. Moreover, test 2 revealed that *B. cockerelli* females are competitive, since they used their signals to cover the playback and to duet with males, while in test 3, the disturbance playback consisting of broadband noises significantly reduced male signalling activity. However, none of the treatments of test 3 negatively affected the mating success of males, which tended to mount the other conspecifics present on the same leaf. The role of vibrations in sexual communication and their potential application as control technique for *B. cockerelli* are discussed as well.

Keywords: behavioural manipulation; vibrational signals; tomato potato psyllid; mating disruption; pest control

1. Introduction

Many insect species rely on vibrational signals to interact with co- and heterospecifics in different behavioural contexts (Hill 2001; Virant-Doberlet and Cokl 2004). Recent studies of applied biotremology demonstrated that vibrations can be used to either manipulate or disrupt insect behaviours in order to control pests' populations (Polajnar et al. 2015; Takanashi et al. 2019). Vibrational techniques are eco-friendly control practices that could be applied in the field to support integrated pest management strategies and decrease the use of pesticides. For instance, the mating behaviour of the leafhopper *Scaphoideus titanus* was successfully disrupted in semi-field conditions by the transmission of the male rivalry signal into grapevine (Polajnar et al. 2016), leading to the realization of the first vibrational vineyard in Italy for the control of grapevine leafhoppers since 2017 (Mazzoni et al. 2019). Another example of the use of vibrations for pest control is the transmission of a female Asian citrus psyllid *Diaphorina citri* playback to small citrus trees to disrupt mating and attract males toward the vibrating traps (Lujo et al. 2016; Mankin 2019). Other applications, currently objects of research, involve the glassy-winged sharpshooter *Homalodisca vitripennis* (Gordon et al. 2017; Gordon and Krugner 2019), and the brown marmorated stinkbug *Halyomorpha halys* (Mazzoni et al. 2017b; Polajnar et al. 2019).

In this research work, we explored the use of vibrational signals to manipulate the mating behaviour of the tomato potato psyllid *Bactericera cockerelli*. This is an important pest of solanaceous crops such as potato (*Solanum tuberosum*), tomato (*Solanum lycopersicum*) and pepper (*Capsicum annum*) (Munyaneza et al. 2007; Butler and Trumble 2012). Psyllid infestations have resulted in severe losses in the United States, Mexico, Central America and New Zealand (Secor and Rivera-Varas 2004; Munyaneza et al. 2007; Teulon et al. 2009; Butler and Trumble 2012). Besides damage due to direct feeding, the psyllid can transmit the bacterium *Candidatus* Liberibacter solanacearum (Liefting et al. 2008; Sengoda et al. 2014), which causes a severe disease known as ''zebra chip'' in commercial potatoes (Munyaneza 2012; Sengoda et al. 2014). Current control strategies are relatively expensive and mainly consist of using pesticides, hence, ongoing research is addressed to design novel approaches for the management of this pest (Butler and Trumble 2012). Alternative techniques could involve the application of vibrations to affect the psyllids' mating behaviours, since they are commonly mediated by vibrational signals (Percy et al. 2006; Lubanga et al. 2014; Liao and Yang 2015). Similar to other psyllid species (Percy et al. 2006), the sexual behaviour of *B. cockerelli* depends upon the establishment of a vibrational duet between a female and a male, in which the latter actively searches for the stationary female on the plant (Avosani et al. 2020). Although the male is usually the first emitter, the female could advertise her presence with the emission of a female attraction signal (FAS), while the female response signal (FRS) is used to duet with the male. The FAS and the FRS have been used to synthesize a female playback, which was already tested on single males placed on a capsicum leaf. It was found that the transmission of the playback to the leaf, not only triggered the male signalling activity, but also attracted the psyllid towards the source of the stimulus (i.e., a mini shaker; (Avosani et al. 2020)).

Besides natural pre-recorded signals, intense sounds can affect insect behaviour, as demonstrated for the green peach aphid *Myzus persicae* (Lee et al. 2012). In this case, sound stimuli with frequencies between 100 and 10 000 Hz transmitted at 66- and 90-dB SPL suppressed the feeding activity of the aphids (Lee et al. 2012). Electronic music was recently used to disrupt insect behaviours as well. The airborne transmission of the song *Scary Monsters and Nice Sprites* by Skrillex

(Skrillex 2010), for instance, negatively influenced feeding, frequency of host attack, and mating in the mosquito *Aedes aegypti* (Dieng et al. 2019). However, *B. cockerelli* uses vibrational signals to communicate, thus vibrational signals may be used to interfere with its behaviour.

Therefore, we performed three experiments using vibrational stimuli transmitted to the plant tissues with the aim of affecting the mating behaviour and/or the persistence of psyllids on the host. In test 1 and 2 (female playback on single males and on pairs, respectively), we used a female playback that was 15 s longer than the stimulus used in the previous work of Avosani and colleagues (Avosani et al. 2020). We hypothesised that a longer signal could be more efficient in attracting the male toward the source, probably by providing more directional information (de Groot et al. 2011). In test 1, the playback was tested on single males to evaluate its reliability in mimicking a real female and leading the insect towards the source of the stimulus. The same playback was then applied on pairs (a female and a male) in test 2 as an experiment of mating disruption. In this case, we assessed whether the playback transmission affected or not the mating success. Finally, in test 3, we investigated if the transmission of other unspecific vibrational signals (i.e., broadband noises and electronic music) to plant tissues could affect some aspects of the psyllid behaviour (i.e., signalling activity, mating success and persistence on the plant).

2. Materials and methods

2.1. Insects' rearing

A colony of *B. cockerelli* was kept under controlled conditions (23 ± 2°C, 60 ± 5% RH, 16:8 L:D) in a climate chamber at Plant and Food Research in Lincoln (Canterbury, New Zealand). The insects were maintained on capsicum (*Capsicum annuum*) inside rearing cages (Bugdorm-6620, MegaView Science Co., Ltd., Taiwan; 60 cm×60 cm×120 cm). each cage contained five plants, which were changed biweekly due to high level of psyllid infestation. In fact, more than 200 adults could be found inside each cage, together with nymphal stages and eggs.

2.2. Recordings

Studies were conducted on capsicum cuttings composed of a stem and either two or three leaves (Figure 7.1) depending on the test. The cutting was placed in a vial filled with water to prevent withering, while a laser vibrometer (PDV 100, Polytec, Waldbrann, Germany) was pointed onto a piece (0.5x0.5 cm) of reflective tape glued to a leaf; the recorded signals were digitized and stored via a sound card (M-Audio Fast Track Pro, M-Audio, United Kingdom) at a 48 kHz sample rate and 24-bit resolution on a laptop computer (HP, EliteBook 8460 p) using Raven Pro 1.6 (The Cornell Lab of Ornithology, Ithaca, NY, USA). The laser was set on a steel plate (1.2mx1.2mx10 mm; Slade Engineering, Christchurch, New Zealand) which was placed on an array of 9 insulating rubber pads over a table. To associate the behaviour and respective emission of vibrational signals, insect movements were continuously followed using two cameras (Logitech Quickcam Pro 9000 webcam, Logitech International S.A., Lausanne, Switzerland) (Microsoft® LifeCam HD-5000, Microsoft Corporation, U.S.A.), pointed on the ad-axial and ab-axial sides of the leaf, respectively. The software Bandicam 4.5. (Bandicam Company 2008-2020, Irvine, California, U.S.A.) was used control the cameras to follow the movement of the psyllids during the trials.

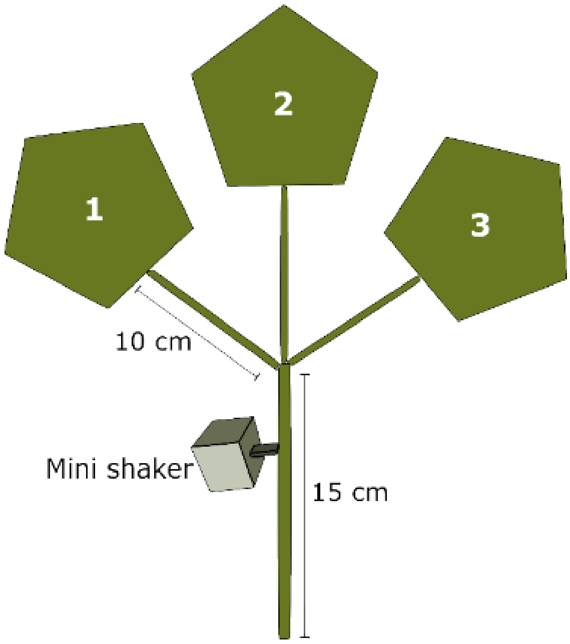

Figure 7.1. Schematic drawing of the experimental setup with a capsicum cutting, which consisted of either two (test 1 and 3) or three (test 2) leaves. The mini shaker was always pointed 2-3 cm below the node.

2.3. Playback trials

The trials were carried out from December 2019 to January 2020 at Plant and Food Research in Lincoln (Canterbury, New Zealand). The playbacks were transmitted to the substrate using an electromagnetic mini shaker (Direct Drive Linear Motor, model DDLM-038-051-01, MotiCont, Los Angeles, California, U.S.A) in direct contact with the stem of the cutting (Figure 7.1). A sharpened steel rod was screwed at the top of the device, which was set on a nearby table using a clamp. In total three experiments were conducted at room temperature (25 ± 2 °C).

Using a procedure similar to an experiment carried out in Avosani et al. (2020), pre-recorded psyllid signals were used to assemble a female playback consisting of a female attraction signal (FAS) followed by a female response signal (FRS) after 25.05 s of silence. The signals were 27.7 s and 53.3 s long, respectively. The repetition time between pulses, the interval between pulse trains, and the frequency ranges of the playback were the same as the natural pre-recorded signals. The duration of the FAS was not modified, while the FRS duration was 15 s longer than the pre-recorded signal (the average duration of the FRS is 42.8 ± 19.4 s (Avosani et al. 2020)). Although *B. cockerelli* is a polygamous insect (Guédot et al. 2013), psyllids were removed from the mass colony and separated by gender two days before performing the test to increase their motivation to mating.

2.3.1. Test 1. Female playback on single males

The female playback was tested on single males (n = 20) as a loop for 20 minutes to assess whether the stimulus could trigger the male search and lead the psyllids towards the device. Single males (n = 20) were left in silence for 20 minutes as control.

2.3.2. Test 2. Female playback on pairs

To evaluate if the female playback could disrupt the mating behaviour, a pair (n = 30) consisting of a male and a female, was released on the cutting. Psyllids were placed on different leaves, which had approximately similar size (12-15x8 cm). In both treated and control groups, if a duet was not established after 5 minutes from the insect release, the signalling activity of the psyllids was stimulated by playing the female playback. The pairs that, despite the use of playback stimulation, did not establish a duet were discarded. The tests ended after 30 minutes from the insect release.

2.3.3. Test 3. Unspecific noise playbacks

Groups (n = 25) consisting of two females and two males were collected from the mass rearing and released on the same leaf of a cutting consisting of two leaves (approximate size: 10-12x8 cm). They were treated for 30 min with the transmission of two different playbacks, which were made using Audacity (Softonic International, Barcelona, Spain). Group 1, the "noise group", was treated with a playback consisting of a sequence of 1.7 s long white noises with 0.3 s of silent intervals and frequency range between 0 and 22000 Hz. Group 2, the "music group" was tested with a playback obtained from the instrumental version of *Scary Monsters and Nice Sprites*. The song was created by music producer Skrillex and was used by Dieng and colleagues (2019) to disrupt the feeding and mating behaviour of the mosquito *A. aegypti* (Dieng et al. 2019). The track was downloaded from the Soundboard website (©2015 Soundboard, https://www.soundboard.com/sb/sound/937286) and then modified using Audacity. The song was cut from minute 1 to 3:40, and the selection was transmitted through the cutting as loop interspaced by 29.5 s of silence. A control group (n = 25) was left in silence for 30 min. To assess whether the playbacks could decrease the psyllids' signalling activity, we measured the number of males that called and those that established a duet with a female. Discerning which male was signalling was possible by direct observation, as the production of vibrations involves the movement of the wings (Avosani et al. 2020). We also counted the number of males that either achieved mating with a female or mounted another male to determine whether the playbacks could interfere with the mating success and interspecific interactions, also in terms of partner's misidentification. The number of psyllids that left the plant during the trials was used to evaluate if the playbacks could repel the insects from the host plant.

2.4. Data Analysis

In test 1, the call latency (i.e., the time from the release of the male to the first call) was compared between treatment and control using the Mann-Whitney two-sample test. In test 2, a chi squared test in contingency tables (2x2), Bonferroni-corrected, was used to compare the number of searching males, the number of matings and the number of males that reached the mini-shaker. The Mann-Whitney two-sample test was applied to compare the time of the search latency (i.e., from the start of the trial to the onset of the male search), the time males took to reach the female, and the time took males to arrive at the node between the leaves. The same test was used for the comparison of the number of times the male visited the female leaf, the number of times the male visited the other two leaves, and the number of times a male moved down the stem. In test 3, the Kruskal–Wallis test followed by Mann–Whitney pairwise, Bonferroni-adjusted, was used to compare the duration of the copulations between groups. The number of calling males between each group was compared using a chi test in contingency tables (2x2), Bonferroni-corrected. A G test in contingency tables (2x3), Bonferroni-corrected, was applied to compare the male mating success, the frequency of male-on-male mounting and the number of insects that jumped off the leaf.

3. Results

3.1. Test 1. Female playback tested on single males

Although the number of calling males was rather similar between the treated and the control groups (11 and 8, respectively, n = 20), the call latency was significantly lower when the males were exposed to the playback (Mann-Whitney test: n = 8, 11; U = 1; $p < 0.001$). Within the treated group, nine of the calling males engaged in a duet with the stimulus and started to search for the source of the signal, reaching the node (n = 4) of the cutting and therefore getting close to the mini shaker. None of the control males moved from the leaf lamina after their release, regardless of their calling activity.

3.2. Test 2. Female playback tested on pairs

In the control and treated groups, 19 and 9 males, respectively, called spontaneously thus triggering the female response and the duet. In the other cases (11 and 21, respectively), the onset of a male-female duet was triggered using the female playback. The duet was established if the male emitted a MCS and the female replied with a FRS. Unexpectedly, most females (72.7% in the control and 66.6% in the treated group) replied to the vibrational stimulus before the males. Females emitted their signals both during and after the end of the playback, while males always called after a female signal, which could be either artificial (playback) or natural (female psyllid). In both control and treatment, duets were established even if the female replied to the playback before the male. Throughout the test, females in the treated group overlapped their signal with both the male and the playback. Few females (9 out of 30) ceased to reply before the male could reach them or before the end of the trial. Similarly, 8 females in the control renounced to duet with the male prior to the end of the test.

Although the number of searching males was similar between the two groups (Chi squared 2x2 in contingency tables, p = 1; Table 7.1) and the male search latency was only marginally statistically different (Mann-Whitney two-sample tests, p = 0.07; Table 7.2), the number of matings was significantly lower in the treated group (23.3%) than in the control (56.7%) (Chi squared 2x2 in contingency tables, $p \leq 0.001$; Table 7.1). However, the time a male took to reach a female was similar between the groups (Mann-Whitney two-sample tests, p = 0.08; Table 7.2), and mating occurred as soon as the male found the partner. Nonetheless, in one trial a treated male continued to call and searched for the female even after he had reached her on the plant. Similarly, the time needed for a male to arrive at the node between the leaves, once he had started to search, was similar between groups (Mann-Whitney two-sample tests, p = 0.08; Table 7.2). In both groups, the searching males visited the three different leaves several times (the one where he was released, the one that hosted the female and the empty leaf). Males moved from the node to the petioles and back, rarely walking into the leaf laminas. The number of times the male visited the female leaf (Mann-Whitney two-sample tests, p = 0.19; Table 7.2) was rather similar between the two groups, while the number of times the male visited the other leaves was significantly higher in the treatment than in the control (Mann-Whitney two-sample tests, p = 0.01; Table 7.2). The number of times a male chose to move towards the stem was also significantly higher in the treated group (Mann-Whitney two-sample tests, p = 0.002; Table 7.2), as well as the number of males that reached the mini shaker (chi squared 2x2 in contingency tables, p = 0.006; Table 7.1). In the treated group, males (26.7%; n = 30) climbed on the device and remained on the device or on the stem in close proximity to it until the end of the trial.

Table 7.1. Results of the test chi squared 2x2 in contingency tables for control and treatment in Test 2. Number of pairs of *Bactericera cockerelli* tested per each group = 30.

Measurement	Number of observations		χ^2	df	*p*
	Control	Playback			
Searching males	27	26	0.16	1	1
Mating events	17	7	6.94	1	≤ 0.001
Males that reached the mini shaker	5	5	7.5	1	p = 0.006

Table 7.2. Results of the test Mann-Whitney two-sample tests for control and treatment in Test 2. Number of pairs of *Bactericera cockerelli* tested per each group = 30.

Measurement	Number of observations		U	*p*
	Control	Playback		
Start of male search	27	26	250	0.07
Time to reach the female	17	7	56.5	0.8
Time to reach the node	17	7	56.5	0.8
Male visits to the female leaf	25	27	274	0.19
Male visits to other leaves	25	27	213	0.01
Males visits to the stem	25	27	195.5	0.002

3.3. Test 3. Unspecific noise playbacks

The number of calling males was significantly different between the two treatments (Chi-squared test: χ^2 = 22.2, df = 1, p ≤ 0.001). In the “noise group” only one male called, while in the “music group”, more than 50% of the males emitted at least one calling signal. The number of calling males in the control was lower (40%), but not significantly different from the music group (Chi-squared: χ^2 = 3.9, df = 1, p = 0.26). The difference was significant when comparing the control to the “noise group” (chi-squared: χ^2 = 9.44, df = 1, p ≤ 0.02). Most of the calling males (58.8%) in the “music group” emitted their signals only during the silence gap, while the remaining 41.2% called both during silence and playback. The male call triggered either female response signals (1 FRS in the “music group”; 2 FRSs in the control group) or calling signals of the other male (3 MCSs in the “music group”). None of the females or males replied to the male call in the “noise group”. In case of a female response, a male-female duet was always established but led to mating only in one case (in the control group). Although female replies and therefore vibrational duets were sporadic, males could achieve mating in all the tested groups without signalling. The male mating success was similar between groups (G test: G = 0.3, df = 2, p = 0.8) since 40% (“music”), 36% (“noise”) and 44% (control) of the males eventually copulated with a female. There was no significant difference in the duration of the copulations between groups (Kruskal–Wallis test: χ^2 = 2.9, df = 2; p = 0.2) and only one (“music group”) and three (control) male attempts failed due to female rejection. Male on male mounting was observed as well, with a similar frequency between groups (G test: G = 3.5, df = 2, p = 0.2), which was of 20% (“music”), 8% (“noise”) and 28% (control) for each group, respectively. Psyllids tended to walk along the leaf but remained on the cutting independent of the transmission of the playback, since the number of insects that jumped off the leaf was similar between groups (G test: G = 0.2, df = 2, p = 0.9).

4. Discussion

The present work demonstrated that the behaviour of *B. cockerelli* is vulnerable to manipulation by means of vibrational signals. In particular, the use of species-specific signals could be potentially used to interfere with the mating behaviour, either as a masking noise or an attractive stimulus. On the contrary, unspecific noises did not have any relevant effect on psyllids' sexual behaviour.

The female playback successfully mimicked a *B. cockerelli* female, since it elicited the male search similarly to the actual female response in the test with pairs. Moreover, the playback prevented the male from visiting the female leaf and led him toward the device, resulting in a lower number of mating events. Although the mini shaker was in direct contact with the stem, *B. cockerelli* males frequently moved along the leaves and reverted their path back towards the node before reaching the leaf lamina. One hypothesis is that the directional cues provided by the playback could keep the male in between the petiole of the leaves and the node. In fact, the intensity of vibrational signals is very variable according to the plant architecture and can be higher at certain transition or bifurcation points than in other parts of the plant (Michelsen et al. 1982; Mankin et al. 2018). Another possibility is that a real female, at short distance, could provide additional stimuli (i.e., chemical and visual) to searching males, and their absence could explain the tendency of males to not go directly to the source of the vibrational stimulus. Indeed, positive phototaxis and negative geotaxis also guided the male towards the top of the plant, where the actual female was placed. In fact, *B. cockerelli* tends to move towards light stimuli, and UV-illumination was recently applied to enhance the attractiveness of sticky traps (Hodge et al. 2019). Notwithstanding, even in the presence of a replying female and natural light, our female playback prevented males from reaching the top of the plant and led them in the opposite direction. Moreover, when a male reached the stem (i.e., close to the mini shaker), he persisted in his search and often mounted on the device without any further reversion of his orientation. On the other hand, whenever the male entered the petiole of the female leaf, he did not turn backwards, thus ignoring the playback. Hence, when the male was on the "right" path towards a potential replying partner, he did not change direction. As a result, it would be very important to investigate the level of search accuracy of *B. cockerelli* males. In fact, this phenomenon could be even more relevant if the male discriminates in favour of high-quality females and assesses the quality of replying females through the perception of their signals (Gwynne 1991). As an example, the accuracy of detection of a potential mate has been demonstrated for the planthopper *Hyalesthes obsoletus* when two different signals were simultaneously transmitted. In this species, the male distinguished perfectly between two different female signals with different frequency patterns and always chose to move towards one of them, which was specifically designed to contain more attractive features than the other (Mazzoni et al. 2015). Similarly, the importance of signals' spectral features in affecting the male responsiveness to a female playback was also indicated in a study of the sharpshooter *H. vitripennis* (Mazzoni et al. 2017a), in which the male preferred synthetic playbacks endowed of certain spectral and temporal parameters. If *B. cockerelli* males can also discriminate between different female signals, then the spectral characteristics of the female playback could be modified to increase the signal attractiveness and lead more males towards the device, even in presence of other replying females on the plant.

Notably, most of the *B. cockerelli* females responded to the playback as well, and often their replies occurred before the male's, which were always emitted after female signals. On the other hand, females signalled both during and after the playback, possibly as a form of rivalry behaviour to mask the vibrational cues provided by the “synthetic rival”. Although female insects are typically less competitive than males (Gwynne 1991), some examples can be found in Pentatomidae (Čokl et al. 2017), in the dance fly *Empis borealis* (Diptera) (Svensson et al. 1989), and in some orthopteran and heteropteran species (Smith 1979; Gwynne 1991). As for psyllids, female competition seems to be an behaviour of *B. cockerelli*. In fact, *D. citri* females ceased to reply to the male when treated with the playback of an artificial female (Lujo et al. 2016). An alternative hypothesis to the intrasexual competition, could be that the *B. cockerelli* females tried to form a cooperative chorus with the playback to attract the male. However, since the device and the female were placed in opposite parts of the plant (the bottom and the top, respectively), their signals were competing to convince the males to take a directional decision and thus make a mating choice. On the other hand, both hypotheses (competition and cooperation) can coexist. According to this, a male could be more stimulated to search from the presence of multiple singing females, while females could establish a chorus as a means of decreasing the risk of being located by eavesdropping predators (Greenfield 1994, 2014). In this case, by overlapping her signal with the rival ones, the female would compete with the rivals in a relatively “safer” way. Besides cooperation or rivalry, the establishment of a chorus could be due either to an excess of sexually receptive females (Gwynne 1991) or to female-biased gender ratio (Čokl et al. 2017). Furthermore, we must remember that psyllid populations can be rather dense and the contemporary presence of more males and females on the same plant is not rare. Nonetheless, there are no reports of intra-sexual rivalry within Psylloidea (Tishechkin et al. 2006). The role of female competition in *B. cockerelli* should be further investigated since similar information could be used to improve a potential behavioural manipulation strategy. For instance, a highly competitive female playback could lead female to cease signalling, as in the case of *D. citri* (Lujo et al. 2016).

The second approach that we tested to interfere with the *B. cockerelli* behaviour was the use of unspecific vibrations. It is known that cerambycids, for instance, respond to generic vibrational signals (Tsubaki et al. 2014; Takanashi et al. 2019), and some of their behaviours such as feeding, walking and oviposition can be disrupted by the transmission of artificial vibrations (Takanashi et al. 2019). In Coleoptera, immobility seems to serve as a strategy to recognise approaching conspecifics or predators (Takanashi et al. 2019). In the Japanese pine sawyer *Monochamus alternatus*, for example, vibrations below 1 kHz induced inactivity responses (Takanashi et al. 2019). Notwithstanding, none of our disturbance playbacks triggered similar responses in *B. cockerelli*. Neither the walking activity nor the physical and vibrational interactions of the psyllids were affected during the playback emissions. However, the possible interference with other behaviours such as feeding and oviposition cannot be excluded. Besides inactivity responses, animals can respond to disturbance noises by modulating their signalling activity and/or leaving the treated area. In some anuran species, for instance, anthropogenic noise affected the calling rate and the spatial displacement of the frogs, which moved away from the source of the disturbance (Caorsi et al. 2017). Although none of our playbacks induced the psyllids to leave the host plant, the males differently adjusted their signalling behaviour in response to either the noise or music. In fact, while the male calling activity was significantly reduced when broadband noises were transmitted through the plant, males did not cease to call when treated with electronic music. Although habituation is more likely to occur when animals are treated with repetitive noises (Gilsdorf et al. 2002), insects commonly avoid signalling in the presence of disturbance sounds and wait for silence (McNett et al. 2010). Thus, the short silent gap between the sequences of white noises (less than 0.5 s) likely discouraged *B. cockerelli* males from signalling, while the calling males in the music group exploited the long silent gap (30 s) within the song playback to start calling. Indeed, besides the temporal pattern, the disruptive potential of a playback can also be determined by spectral features. In the fruitfly *Drosophila montana*, for instance, the transmission at high intensities of a background noise with similar frequency to the male courtship signal resulted in a decrease of both female replies and male mating success (Samarra et al. 2009). Similarly, the most efficient disturbance noise to disrupt mating in *Empoasca vitis* was a playback set on the dominant frequency of the male call (Nieri and Mazzoni 2019). In *H. vitripennis*, the transmission of a noise with the dominant frequency of the female call (80 Hz) reduced the male replies and, consequently, the mating (Mazzoni et al. 2017a). The music playback did not prevent *B. cockerelli* males from calling, probably because the song consisted of a mixture of sounds with discrete frequencies, which were less efficient in masking the spectral contents of psyllids' calls than the broadband noise. In fact, although males tended to start calling during the silent gap, some individuals emitted their signal during the music playback. Conversely, the sequence of noises completely covered the frequency range of *B. cockerelli*'s signals, which are composed of broadband pulses ranging from 190 to 1125 Hz with the dominant at 548.5 ± 257.6 Hz (Avosani et al. 2020). Although this disturbance noise could be deployed to suppress the male calling activity in crops, the broad frequency range could also affect other insect species (pest and/or beneficial insects) responding to vibrational signals and present on the plant. Accordingly, only the frequencies that are in the sensitivity range of insect vibro-receptors would have an impact on the pest's behaviour and communication. In insects, the detection of vibrations is mainly achieved by chordotonal organs and among these, the subgenual organ is the most sensitive and responds to a frequency range between 50 and 1000 Hz (Čokl et al. 2005). Consequently, the signals used to communicate must also lie in the same frequency range. The noise playback, with its wide frequency range, would mask these signals and interfere with intra-specific communication of other insects than *B. cockerelli*. As example, green lacewings (Chrysopidae: Neuroptera) are responsive to vibrations and use substrate-borne signals to achieve mating (Henry 1984; Devetak and Amon 1997). These insects are effective predators and feed on many important agricultural pests, and for these reasons they are used in biological pest control (Senior et al. 2001).

Therefore, more tests are needed to both assess how background signals could affect the vibrational communication of useful insects in the field, and better understand which features best comply with the *B. cockerelli* behaviour to design species-specific signals to be used for population control. Besides mating disruption, disturbance vibrational signals could be used to disrupt the feeding behaviour of the psyllids, as has been reported for cerambycids (Takanashi et al. 2019). It would be therefore worthwhile to evaluate whether the playback can interfere with feeding by using an Electrical Penetration Graph (EPG) technique during the emission of the stimulus.

A control strategy against *B. cockerelli* based on the use of disturbance vibrations should be applied as a tool within a wider strategy of IPM to keep the infestations low. At high population density, the efficacy of the method would be probably lower, as suggested by the fact that *B. cockerelli* pairs occurring in close vicinity (in our trials we released four psyllids on the same leaf), managed to mate. Therefore, the mating success due to causal encounters was not negatively affected by the disturbance playbacks. In this regard, we hypothesise that vibrational signals are not always necessary to achieve mating, since the "silent" copulations seem to be common in *B. cockerelli* as well as in other Psylloidea. In fact, in some species a male can approach the female and successfully achieve copulation without any preliminary signals (Tishechkin 2007; Lubanga et al. 2016; Oppedisano et al. 2019). In other hemipterans such as leafhoppers (Cicadomorpha), pair formation is strictly dependent on the exchange of different vibrational signals that are associated to different phases of the mating process (i.e., identification, localisation, courtship) (Nuhardiyati and Bailey 2005; Polajnar et al. 2014). Unlike leafhoppers, the psyllids' repertoire of vibrational signals is typically limited to the male call and female reply, which are used invariantly during pair formation (Percy et al. 2006; Tishechkin 2007). This can be explained by the fact that psyllids can live in dense colonies on the plant (Burckhardt et al. 2014) and, in these circumstances, intra-specific interactions can minimally rely on exchanges of vibrational signals. In fact, we observed frequent mating and male-male mountings when both males and females were released on the same leaf in test 3. Same-sex sexual interactions are probably a result of high sexual responsiveness and could be explained as mistaken identification by the mounting male. As suggested for many other arthropods, the benefit of attempting mating with a conspecific (regardless of the gender) exceeds the costs of a misidentification (Scharf and Martin 2013). Although it is possible that there is an adaptive explanation for same-sex sexual interactions, unveiling the role of this behaviour in *B. cockerelli* was not the aim of this work. Nonetheless, future research should be addressed in this regard.

Further studies should also assess if other signal modalities are involved in the mating process and that can be synergistic or complementary to vibrations to accomplish important tasks (i.e., directionality, mate choice, female acceptance) (Lubanga et al. 2014). Accordingly, the role of odours in the mating behaviour of *B. cockerelli* has been demonstrated by Guédot and colleagues (Guédot et al. 2010). In their experiments, males were attracted by both live females and female odorants, which are likely sex attractants. Since the researchers did not evaluate whether the psyllids were also emitting vibrations, further trials should assess the use of multiple cues in the mating behaviour of *B. cockerelli*. If pair formation depends on both vibrational and chemical signals, these stimuli could be coupled to design a future technique to trap the insects and/or disrupt their mating behaviour. Accordingly, an example of manipulation of psyllids' behaviour by using multiple cues has been recently proposed for *Cacopsylla picta,* an important pest of apple (Oppedisano et al. 2019). In fact, the sexual communication of the genus Cacopsylla seems to rely on both vibrational and chemicals signals, and plant volatiles play a role as well (Soroker et al. 2004; Horton and Landolt 2007; Oppedisano et al. 2019).

5. Conclusions

To summarise, our study demonstrated that the mating behaviour of *B. cockerelli* can be affected by the transmission of a female playback, when a female and a male occur on different leaves. Furthermore, the broadband playback negatively affected the psyllids' calling behaviour. On the other hand, the disturbance test also demonstrated that *B. cockerelli* pairs that occur on the same leaf can meet and copulate even in the presence of background noises. Therefore, a vibrational mating disruption approach is likely to be efficient if applied before high rates of infestations are reached and when, therefore, the partner location can depend on vibrational communication. A remarkable outcome is that females can establish choruses, a phenomenon that was never reported in *B. cockerelli*, although at this stage of research, their role is not yet clear.

Indeed, even if vibrational manipulation of *B. cockerelli*'s behaviour has the potential to become a tool for an integrated pest management approach, technical constraints must be considered before the method can be used in the field. Although disruptive signals for the control of

S. titanus were transmitted to grapevine plants by exploiting the wires in the vineyard (Mazzoni et al. 2019), a similar strategy may not be suitable for potato crops. A possible option could be the induction of vibrations in host plants by means of sound-speakers. For instance, mating was disrupted in *Amrasca devastans* and *Nilaparvata lugens* by aerially transmitting disturbance sounds of 80 dB, 200–300-Hz (Saxena and Kumar 1980). Similar approaches should therefore be tested in field environment for a future use of the female playback and other disturbance signals to disrupt the mating behaviour in *B. cockerelli*.

References

Avosani S, Sullivan TES, Ciolli M, et al (2020) Vibrational communication and evidence for vibrational behavioural manipulation of the tomato potato psyllid, *Bactericera cockerelli*. Entomol Gen 40:351–363. https://doi.org/10.1127/entomologia/2020/0984

Burckhardt D, Ouvrard D, Queiroz D, Percy D (2014) Psyllid hostplants (Hemiptera: Psylloidea): Resolving a semantic problem. Florida Entomol 97:242–246. https://doi.org/10.1653/024.097.0132

Butler CD, Trumble JT (2012) The potato psyllid, *Bactericera cockerelli* (Sulc) (Hemiptera: Triozidae): life history, relationship to plant diseases, and management strategies. Terr Arthropod Rev 5:87–111. https://doi.org/10.1163/187498312x634266

Caorsi VZ, Both C, Cechin S, et al (2017) Effects of traffic noise on the calling behavior of two Neotropical hylid frogs. PLoS One 12:1–14. https://doi.org/10.1371/journal.pone.0183342

Čokl A, Dias AM, Moraes MCB, et al (2017) Rivalry between stink bug females in a vibrational communication network. J Insect Behav 30:741–758. https://doi.org/10.1007/s10905-017-9651-z

Čokl A, Virant-Doberlet M, Zorović M (2005) Sense organs involved in the vibratory communication of bugs. In: Insect sounds and communication. CRC Press, pp 80–89

De Groot M, Čokl A, Virant-Doberlet M (2011) Search behaviour of two hemipteran species using vibrational communication. Cent Eur J Biol 6:756–769. https://doi.org/10.2478/s11535-011-0056-2

Devetak D, Amon T (1997) Substrate vibration sensitivity of the leg scolopidial organs in the green lacewing, *Chrysoperla carnea*. J Insect Physiol 43:433–437. https://doi.org/10.1016/S0022-1910(96)00121-7

Dieng H, The CC, Satho T, et al (2019) The electronic song “Scary Monsters and Nice Sprites” reduces host attack and mating success in the dengue vector *Aedes aegypti*. Acta Trop 194:93–99. https://doi.org/10.1016/j.actatropica.2019.03.027

Gilsdorf JM, Hygnstrom SE, VerCauteren KC (2002) Use of frightening devices in wildlife damage management. Integr Pest Manag Rev 7:29–45. https://doi.org/10.1023/A:1025760032566

Gordon SD, Krugner R (2019) Mating Disruption by Vibrational Signals: Applications for Management of the Glassy-Winged Sharpshooter. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 355–373

Gordon SD, Sandoval N, Mazzoni V, Krugner R (2017) Mating interference of glassy-winged sharpshooters, *Homalodisca vitripennis*. Entomol Exp Appl 164:27–34. https://doi.org/10.1111/eea.12594

Greenfield MD (1994) Cooperation and conflict in the evolution of signal interactions. Annu Rev Ecol Syst 25:97–126. https://doi.org/10.1146/annurev.es.25.110194.000525

Greenfield MD (2014) Signal interactions and interference in insect choruses: singing and listening in the social environment. J Comp Physiol A Neuroethol Sensory, Neural, Behav Physiol 201:143–154. https://doi.org/10.1007/s00359-014-0938-7

Guédot C, Horton DR, Landolt PJ (2010) Sex attraction in *Bactericera cockerelli* (Hemiptera: Triozidae). Environ Entomol 39:1302–1308. https://doi.org/10.1603/en10048

Guédot C, Horton DR, Landolt PJ, Munyaneza JE (2013) Effect of mating on sex attraction in *Bactericera cockerelli* with evidence of refractoriness. Entomol Exp Appl 149:27–35. https://doi.org/10.1111/eea.12104

Gwynne DT (1991) Sexual competition among females: What causes courtship-role reversal? Trends Ecol Evol 6:118–121. https://doi.org/10.1016/0169-5347(91)90089-G

Henry CS (1984) Sexual behavior of green lacewings [Chrysopidae]. Ser Entomol 101-110

Hill PSM (2001) Vibration and animal communication: A review. Am Zool 41:1135–1142. https://doi.org/10.1668/0003-1569(2001)041[1135:VAACAR]2.0.CO;2

Hodge S, Bennett J, Merfield CN, Hofmann RW (2019) Effects of sticky trap colour, UV illumination and within-trap variation on tomato potato psyllid captures in glasshouses. New Zeal J Crop Hortic Sci 47:48–62. https://doi.org/10.1080/01140671.2018.1508043

Horton DR, Landolt PJ (2007) Attraction of male pear psylla, *Cacopsylla pyricola*, to female-infested pear shoots. Entomol Exp Appl 123:177–183. https://doi.org/10.1111/j.1570-7458.2007.00537.x

Lee Y, Kim H, Kang TJ, Jang Y (2012) Stress response to acoustic stimuli in an aphid: A behavioral bioassay model. Entomol Res 42:320–329. https://doi.org/10.1111/1748-5967.12000

Liao YC, Yang MM (2015) Acoustic communication of three closely related psyllid species: A case study in clarifying allied species using substrate-borne signals (Hemiptera: Psyllidae: Cacopsylla). Ann Entomol Soc Am 108:902–911. https://doi.org/10.1093/aesa/sav071

Liefting LW, Perez-Egusquiza ZC, Clover GRG, Anderson JAD (2008) A New ‘*Candidatus* Liberibacter’ Species in Solanum tuberosum in New Zealand. Plant Dis 92:1474. https://doi.org/10.1094/PDIS-92-10-1474A

Lubanga UK, Guédot C, Percy DM, Steinbauer MJ (2014) Semiochemical and vibrational cues and signals mediating mate finding and courtship in psylloidea (Hemiptera): A synthesis. Insects 5:577–595. https://doi.org/10.3390/insects5030577

Lubanga UK, Peters RA, Steinbauer MJ (2016) Substrate-borne vibrations of male psyllids vary with body size and age but females are indifferent. Anim Behav 120:173–182. https://doi.org/10.1016/j.anbehav.2016.07.033

Lujo S, Hartman E, Norton K, et al (2016) Disrupting mating behavior of *Diaphorina citri* (Liviidae). J Econ Entomol 109:2373–2379. https://doi.org/10.1093/jee/tow202

Mankin RW (2019) Vibrational Trapping and Interference with Mating of *Diaphorina citri*. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 399–413

Mankin RW, Stanaland D, Haseeb M, et al (2018) Assessment of plant structural characteristics, health, and ecology using bioacoustic tools. In: Proceedings of Meetings on Acoustics 175ASA.

Acoustical Society of America, p 10003

Mazzoni V, Gordon SD, Nieri R, Krugner R (2017a) Design of a candidate vibrational signal for mating disruption against the glassy-winged sharpshooter, *Homalodisca vitripennis*. Pest Manag Sci 73:2328–2333. https://doi.org/10.1002/ps.4619

Mazzoni V, Nieri R, Eriksson A, et al (2019) Mating Disruption by Vibrational Signals: State of the Field and Perspectives. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 331–354

Mazzoni V, Polajnar J, Baldini M, et al (2017b) Use of substrate-borne vibrational signals to attract the Brown Marmorated Stink Bug, *Halyomorpha halys*. J Pest Sci (2004) 90:1219–1229. https://doi.org/10.1007/s10340-017-0862-z

Mazzoni V, Polajnar J, Virant-Doberlet M (2015) Secondary spectral components of substrate-borne vibrational signals affect male preference. Behav Processes 115:53–60. https://doi.org/10.1016/j.beproc.2015.02.019

McNett GD, Luan LH, Cocroft RB (2010) Wind-induced noise alters signaler and receiver behavior in vibrational communication. Behav Ecol Sociobiol 64:2043–2051. https://doi.org/10.1007/s00265-010-1018-9

Michelsen A, Fink F, Gogala M, Traue D (1982) Plants as transmission channels for insect vibrational songs. Behav Ecol Sociobiol 11:269–281

Munyaneza JE (2012) Zebra Chip Disease of Potato: Biology, Epidemiology, and Management. Am J Potato Res 89:329–350. https://doi.org/10.1007/s12230-012-9262-3

Munyaneza JE, Crosslin JM, Upton JE (2007) Association of *Bactericera cockerelli* (Homoptera: Psyllidae) with “Zebra Chip,” a New Potato Disease in Southwestern United States and Mexico. J Econ Entomol 100:656–663. https://doi.org/10.1603/0022-0493(2007)100[656:aobchp]2.0.co;2

Nieri R, Mazzoni V (2019) Vibrational mating disruption of *Empoasca vitis* by natural or artificial disturbance noises. Pest Manag Sci 75:1065–1073. https://doi.org/10.1002/ps.5216

Nuhardiyati M, Bailey W (2005) Calling and duetting behavior in the leafhopper *Balclutha incisa* (Hemiptera: Cicadellidae: Deltocephalinae): Opportunity for female choice? J Insect Behav 18:259–280. https://doi.org/10.1007/s10905-005-0479-6

Oppedisano T, Polajnar J, Kostanjšek R, et al (2019) Substrate-borne vibrational communication in the vector of apple proliferation disease *Cacopsylla picta* (Hemiptera: Psyllidae). J Econ Entomol 1–8. https://doi.org/10.1093/jee/toz328

Percy DM, Taylor GS, Kennedy M (2006) Psyllid communication: Acoustic diversity, mate recognition and phylogenetic signal. Invertebr Syst 20:431–445. https://doi.org/10.1071/IS05057

Polajnar J, Eriksson A, Lucchi A, et al (2015) Manipulating behaviour with substrate-borne vibrations - Potential for insect pest control. Pest Manag Sci 71:15–23. https://doi.org/10.1002/ps.3848

Polajnar J, Eriksson A, Rossi Stacconi MV, et al (2014) The process of pair formation mediated by substrate-borne vibrations in a small insect. Behav Processes 107:68–78. https://doi.org/10.1016/j.beproc.2014.07.013

Polajnar J, Eriksson A, Virant-Doberlet M, Mazzoni V (2016) Mating disruption of a grapevine pest using mechanical vibrations: from laboratory to the field. J Pest Sci (2004) 89:909–921. https://doi.org/10.1007/s10340-015-0726-3

Polajnar J, Maistrello L, Ibrahim A, Mazzoni V (2019) Can Vibrational Playback Improve Control of an

Invasive Stink Bug? In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Studying Vibrational Behavior. Springer International Publishing, Cham, pp 375–398

Samarra FIP, Klappert K, Brumm H, Miller PJO (2009) Background noise constrains communication: Acoustic masking of courtship song in the fruit fly *Drosophila montana*. Behaviour 146:1635–1648. https://doi.org/10.1163/156853909X463713

Saxena KN, Kumar H (1980) Interruption of acoustic communication and mating in a leafhopper and a planthopper by aerial sound vibrations picked up by plants. Experientia 36:933–936

Scharf I, Martin OY (2013) Same-sex sexual behavior in insects and arachnids: prevalence, causes, and consequences. Behav Ecol Sociobiol 67:1719–1730. https://doi.org/10.1007/s00265-013-1610-x

Secor G a, Rivera-Varas V V (2004) Emerging diseases of cultivated potato and their impact on Latin America. Rev Latinoam la Papa 1:1–8

Sengoda VG, Cooper WR, Swisher KD, et al (2014) Latent period and transmission of "*Candidatus* liberibacter solanacearum" by the potato psyllid *Bactericera cockerelli* (Hemiptera: Triozidae). PLoS One 9:1–10. https://doi.org/10.1371/journal.pone.0093475

Senior LJ, McEwen PK, McEwen P, et al (2001) The use of lacewings in biological control. Lacewings Crop Environ 296–302

Skrillex (2010) Scary Monsters and Nice Sprites. Big Beat Records

Smith RL (1979) Paternity assurance and altered roles in the mating behaviour of a giant water bug, *Abedus herberti* (Heteroptera: Belostomatidae). Anim Behav 27:716–725. https://doi.org/https://doi.org/10.1016/0003-3472(79)90007-1

Soroker V, Talebaev S, Harari AR, Wesley SD (2004) The role of chemical cues in host and mate location in the pear psylla *Cacopsylla bidens* (Homoptera: Psyllidae). J Insect Behav 17:613–626. https://doi.org/10.1023/B:JOIR.0000042544.35561.1c

Svensson BG, Petersson E, Forsgren E (1989) Why do males of the dance fly *Empis borealis* refuse to mate? The importance of female age and size. J Insect Behav 2:387–395. https://doi.org/10.1007/BF01068063

Takanashi T, Uechi N, Tatsuta H (2019) Vibrations in hemipteran and coleopteran insects: Behaviors and application in pest management. Appl Entomol Zool 54:21–29. https://doi.org/10.1007/s13355-018-00603-z

Teulon DAJ, Workman PJ, Thomas KL, Nielsen MC (2009) *Bactericera cockerelli*: Incursion, dispersal and current distribution on vegetable crops in new zealand. New Zeal Plant Prot 62:136–144. https://doi.org/10.30843/nzpp.2009.62.4783

Tishechkin DY (2007) New data on vibratory communication in jumping plant lice of the families Aphalaridae and Triozidae (Homoptera, Psyllinea). Entomol Rev 87:394–400. https://doi.org/10.1134/S0013873807040021

Tishechkin DY, Drosopoulos S, Claridge MF (2006) Vibratory communication in Psylloidea (Hemiptera). Insect sounds Commun Physiol Behav Ecol Evol Ed by S Dros MF Claridge CRC Press Boca Raton, Florida 357–363

Tsubaki R, Hosoda N, Kitajima H, Takanashi T (2014) Substrate-Borne Vibrations Induce Behavioral Responses in the Leaf-Dwelling Cerambycid, *Paraglenea fortunei*. Zoolog Sci 31:789–794. https://doi.org/10.2108/zs140029

Virant-Doberlet M, Cokl A (2004) Vibrational communication in insects. Neotrop Entomol 33:121–134. https://doi.org/10.1590/s1519-566x2004000200001

Conclusions and future perspectives

In the last decades, the urgent need for alternative pest control strategies called on science to explore innovative ways of research. To provide fundamental knowledge regarding the biology, ecology, and ethology of a target species, previously unexplored techniques and emerging field of science are accordingly investigated. In this thesis, biotremology techniques were applied to characterise the mating process and associated vibrational signals emitted by two important insect vectors: the meadow spittlebug *Philaenus spumarius* and the tomato potato psyllid *Bactericera cockerelli*. Information regarding the sexual behaviour and ecology of these pests is indeed required to provide insights on their life cycle, dispersal, and future management techniques.

In the case of *P. spumarius*, its vibrational communication was deeply investigated, leading the identification of the pair formation process and associated substrate-born vibrational signals. In this regard, even if males emit calling signals as soon as they emerge as adults in spring, the mating process is controlled by females, which only begin to call with the arrival of autumn. Prior to this period, if the male attempts to copulate with a female, she emits a rejection signal as deterrent. A vibrational duet is eventually established when the male courtship signal - emitted in response to the female call - elicits the female's positive reply. However, mating is only achieved by the male if the duet is maintained until successful localisation of the signalling partner. The female response is a simple signal that provides localisation cues, whilst the complex male courtship likely contains information regarding male quality and is used to maintain the female's interest in mating. Once the male approaches the partner, he immediately attempts to copulate, contrarily to other auchenorrhynchan species, in which a specific signal is required to trigger female acceptance before mating. The duet is the stage of pair formation of *P. spumarius* most susceptible to vibrational interference. Mating disruption techniques for *P. spumarius* should hence focus on this phase to prevent mate localisation. Although males could interact with the emission of a male-male signal, the latter was not used to interfere with an ongoing duet, suggesting that other rivalry strategies may potentially occur in *P. spumarius*. Some male insects, for example, display satellite behaviours, such as eavesdropping on an ongoing duet to find the female before the signalling male. Given that similar knowledge could lead to development of vibrational traps against *P. spumarius,* additional trials should be realised to confirm this hypothesis.

The physiology of *P. spumarius* females was investigated to assess the potential relationship between calling behaviour and ovarian development. Our innovative protocol coupled biotremology trials and morphological inspections, performed throughout the entire adult stage of the insect. It was thus possible to demonstrate that female proceptivity was associated with the development of her ovaries, since females only called and established duets when they started to develop eggs. This trend increased throughout the season, and the peak of calling activity was accordingly reached when females carried many mature eggs. The role of photoperiod and of ovarian parapause was discussed, together with the potential role of the juvenile hormone. The delayed female calling activity implies that a vibrational mating disruption technique against *P. spumarius* could be effective only if applied when females are receptive to mating, but further research is required to better understand crucial elements of the mating process. For instance, we need to know where mating generally occurs, and if and how disruptive vibrations could be transmitted to both the ground cover and olive canopies to prevent mate finding. Given that males can force females to copulate and that females have a spermatheca, future studies should investigate sperm usage and potential sperm competition in *P. spumarius*. Similar information is indeed required to understand if mating disruption could be an effective strategy for the control of this pest.

The introduction of *Xylella fastidiosa* in Italy in 2013 primed numerous surveys aimed at describing the abundance of *P. spumarius* in European agro-habitats. Available sampling protocols were refined until generalisation of a technique that was used to investigate spittlebug presence in parts of Italy, Spain, and Portugal. Even if these studies provided strong and crucial evidence regarding *P. spumarius* ecology, its detectability was never considered, potentially leading to estimation biases. In this work, surveys were conducted in Northern Italy (Trentino), and data were used to run occupancy models, providing new information regarding the probability of detecting *P. spumarius* and other auchenorrhynchan species in olive orchards and vineyards. In fact, even if the presence of the spittlebug was high in all visited sites, its detectability depended on site covariates. For instance, factors as presence of vineyards and high plants in the ground cover enhanced the detectability of *P. spumarius*. On the contrary, high presence of forests and increasing hours of sunlight resulted in a lower probability to find the insect, even when using the most efficient sampling methods. Thus, a high ground coverage may lead to an overestimation of *P. spumarius* abundance, and the effect of the covariate on spittlebug detection could be erroneously ascribed to its presence. Given these results, even if occupancy models are more commonly used to study the ecology of vertebrates, they are valuable tools that should be applied when considering the distribution of insect vectors. Data collected from agro-habitats of Trentino were used to investigate the feeding preferences of *P. spumarius,* by studying the association with its host plants. Similar information could suggest how to shape the ground cover of crops vulnerable to *X. fastidiosa* to reduce the presence of its main vector in this Italian region, which is still a *X. fastidiosa*-free area. Even so, crop vegetation should be monitored and shaped to avoid the possible dramatic outbreak of diseases transmitted by *P. spumarius* and other auchenorrhynchans.

An innovative approach for the management of insect-vectored diseases proposed in this thesis is the vibrational disruption of the feeding behaviour. A substrate-borne stimulus designed on *P. spumarius* female rejection signal reduced both probing and xylem ingestion, potentially owing to a combination of direct and indirect effects on both insects and plants. The stimulus significantly impeded the feeding activity of the spittlebug on sunflower, especially when transmitted at high intensities, whilst no behavioural effects were observed on olive. This could be linked to the dramatic decrease in signal intensities, notably in the frequency spectrum below 500 Hz. As mentioned before, the phenology and sexual behaviour of *P. spumarius* depends on the season, and additional studies should evaluate if other modified *P. spumarius* signals can disrupt feeding, and if the effect could be influenced by the period of the season. Indeed, research needs to be addressed at designing the most efficient transmission methods for olive and other plants susceptible to *X. fastidiosa.* This work is only the preamble of the development of vibrational strategies aimed at disrupting *P. spumarius'* feeding behaviour, but it already provided interesting insights in this regard. If pursued and implemented, this approach could provide to farmers a useful tool to prevent the transmission of *X. fastidiosa*, supporting the management of the disease. Moreover, a similar vibrational inhibition of the feeding activity could be tested on other vector-plants pathogens systems, especially when the susceptible plant is herbaceous and/or supported by wires and poles, which may facilitate the transmission of vibrational stimuli.

The psyllid *B. cockerelli* is an important pest of tomato and potato crops, not only because it can directly damage the plants, but also given its role in transmitting the bacterium *Candidatus* Liberibacter solanacearum. In this thesis, to assess whether substrate-borne stimuli may interfere with relevant behaviours, the vibrational communication of the psyllid was characterised, together with the pair formation process. The vibrational repertoire of *B. cockerelli* was less complex and diverse than the one of *P. spumarius,* potentially because psyllids use multiple cues to interact with conspecifics and the environment, whilst Auchenorrhyncha communication solely relies on vibrational signals. Contrarily to *P. spumarius,* the mating process starts with the male calling signal, to which a female replies by emitting a long signal that partially overlaps with the last part of the male signal. The first vibrational interaction underlies species identification, while mate localisation involves the establishment of a duet, namely the continuous exchange of male calls and female replies. The latter signal is likely long enough to provide to the male the necessary localisation cues. As observed in *P. spumarius,* the male immediately copulates with the female when he happens to be in close proximity, not only after localisation of the signalling female but also after randomly encountering the female. In rare occasions, a female triggered the pair formation process by emitting her response signal before the male call, suggesting that a trapping approach could be tested. A vibrational stimulus based on the female response was designed to trigger a duet between the male and the playback, and potentially lead the male towards the signal's source. The stimulus attracted *B. cockerelli* males to the source and reduced the mating rate, when used in pair trials. Given that this insect can reach high population densities, a similar approach should be implemented according to the crop of interest and used before high rates of infestations, to both monitor and control psyllid populations. Again, this case provided evidence that behaviours of insects can be manipulated by transmitting vibrational signals to host plants.

To conclude, this thesis suggests that by studying the vibrational communication, the ecology, and the feeding behaviour of an insect vector with innovative approaches, it is possible to provide a better understanding of the target species. Similar knowledge is nowadays crucial, because it represents the first step towards sustainable pest control strategies alternative to pesticides.

Sabina Avosani

University of Trento
Doctoral School in Civil, Environmental and Mechanical Engineering
http://web.unitn.it/en/dricam
Via Mesiano 77, I-38123 Trento
Tel. +39 0461 282670 / 2611 - *dicamphd@unitn.it*

In this work, we investigated the mating behaviour and vibrational communication of two insect vectors having an important economic impact: *Philaenus spumarius* (Hemiptera: Aphrophoridae) and *Bactericera cockerelli* (Hemiptera: Triozidae). We also provided novel insights regarding the vibrational manipulation of their behaviour.

We characterised the sexual behaviour and associated vibrational signals of *P. spumarius*. We evaluated the correlation between the female signalling activity and development of the reproductive organs, suggesting that a vibrational mating disruption could be effective only if applied from the onset of egg maturation. We used occupancy models to estimate *P. spumarius* presence and detection probability in Trentino (Northern Italy), a rarely deployed approach to study insect distribution. Site covariates impacted *P. spumarius* detectability, and we also studied the association between *P. spumarius* and its host plants. Such information can support monitoring programs and control strategies aimed at reducing *P. spumarius* population in crops of interest by shaping the plant composition of the ground cover. We also attempted to disrupt its feeding activity using vibrations, opening the gates for future research.

The tomato potato psyllid *Bactericera cockerelli* is an important threat to crops in New Zealand. Besides the characterisation of pair formation process and associated vibrational signals, we used vibrations to disrupt mate finding by transmitting vibrations to the host plant. Given that the stimulus attracted male psyllids in small scale experiments, this strategy is a feasible approach that could be implemented for use in fields and greenhouses

In conclusion, we provided new information regarding the ethology of two insect vectors and suggested that there is room for applied biotremology to support sustainable management strategies.

Sabina Avosani

October 2017 – October 2020. Doctor of Philosophy–Agricultural Entomology, University of Trento, Department of Civil, Environmental and Mechanical Engineering,
in collaboration with Fondazione Edmund Mach

October 2015 - July 2017. Master of Science, Plant, Food and Agri-Environmental Biotechnologies, Università degli Studi di Milano, in collaboration with Fondazione Edmund Mach

September 2011 - November 2014. Bachelor’s degree; Biological Sciences, Università degli Studi di Milano-Bicocca

October 2020 - June 2021. Consultant, Plant and Food Research, New Zealand

March 2017 - October 2017. Technical and scientific assistant, Centre International de Hautes Études Agronomiques Méditerranéennes, Italy