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Coupling computational models and greenhouse bioassays to develop an innovative pest control strategy against the greenhouse whitefly *Trialeurodes vaporariorum*

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Abstract

In applied biotremology, vibrational signals or cues are exploited to manipulate the target species behaviour. To develop an efficient pest control strategy, other than a detailed investigation into the pest biology and behaviour, the role of the substrate used to transmit the signal is an important feature to be considered, since it may affect vibrations spreading and effective signal transmission and perception. Therefore, we used a multidisciplinary approach to develop a control technique against the greenhouse whitefly, *Trialeurodes vaporariorum.* Firstly, an *ad hoc* vibrational disruptive noise has been developed, based on the acquired knowledge about the mating behaviour and vibrational communication of the mated species. Subsequently, we employed finite element models (FEM) to investigate the tomato plant response to the aforesaid noise. Modelling how vibrations spread along the plant allowed us to set up a greenhouse experiment to assess the efficacy of the vibrational treatment, which was administrated through vibrational plates. plant. The methodology applied in this study represents an innovative, environmentally sound alternative to the usage of synthetic pesticides.

Keywords: *biotremology, pest control, FEM, vibration, substrate*

Introduction

Animals can produce and perceive substrate borne vibrations to communicate. Vibrational communication is one of the most ancient and widespread communication channels and yet one of the less studied (Cocroft et al. 2014). In particular, insects' vibrational communication is studied in applied biotremology to find alternative and ecological strategies for controlling insect pests (Takanashi and Nishino 2019) that involve "behavioural manipulation" (BM) of the target insect, which means to disrupt intra-specific communication and thus to affect its ability to reproduce and proliferate (Strauß et al. 2021). This is possible by providing the characterization of the insect vibrational communication, thanks to the measurements of the spectral and temporal parameters of the involved signals, and then by associating each signal to the receiver behavioural responses (Hill et al. 2019). This knowledge may allow us to manipulate the insect's behaviour by means of artificial stimuli, such as disruptive noises or simulated calling signals (Foster and and Harris 1997; Mazzoni et al. 2017; Takanashi and Nishino 2019; Strauß et al. 2021). A vibrational device is needed for these purposes, such as vibrational traps that release attractive signals or transducers that reproduce specific vibrations able to mask insects' signals, disturb their biological activities (i.e., feeding, oviposition) or repel them from a crop (Polajnar et al. 2015). For example, vibrational mating disruption (exploiting males interference signals) has been successfully applied against the leafhopper *Scaphoideus titanus* n a commercial vineyard in Northern Italy (Mazzoni et al. 2019). In another case, a vibration exciter has been developed using a magneto restrictive material, capable of inducing a startle response in the insect target (Takanashi et al. 2019).

Because many insects live on plants and use them as a substrate for their communication, our approach considers the substrate properties as an important feature for a successful vibrational control. The interaction between insects and host plant substrates has been studied in the past decades to better understand the way of propagation of vibratory signals along the stem and the leaves (Michelsen et al. 1982; Cocroft et al. 2000; Magal et al. 2000; Cokl et al. 2004; Cokl 2005; Casas et al. 2007; Polajnar et al. 2012). Vibratory signals propagate in the stem as bending waves, which can reflect both at the top and at the root of the plant. Plants generally act as low pass filters, and the energy loss of bending waves in plant stems by friction at frequencies below some thousands of Hz (kHz) is relatively low (Hager and Kirchner 2013; Michelsen 2014). For these reasons, to have effective vibrational pest control, the role of the substrate used to transmit the signal is an important feature to be considered. Indeed, plants present a complicated architecture with different tissue and organ geometry, and therefore mechanical properties (Strauß et al. 2021), and they change shape and structure during the growth and life cycle (James et al. 2014). All these aspects may affect vibration spreading and effective signal transmission. In order to consider all these variables, in the last few years numerical tools, such as finite element models (FEM), have been developed and coupled with experiments to provide additional information and forecast the effects of different vibrating systems on trees or plants (Der Loughian et al. 2014; Hoshyarmanesh et al. 2017). FEM approach consists of dividing a structure into an appropriate number of elements, whose sizes may vary, with assigned material properties and boundary conditions. The material formulation is fundamental to describe the constitutive relationship between applied deformations and resulting stresses. Among the advantages of FEM, it is possible to model complex scenarios such as dynamics of plant-like structures. Indeed, FEM for trees and plants have been used especially to study the influence and possible damage of the wind (Sellier et al. 2006; Hu et al. 2008; Rodriguez et al. 2012; Jackson et al. 2019), by computing their natural frequencies and modes.

A mode of vibration is defined as a particular shape (i.e., modal shape) of free motion that can oscillate in time, eventually fading out due to damping. Vibration modes are observed when the system is free to oscillate after an initial perturbation, and the associated frequencies are called natural frequencies. Usually, a real system is characterized by several modes, which can combine together to respond to a certain stimulus and they can be used to reconstruct and forecast the system response. Well-known theories and analytical formulations from linear dynamics can be used when dealing with pole-like vibrating systems (Fertis 1995); however, when a more complex geometry is adopted, vibrational modes and the dynamic response to a vibrational perturbation are usually extracted by numerical methods as FEM (Sellier et al., 2006, Hu et al., 2008, Rodriguez et al., 2012, Jackson et al., 2019,), as within this work. Many examples are also reported in a recent review by E. de Langre (de Langre 2019), where the basics of plant vibrations, theory and models have been discussed.

In the present study, FEM analyses have been used to describe a tomato plant when subjected to an external vibrational disruptive noise, a technique applied for the first time to one of the most critical pests in the greenhouse. Numerical results have been compared with experimental measures, to validate the model and then to study the efficacy of the stimulus during plant's growth. The computational approach can be a useful tool for understanding the amount of signal that reaches the leaves and thus covers the plant, while the bioassay was necessary to verify the efficacy of the signal on greenhouse whitefly (GW) population and its disruptive ability.

By combining biotremology with engineering, a new technique based on vibrations was proposed to manage tomato plant pests. Our target insect was the GW *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae), which is considered one of the most harmful and economically relevant insect pests in greenhouses worldwide. The GW can cause both direct (by subtracting nutrients during the feeding activity) and indirect (by transmitting viruses and producing honeydew that reduces plant transpiration) damage to plants.

In conventional farming insecticides are used for GW control, such as imidacloprid, fenpropathrin and deltamethrin, even though many strains became resistant to some of these compounds (Gorman et al. 2002, 2007). Another option, mainly adopted in IPM and organic farming, is represented by biological control, which has been widely used in greenhouses and it is mainly based on the chalcid wasp *Encarsia formosa* (Gahan, 1924, . Successful control can be obtained if the parasite is established on plants when natural infestations are small. Therefore, the efficacy of these technique depends upon different factors such as host plant quality, temperature, usage of fertilizer, dimension of the greenhouse, stage of infestation (Hoddle et al. 1998). We consider here a third option: the possibility of interfering with the mating behaviour of our target species.

The GW mating behaviour is structured into 5 stages (namely: Call, Alternated Duet, Courtship, Overlapped Duet and Mating/Failed Mating Attempt), where the Courtship stage plays a crucial role in eliciting the female acceptance, leading to the Overlapped Duet stage, which precedes the actual mating. During this process, several different vibrational signals as described in Fattoruso et al. (2021) are involved; therefore, we hypothesize that a disruptive noise, designed to cover the specific frequency range used by GW to communicate, would significantly reduce mating and preserve the plants and their growth. In the case of the GW, in fact, it was not possible to exploit the insects natural signals (i.e., male and female calls) to interfere with mating, because of males' "stubbornness" in attempting mating despite the presence of a rival male or of a rejecting female (Fattoruso et al. 2021). Therefore, the best strategy would consist in impairing males' ability to locate the female and elicit her acceptance, by interfering with their communication by means of a synthetic signal capable of perfectly masking the natural signals thus preventing their perception between conspecifics. Especially, the courtship stage usually plays a crucial role for successful mating, in that only at this stage the female might accept or reject the male, and the acceptance is mediated by a male-female duet.

Therefore, in the present study a disruptive noise was designed to specifically disturb the whiteflies signal involved in this stage (Chirp, Pulse Train and Female Responding Song). The signal was tested for two months trial on tomato plants in the greenhouse after plant infestation. In parallel, a finite element model was realized to provide a tool for future applications: the model could be used to simulate different scenarios (e.g., plant growth), add information about signal spreading (thanks to the colour maps which describe e.g., the velocity along the plant) and signal concentrations, thus hopefully leading to exploit the proposed system to other greenhouse crops.

Materials and Methods

3D Finite element modelling and analysis:

For the tomato plant dynamics, a 3D model of a real plant was developed from a free 3D model downloaded from Sketchfab, which was then adapted and finally imported in the numerical solver Abaqus Standard 2018 (Dassault Systemes Simulia Corp., Providence, RI), as shown in Figure 1b. Both the stem and the leaves were created, and the model was discretized in a fine mesh of linear triangular, quadrilateral and tetrahedral elements resulting in about 18800 elements and 10000 nodes. The stem was assumed as a 3D solid model with varying section diameter from the bottom (about 10 mm) to the top, varying along the total plant height h (average height of the plants, equal to 670 mm), while the leaves were described as shell parts, with a fixed thickness of 0.5 mm and 5 integration points. Internal constraints type “tie” was defined to couple the stem with the leaves. In order to mimic different plants or different stages during growth, and highlight possible changes within the vibrational modes and signal spreading, two additional plants were modelled, scaling the dimensions by a factor of 0.45 or 1.30 with respect to the reference plant, resulting in smaller (h *lower*=300 mm) and higher (h upper=870 mm) plants, which were examined with the same analyses,

The mechanical behaviour of the tomato plant was defined by means of a linear elastic constitutive formulation since the phenomenon can be assumed to be in the range of small displacements and small strains (applied vibrations caused plant displacements of a few micrometres, thus they are small enough, in comparison with the size of the plant, justifying the choice of linear dynamics) (de Langre, 2019). Viscoelasticity was also neglected since the vibrational stimulus is sudden and does not allow the biological material to display viscosity behaviour. Mechanical properties were chosen according to previous studies (Blahovec 1988; Zhang et al. 2016; Kang et al. 2016), thus for the stem and leaves a density ρ equal to 800 kg/m^3 and 700 kg/m^3, respectively, an elastic modulus E of 1 GPa and 0.8 GPa, and for both a Poisson coefficient ν of 0.2. The bottom part of the plant was fixed by imposing null displacement in the global system. Both the linear perturbation frequency analysis and the modal dynamics analysis were performed. The first step (linear perturbation, frequency) allows the calculation of the natural frequencies of the plant and the associated modes. All the modes involved in the frequency range of the stimulus (0-400 Hz) were considered. The second step (linear perturbation, modal dynamics) accounts for the results obtained from the previous step and simulates the effects of a vibrating plate by imposing a velocity base motion along with one of the two horizontal directions. Stimulus amplitude was given to the model during the entire second step, for a duration of 0.4 s. By applying to the system an imposed oscillating velocity (the disruptive noise), the only parameter to modify within the simulation was the critical damping fraction of the whole system, by comparing the model results with three different control points, namely S4, L1 and L3. Then, the numerical spectra in the other 7 measure points were compared with experiments, both for leaves and for stems.

After assessing the model, the influence of leaves (Stem-Leaves plant) in the total response of the model was evaluated with reference to the only stem (Stem plant), i.e., the plant modelled without leaves, but only the main stems. In order to analyse both the natural frequencies and the plant behaviour when subjected to an external vibrational stimulus, the numerical model reported in Figure 1b was used, thus considering both the stem and the leaves (SL plant). However, leaves can be usually neglected and considered as local, independent subsystems (Vogel 2013), due to their small masses, compared with the whole plant (de Langre, 2019). This means that they should not affect the global (trunk) or even semi-global (branch) modes. Moreover, modeling a plant considering the sole stem would strongly simplify the problem. For this reason, we also analyzed a tomato plant modeled only by its stem (S plant), to compare the differences both in terms of natural frequencies and substrate velocities with the SL plant.

Signal spreading and measures on tomato plants:

Measures of signal propagation and characteristics were conducted firstly in the biotremology laboratory at Fondazione Edmund Mach (Trentino, Northern Italy), in a sound insulated chamber at a temperature of 22 ± 1 °C and 65% RH, where plates and tomato plants were placed on an anti-vibrational table (Astel s.a.s., Ivrea, Italy). Two laser vibrometers (VQ-500-D-V, Ometron Ltd., Harpenden, UK and OM-DS VibroGo E 52039, Polytec GmbH, Waldbronn, Germany) were used to measure the substrate vibrations generated by the plates. Lasers were pointed on multiple measure points, as reported in Figure 1a (where small pieces of reflective tape of about 0.5×0.5 cm were placed) on both stems (6 points) and leaves (4 points), and vibrations were simultaneously recorded by setting the laser sensitivity to 5 mm/s/V. Signals were acquired with a hard drive multichannel LAN-XI data acquisition device (Brüel and Kjær Sound and Vibration A/S), sample rate of 8192 Hz. Measurements were repeated twice on two plants simultaneously.

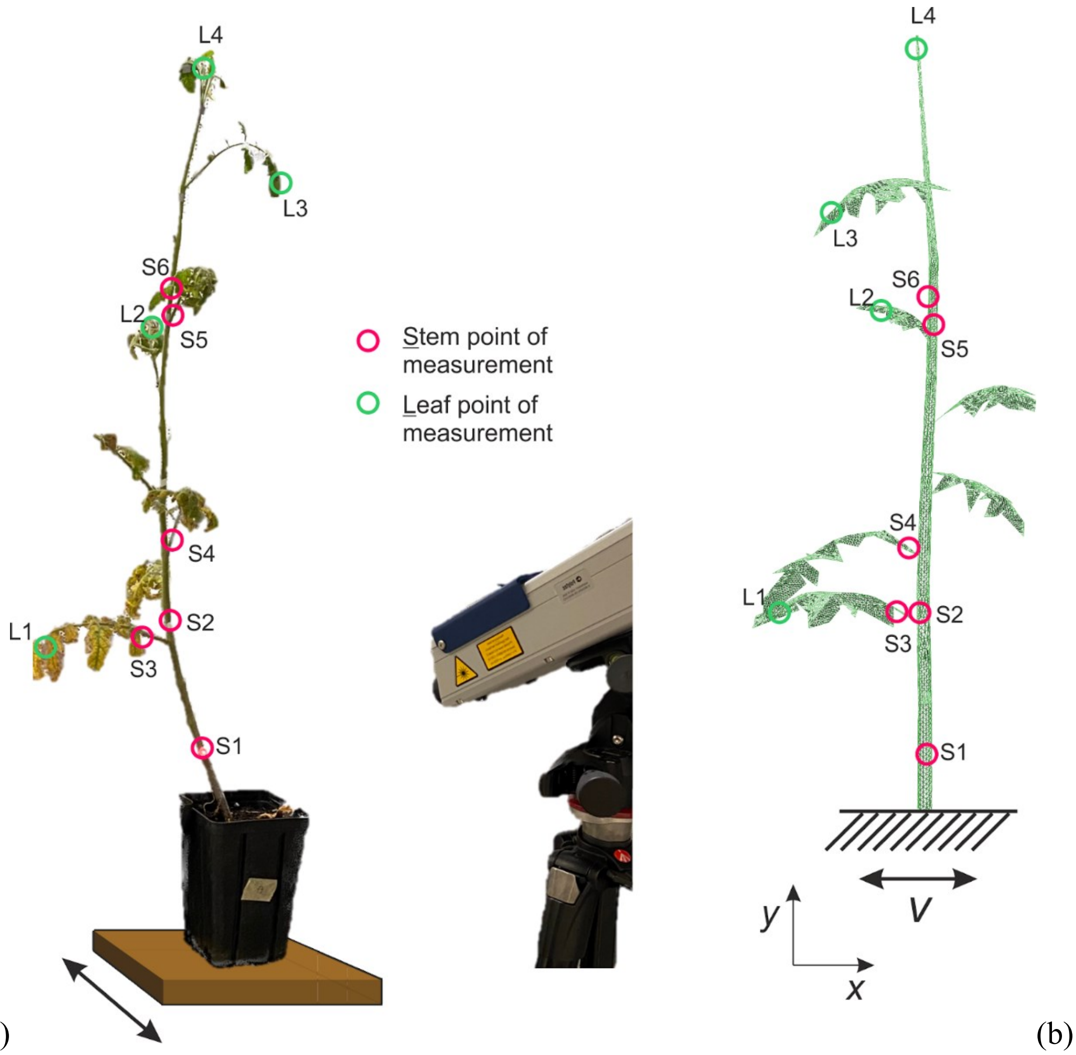

Figure 1. a) Experimental setup to record signal propagation along the plant by means of laser vibrometer. Six points of measure were chosen along the stem (from S1 to S6, red circles), and four points on the leaves (L1-L4, green circles). b) 3D finite element model of a tomato plant, on which the same points were checked and compared with experiments.

Fast Fourier transform and data analysis:

Recordings were post-processed with Matlab 2020 user-developed script (1994-2021 The MathWorks, Inc.) to compute the fast Fourier transform (FFT) with a window length of 1024 samples, frequency resolution of 8 Hz, 66.7% overlap, and Hann window. The spectra of the recorded signals were then extracted, visualized and compared.

Insect rearing:

The whiteflies used for the experiment (*T. vaporariorum*) were obtained from a colony maintained at the Biobest company (Westerlo, Belgium) and shipped to the Fondazione Edmund Mach laboratory (San Michele all' Adige, Trento, Italy). They were reared in the greenhouse at 25 ± 2 °C, 70 ± 5% RH and 16:8 (L:D), in mesh cages (Bugdorm-6620, 60 × 60 × 120 cm, MegaView Science Co., Ltd., Taiwan) containing seedlings of tomato (*Solanum lycopersicum* var. Cuore di bue). All plants used for insect rearing were grown in the greenhouse at controlled conditions and no treatments were applied. Trials were carried out in the biotremology lab of Fondazione Edmund Mach (FEM) from August to October 2020.

Plant rearing:

All the seedlings used for the experiment were grown in 1L pots in the greenhouse at 25 ± 2 °C, 70 ± 5% RH and 16:8 (L:D). When they reached an average height of 43±10 cm, we proceeded with introducing the whiteflies in the cages.

Test products application:

We applied three different treatments: water as a negative control, the disturbing signal and a pesticide (Decis Jet 2.5 mL/L) as a positive control. Before starting the infestation, all plants were placed in 4 large mesh cages. Around 500 adult insects were released and kept in each cage, free to lay eggs. After 24 h, all the adults were carefully removed from each leaf using a manual aspirator. We treated the plants when the nymphs reached the 3rd or 4th[V3] instar (after 15-17 days, assessed by leaf inspection with stereoscopic microscope). The test items were applied by spraying the plants. The plants were sprayed evenly, and the application stopped just before reaching the run-off point. After each treatment, the plants were divided placing three of them per cage (BugDorm-4S2260, W24.5 x D24.5 x H63.0 cm); for each treatment there were 4 cages. Regarding the vibrational noise, a vibrational device was placed under each cage. The vibrational device ("vibroplate") developed to control the GW consisted of a square plate made of wood (side length: 20 cm, thickness: 1 cm). The plate was provided with 4 iron legs (h: 6.5 cm). Under the plate centre, a mini-shaker (Tremos, CBC Europe S.r.l.), which was electrically powered and generated a continuous horizontal stimulus, was placed. The vibrational signal designed to disrupt the GW communication was characterized by 5 peaks of amplitude corresponding with the fundamental frequency of the signals used by insects to communicate 150, 200, 250, 300, 350 Hz (Fattoruso et al. 2021) (Figure 2). The choice of this signal design was to maintain a narrow frequency band with the aim to minimize any interference towards non-target species (i.e., pollinators and antagonists commonly used as biocontrol agents of whiteflies). Peaks of amplitude at 300 and 350 Hz were slightly increased to compensate the plant filtering effect. The created signal has a total duration of 1 second and then was played back in loop 24/7. Plants' weight and signal propagation through the plants was assessed at the beginning and at the end of the trial.

Whiteflies infestation and data analysis:

The GW infestation was assessed by randomly sampling nine leaves per cage, three from the upper, three from the middle and three from the lower canopy, respectively. The number of eggs, nymphs and pupae was counted using a stereoscopic microscope. The survey was repeated for three times: after 15, 36 and 57 days from the treatment. To evaluate the effectiveness of the vibrational treatment, compared to the negative (water) and positive (Decis Jet) controls, a full factorial two-way ANOVA (treatment x date of survey) was followed by a Tukey post-hoc test used to ascertain significant differences between means. Data were previously assessed, and Log transformed to respect the assumptions for parametric analysis assessed by means of Shapiro-Wilk test (normality) and Hartley F-max (homogeneity of variance).

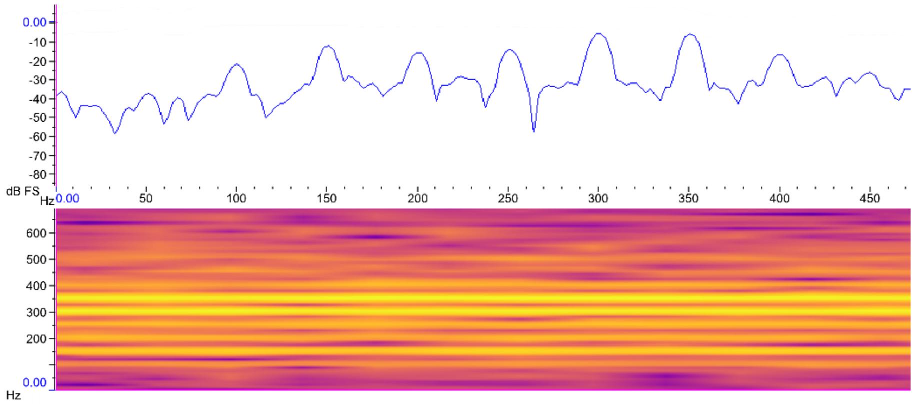

Figure 2. Background noise implemented with peaks of the amplitude corresponding to the frequencies of 150, 200, 250, 300, 350 Hz, length of the signal 1s.

Results

In Figures 3 and 4, the comparison between the experimental spectra obtained from measurements of plants and the numerical ones computed by means of numerical simulations are reported. From simulations, the velocity versus time was exported for each measuring point, considering the direction that was acquired with the laser vibrometer, thus v_x for S points and v_y for L points.

The main interest is focussed on leaves, since they represent the habitat of the target species, a detailed comparison between L1 to L4 for all the signal fundamental frequencies (from 150 to 350 Hz) is reported in Figure 5.

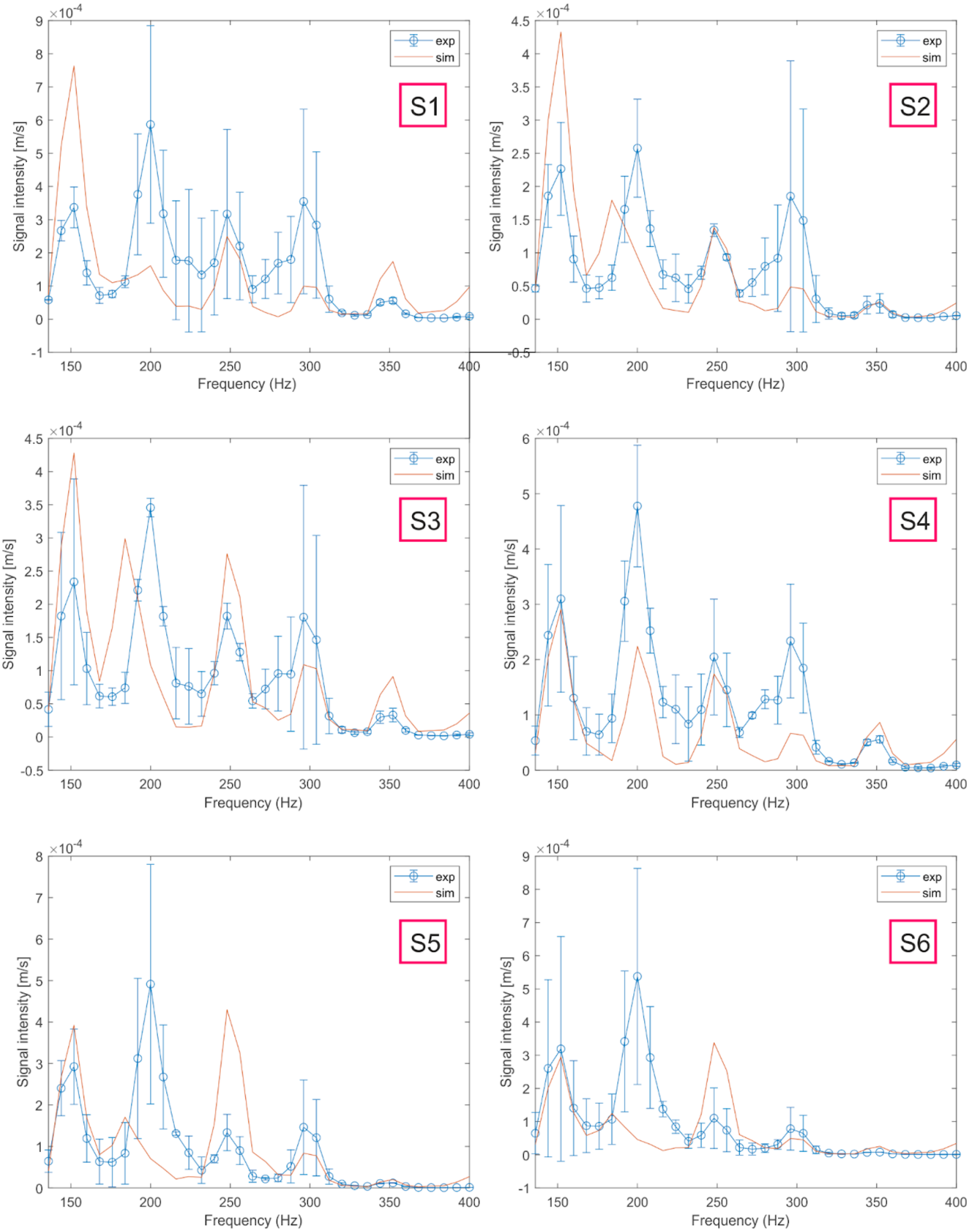

Figure 3. Comparison between numerical and experimental spectra of the recorded disruptive signal on the stems of the plant (orange lines and blue line with circles, respectively) for points S1 to S6.

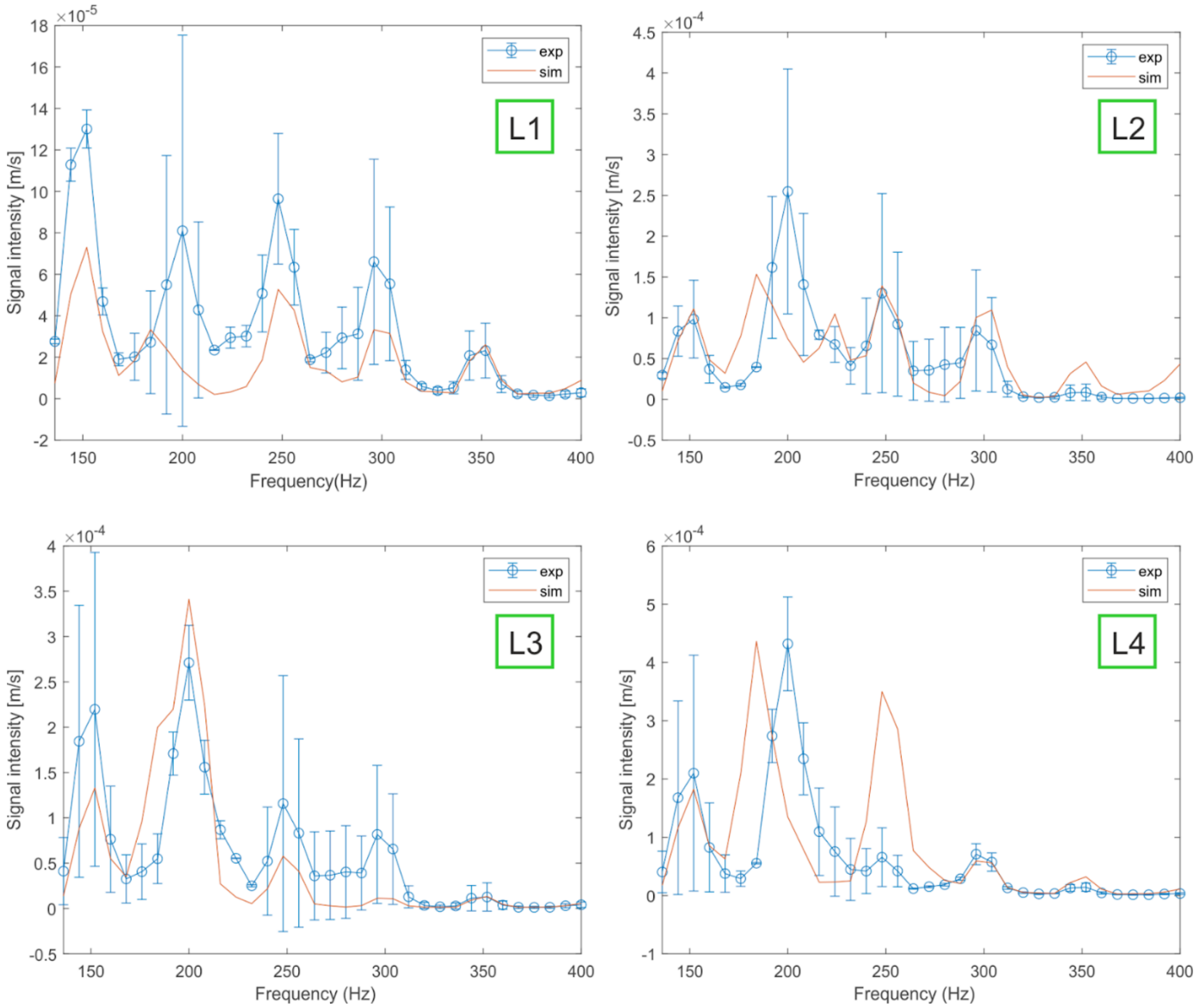

Figure 4. Comparison between numerical and experimental spectra of the recorded disruptive signal on the leaves of the plant (orange lines and blue line with circles, respectively) for points L1 to L4.

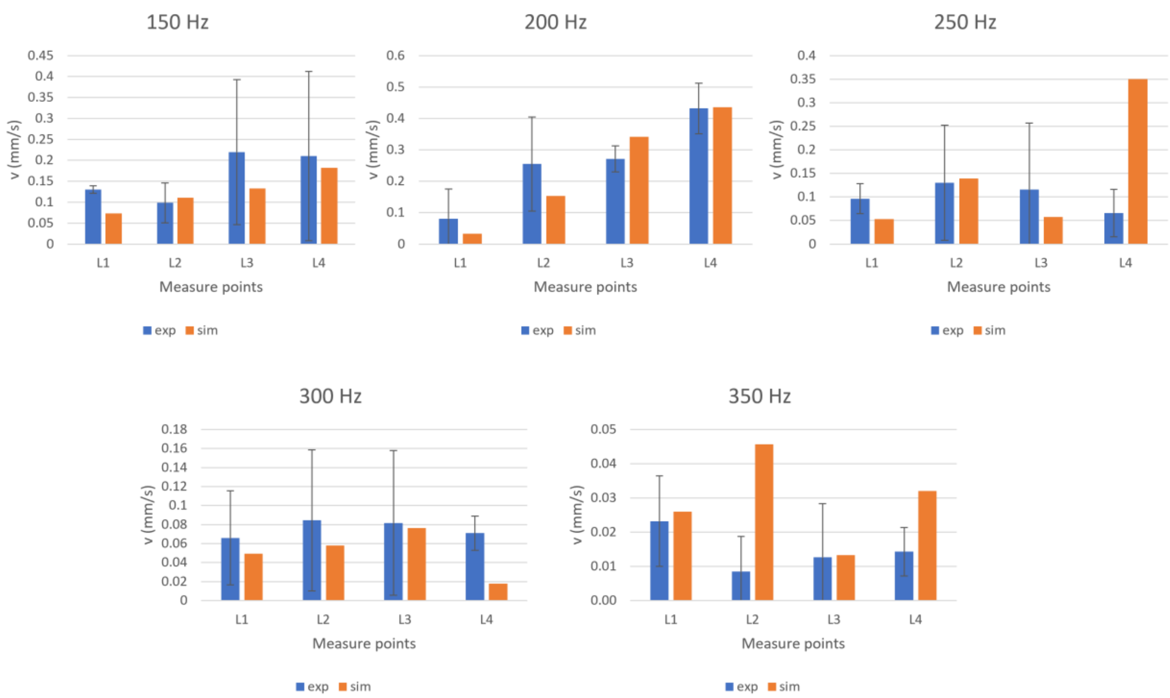

Figure 5. Average and standard deviations of measures on leaves and comparison with simulated results with respect to the different signal peaks.

Stem-Leaves plant VS Stem plant dynamics

From Figure 6b it is notable that the SL and S plants are characterized by close natural frequencies and modes, thus confirming this assumption. Associated vibrational modes are also reported with simple schemes (Figure 6a). When considering imposed base excitation, the total velocities that involve both the modes have similar path and intensity, thus showing good approximations also in the case of S plant (Figure 6c, as reported by the contour maps), as well as for the natural frequencies, with a relative error from about 0.1% (mode 4) up to a maximum of 8% (mode 1) . However, the missing information in this last model is the total amount of signal reaching the leaves, which represents the key factor for the efficiency of the vibrational disruption. In this particular application, the intensity of the disruptive signal should be greater than a threshold value, assumed to be equal to 0.01 mm/s as a precautionary lower bound (Polajnar et al. 2016). For this reason, we decided to fully analyse the SL plant model also in other configurations.

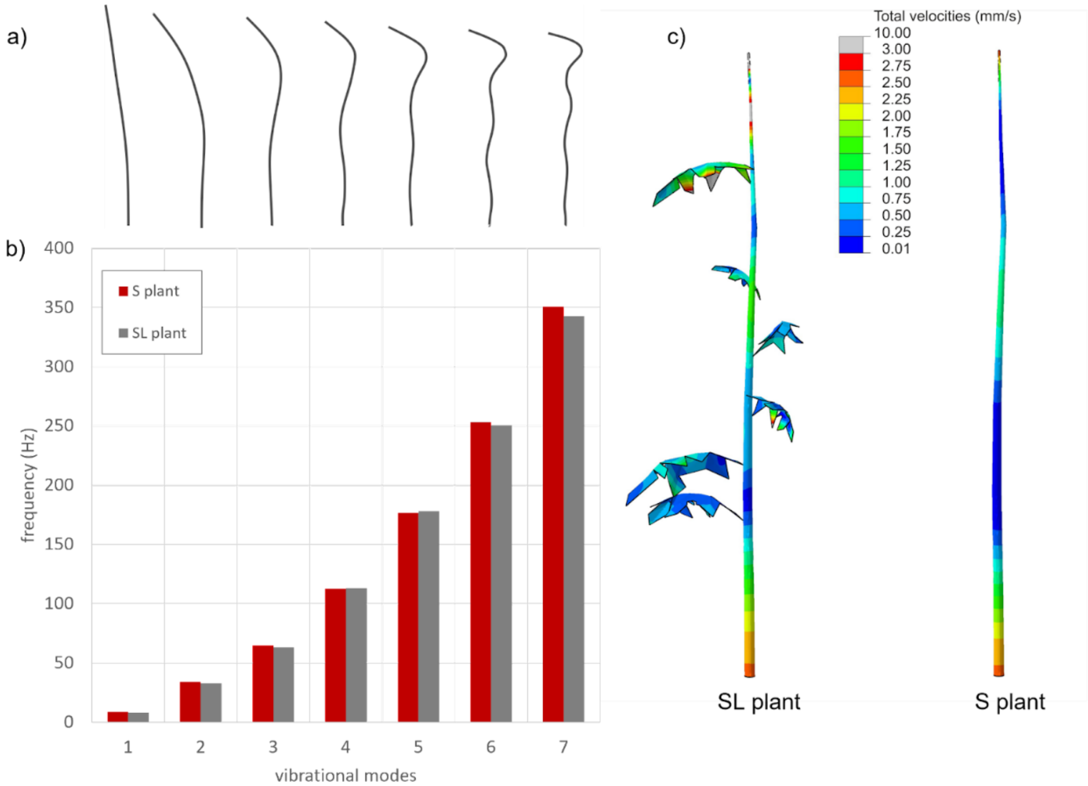

Figure 6. a) Numerical vibrational modes of S plant, from mode 1 to 7. b) Natural frequencies of S plant and comparison with SL plant (red and grey bars, respectively). c) Vibrational velocity path through the plant by adopting a SL plant or a S plant model.

Influence of plant growth on vibrations distributions

The proposed vibrational disruption method to avoid GW mating and proliferation on tomato plants has been designed to be applied in the greenhouse and thus should accompany the plants from their early stage until the complete growth. Throughout this interval, the plant changes its mass, but especially its height, which strongly modifies the associated natural frequencies and vibrational modes.

When assuming the plant as a uniform beam (both the mass and the stiffness are not uniformly distributed in a plant, so this approach is only a first-order approximation), its natural frequency f is $\propto h^{-2}\sqrt{K/m}$ where m is the mass per unit length of the beam, K is the bending rigidity (Young's modulus multiplied by moment of inertia), and h is the plant height (Fertis, 1995). The density and the material stiffness of the tissue are not expected to vary much across the space (position) and time (growth), while the height and diameter (D) widely change. Accordingly, m scales as the cross-sectional area of the plant (i.e. as D^2), K scales as D^4, f should vary as D/h^2. Moreover, $D << h$ and its range of variation is more limited, so that the most influential parameter is h, and in particular, when h increases, the frequency decreases more rapidly. This being the case, we considered two other additional cases, one associated with a young plant of about 300 mm high (average plant height when the experiments started, namely *h lower*) and the other representing the maximum height reached in the experiments, i. e., 870 mm (*h upper*). Results are reported in Figure 7a, where the natural frequency variation is clearly evident, in particular it raised faster for *h lower*, with a constant frequency increase of 250% with respect to the same mode frequency of the reference plant height (h), while, on the contrary, a decrease in the frequency for *h upper* of about 60% with respect to the reference plant. Furthermore, in this case the signal covering throughout the plants was investigated to check whether, during the growth, the efficacy of the vibrational treatment could be compromised.

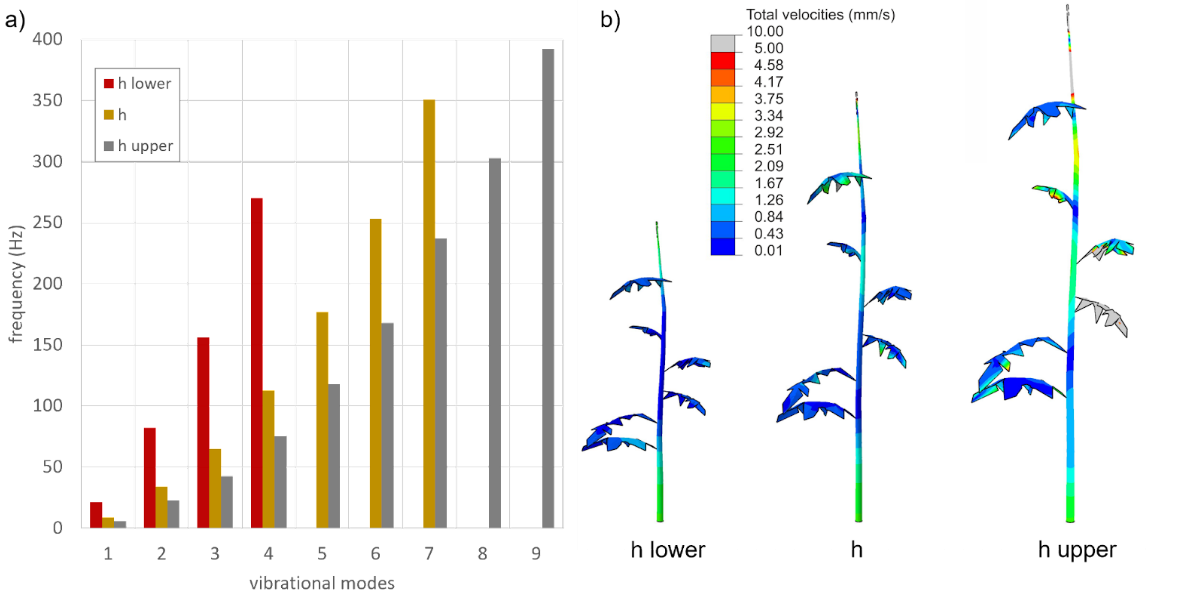

Figure 7. a) Natural frequencies of S plant (height *h*) compared with the lower (*h lower*) and upper (*h upper*) case studies. Natural frequencies were extracted in the range of 0-400 Hz. c) Comparison of the total velocity path through the plant by adopting one of the previous models.

<u>Whiteflies infestation:</u>

Both treatments (Decis Jet and vibrations) were associated with a significantly lower whitefly population than the water control (Two-Way Anova: treatment: $F_{2,27}$.= 13,95, P < 0.001). Factor date of survey was also significant, with an increase in population (date: $F_{2,27}$.= 8,22, P = 0.002). Although the interaction treatment x date of survey was not significant ($F_{4,27}$.= 0,54, P = 0.71), the GW population increase was rather constant from the first to the third survey in the case of water control and Decis Jet, while it was observed only between the first and second period (36 days after the infestation) in the case of the vibrational treatment (Figure 8). Post hoc analysis (Tukey test) indicated that the number of individuals collected from the water control was significantly lower than both vibrations and insecticide (water vs vibrations: p = 0.008; water vs Decis Jet: p < 0.001; vibrations vs Decis Jet: p = 0.15). As for the date, the 1st sampling was associated to a GW population significantly lower than the 2nd and the 3rd (1st vs 2nd: p = 0.04; 1st vs 3rd: p = 0.001; 2nd vs 3rd: p = 0.32).

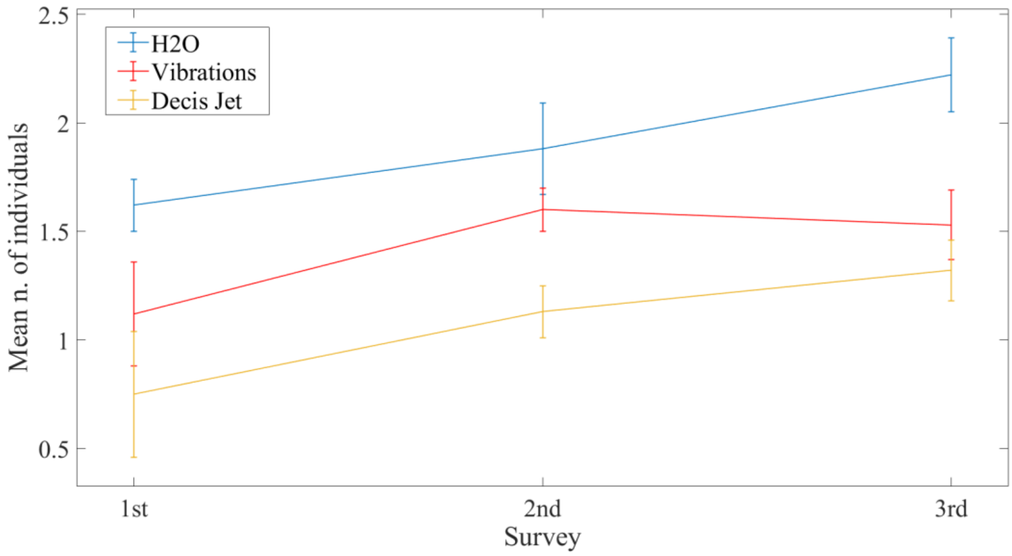

Figure 8. Average (log transformed) number (± SE) of individuals counted per cage in the first (1st), second (2nd) and third period (3rd) of the *T. vaporariorum* survey.

Discussion

Within this work, we employed computational tools to investigate the tomato plant response, when subjected to a continuous vibrational stimulus (disruptive noise) with spectral characteristics specifically designed to interfere with the GW mating communication. This particular vibratory system (i.e., tomato plant) is assumed to respond elastically, since the spatial and temporal variations of deformations could result in moving elastic waves, when a local deformation is propagated (i.e., the ones produced and used by insects to communicate), or let the whole system oscillate in place, such as trees due to wind.

The here developed finite element model reproduces a typical tomato plant, with average size and shape. In order to mimic a real plant behaviour, we firstly compared the numerical predictions (in terms of signal velocities) with experimental results obtained from real plants. Spectra of these velocities in the frequency domain are reported in Figures 3 and 4. On average, the model was able to predict qualitatively the plant behaviour, and also quantitatively for results between 150 and 300 Hz (Figure 5). In reality, tomato plants are subjected to a huge variability during their life, due to many variables such as water content, presence of insects, age of the plant and others; this variability is reflected in the plant response to vibrational stimuli. For these reasons, we can state that the simulated response is a good approximation of the reality, where fundamental and dominant frequencies are correctly identified in those regions in which the insects live and mate (i.e., the leaves, L1 to L4 of Figure 4 and 5). Due to some simplifications related to mass and stiffness distribution, small deviations of the signal frequencies can be observed, due to local approximation of the real system. However, thanks to the computational approach, we modelled not only the stem (which, as a first approximation, could be assumed as a flexible beam fixed in only one of the extremities), but also its coupled behaviour with the leaves. In particular, both experimentally and numerically, all the dominant frequencies between 150 and 300 Hz were measured as largely higher than the safety threshold (0.01 mm/s), with the only exception for 350 Hz, however suggesting that the signal coverage on the plant was strong enough in amplitude to impair insect's communication and thus mating. In addition, tomato plants grow mainly in the vertical direction, which positively affects the signal spreading, since the natural frequencies decrease and become closer to the fundamental frequencies of the signal. This aspect could lead to a resonating system, damped by the vase and the ground, but that amplifies the effects of the disruptive noise on the entire plants and especially on the top leaves, which are known to be the preferred reproductive site for the GW (Bi and Toscano 2007). This insight suggests a major efficiency of the disruptive noise on medium and high plants, thus after a few weeks when starting with young plants (about 40 cm height), due also to more numerous modes that contribute to the overall plant response. Our hypothesis was corroborated by the data acquired from the greenhouse bioassay in that we observed an increase in the treatment efficacy from the 5th week (Figure 8). In fact, both the whitefly population of the water (negative) control and the Decis Jet treatment (positive control) showed a constant increasing trend starting from the first survey. A difference between them was that while the GW population treated with water has been consistently higher than the others since the first survey, the positive control was initially the lowest one, presumably because of the immediate effect of the pesticide. However, the survived population started to increase in size in the absence of any further treatments and maintained a growth trend similar to the water control. Remarkably, the whitefly population of the vibrational treatment, continued growing with a similar trend to the others until the second survey (36 days = 5 weeks) whereas in the last period no further increase was observed. These results seem to confirm the temporal prediction given by the model and also suggest that the disruptive noise seems to be amplitude dependent. This aspect, however, should be object of future research, to design a dose-response curve based on the amplitude of the disruptive noise. This method should be tested in further experiments on a larger scale also to evaluate potential long-term side effects. For instance, the application of a constant vibration to the plant might cause habituation of the whitefly population, thus reducing the disruptive effect. On the other hand, we cannot exclude that the same plants, exposed for a long period to constant vibrations, could change a part of their gene expression with significant consequences in terms of physiology but also resistance to pathogens and pests (Brenya et al. 2022) . As far as the disruptive noise consists of few selected harmonics that perfectly cover the whitefly mating signal, this does not exclude that some plant regulatory mechanisms might be affected by the prolonged exposure to it.

Due to the complexity of the analysed system, some assumptions have been made, such as the simplification of the shape of a 3D tomato plant, especially for the leaves. Adding many parts and constructs to the model would generate a very specific output, which would lack in the response of an average tomato plant. For this reason, we decided to adopt a semi-real shaped plant, thus able to limit the unknown parameters to include within the model. Other assumptions have been made for the material behaviour, which has been supposed to be linear elastic, with homogeneous and isotropic mechanical properties. These hypotheses are justified by the peculiar type of vibration, characterised by a small amplitude (about units-tens of μm) and which results in infinitesimal displacements. Even the involved stresses on the plant are extremely small (few kPa), thus allowing the adoption of the here reported model. Moreover, it cannot consider many biological aspects that influence the plant also within a day, such as the water content, temperature, humidity, and other factors. However, the application of this new technology has been designed and proposed for the greenhouse, in which the surrounding environment is precisely controlled, so that the model actually represents a generic plant in a specific environment. Since our main interest was to study the entire response of a tomato plant, we considered both stem and leaves, but our results suggest the possibility to adopt S plant models, if other quantitative information is needed, e.g., the behaviour of multiple plants together.

Future developments could integrate the model considering the changes of the plant mass not only from a geometrical perspective (volume variation) but also due to different water contents or different phenological states of the plant (fruitification). Moreover, more studies are needed to better understand the specific effect of the disruptive noise on insect behaviour and possible habituation to the stimulus (Yanagisawa et al. 2021). The adopted disruptive noise can be improved in the spectral characteristics, evaluating which of the different frequency picks impairs insect's communication through behavioural bioassays. Future applications could also involve the usage of this technique for other greenhouse crops and different pest insects. It would also be interesting to test the efficacy of the combination of vibrations and insecticides. In fact, a possible synergistic effect could significantly reduce the GW population thus leading to a substantial reduction of chemical treatments in greenhouses when associated to disruptive vibrations. From this descends that the extension of the method to other crops and possibly to other pests that communicate by vibrational signals such as leafhoppers and stink bugs could open new perspectives in the context of integrated pest management and replacement of controversial tools such as broad-spectrum insecticides (Desneux et al. 2007; Guedes et al. 2016; Nieri et al. 2022). Additionally, an increasing number of studies are being conducted on the effect of sound and vibrations on plants physiology showing in particular how these stimuli can have the ability to increase plants defence efficacy against a number of pathogens (Mishra et al. 2016).

To conclude, in the framework of this research, we combined biotremology with engineering concepts and tools to develop a new strategy for the control of pests in greenhouses. The multidisciplinary approach of this experimentation allowed us to consider different aspects related to both pest biology and substrate vibration propagation properties, using numerical modelling and empirical data to assess the first trial of this innovative and environmentally sound alternative to the usage of synthetic pesticides. By adding the model contribution, we verified the signal amplitude along the plant and, moreover, we were able to confirm that plant growth can play a significant role in the signal spreading (improving when increasing plant height). If this method or other similar methods based on principles of biotremology will be adopted by industries as a tool of pest control will depend on several factors. At this preliminary stage is not yet possible to make a proper benchmark analysis, by comparing the vibrational approach with other consolidated methods. However, since other pest control methods based on vibrations are currently under study or even already used by farmers (Nieri et al., 2022), it looks reasonable to consider our approach and method as a promising tool of IPM that could work at least as a synergist to reinforce other sustainable methods of pest control.

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Contributions

A.B., V.F. and V.M. contributed to the study conception and design. A.B. conducted the numerical simulations and plant measurements, V.F. conducted the experiments. A.B. and V.M. analysed the data. A.B and V.F. wrote the first draft of the manuscript, then revised based on all authors' comments . N.M.P. supervised the project. All authors read and approved the final manuscript.

Data Availability

The raw/processed data required to reproduce these findings cannot be shared at this time due to technical issues, but are available upon direct request to the corresponding authors.

Competing interests

The authors declare no competing interests.

References

Bi Jian L., Toscano Nick C. (2007) Current status of the greenhouse whitefly, Trialeurodes vaporariorum, susceptibility to neonicotinoid and conventional insecticides on strawberries in southern California. Pest Manag. Sci 8:747-752.

Blahovec J (1988) Mechanical properties of some plant materials. J Mater Sci 23:3588–3593. https://doi.org/10.1007/BF00540499

Brenya E, Pervin M, Chen Z-H, et al (2022) Mechanical stress acclimation in plants: linking hormones and somatic memory to thigmomorphogenesis. Plant Cell Environ 45:989–1010. https://doi.org/10.1111/pce.14252

Casas J, Magal C, Sueur J (2007) Dispersive and non-dispersive waves through plants: implications for arthropod vibratory communication. Proc R Soc B Biol Sci 274:1087–1092. https://doi.org/10.1098/rspb.2006.0306

Cocroft RB, Gogala M, Hill PSM, Wessel A (2014) Fostering research progress in a rapidly growing field. In: Studying vibrational communication. Springer-Verlag Berlin Heidelberg 2014, pp 3–12

Cocroft RB, Tieu TD, Hoy RR, Miles RN (2000) Directionality in the mechanical response to substrate vibration in a treehopper (Hemiptera: Membracidae: Umbonia crassicornis). J Comp Physiol A 186:695–705. https://doi.org/10.1007/s003590000123

Cokl A (2005) Tuning of host plants with vibratory songs of Nezara viridula L (Heteroptera: Pentatomidae). J Exp Biol 208:1481–1488. https://doi.org/10.1242/jeb.01557

Cokl A, Presern J, Virant-Doberlet M, et al (2004) Vibratory signals of the harlequin bug and their transmission through plants. Physiol Entomol 29:372–380. https://doi.org/10.1111/j.0307-6962.2004.00395.x

de Langre E (2019) Plant vibrations at all scales: a review. J Exp Bot 70:3521–3531. https://doi.org/10.1093/jxb/erz209

Der Loughian C, Tadrist L, Allain J-M, et al (2014) Measuring local and global vibration modes in model plants. Comptes Rendus Mécanique 342:1–7. https://doi.org/10.1016/j.crme.2013.10.010

Desneux N, Decourtye A, Delpuech J-M (2007) The sublethal effects of pesticides on beneficial arthropods. Annu Rev Entomol 52:81–106. https://doi.org/10.1146/annurev.ento.52.110405.091440

Fattoruso V, Anfora G, Mazzoni V (2021) Vibrational communication and mating behavior of the greenhouse whitefly Trialeurodes vaporariorum (Westwood) (Hemiptera: Aleyrodidae). Sci Rep 11:6543. https://doi.org/10.1038/s41598-021-85904-0

Fertis DG (1995) Mechanical and structural vibrations. John Wiley & Sons Inc (US), New York

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

Gorman K, Devine G, Bennison J, et al (2007) Report of resistance to the neonicotinoid insecticide imidacloprid in Trialeurodes vaporariorum (Hemiptera: Aleyrodidae). Pest Manag Sci 63:555–558. https://doi.org/10.1002/ps.1364

Gorman K, Hewitt F, Denholm I, Devine GJ (2002) New developments in insecticide resistance in the glasshouse whitefly (Trialeurodes vaporariorum) and the two-spotted spider mite (Tetranychus urticae) in the UK. Pest Manag Sci 58:123–130. https://doi.org/10.1002/ps.427

Guedes RNC, Smagghe G, Stark JD, Desneux N (2016) Pesticide-induced stress in arthropod pests for optimized integrated pest management programs. Annu Rev Entomol 61:43–62. https://doi.org/10.1146/annurev-ento-010715-023646

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

Hill PSM, Mazzoni V, Narins P, et al (2019) Quo vadis, biotremology? In: Biotremology: Studying Vibrational Behavior. Springer Nature Switzerland, Cham, pp 3–14

Hoddle MS, Van Driesche RG, Sanderson JP (1998) Biology and use of the whitefly parasitoid *encarsia formosa*. Annu Rev Entomol 43:645–669. https://doi.org/10.1146/annurev.ento.43.1.645

Hoshyarmanesh H, Dastgerdi HR, Ghodsi M, et al (2017) Numerical and experimental vibration analysis of olive tree for optimal mechanized harvesting efficiency and productivity. Comput Electron Agric 132:34–48. https://doi.org/10.1016/j.compag.2016.11.014

Hu X, Tao W, Guo Y (2008) Using FEM to predict tree motion in a wind field. J Zhejiang Univ-Sci A 9:907–915. https://doi.org/10.1631/jzus.A0720035

Jackson T, Shenkin A, Wellpott A, et al (2019) Finite element analysis of trees in the wind based on terrestrial laser scanning data. Agric For Meteorol 265:137–144. https://doi.org/10.1016/j.agrformet.2018.11.014

James K, Dahle G, Grabosky J, et al (2014) Tree biomechanics literature review: dynamics. Arboric Urban For 40:. https://doi.org/10.48044/jauf.2014.001

Kang J-H, Campos ML, Zemelis-Durfee S, et al (2016) Molecular cloning of the tomato Hairless gene implicates actin dynamics in trichome-mediated defense and mechanical properties of stem tissue. J Exp Bot 67:5313–5324. https://doi.org/10.1093/jxb/erw292

Magal C, Schöller M, Tautz J, Casas J (2000) The role of leaf structure in vibration propagation. J Acoust Soc Am 108:2412–2418. https://doi.org/10.1121/1.1286098

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 90:1219–1229. https://doi.org/10.1007/s10340-017-0862-z

Michelsen A (2014) Physical aspects of vibrational communication. In: Cocroft RB, Gogala M, Hill PSM, Wessel A (eds) Studying Vibrational Communication. Springer, Berlin, Heidelberg

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

Mishra RC, Ghosh R, Bae H (2016) Plant acoustics: in the search of a sound mechanism for sound signaling in plants. J Exp Bot 67:4483–4494. https://doi.org/10.1093/jxb/erw235

Nieri R, Anfora G, Mazzoni V, Rossi Stacconi MV (2022) Semiochemicals, semiophysicals and their integration for the development of innovative multi-modal systems for agricultural pests' monitoring and control. Entomol Gen 167–183. https://doi.org/10.1127/entomologia/2021/1236

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 89:909–921. https://doi.org/10.1007/s10340-015-0726-3

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

Rodriguez M, Ploquin S, Moulia B, de Langre E (2012) The multimodal dynamics of a walnut tree: experiments and models. J Appl Mech 79:044505. https://doi.org/10.1115/1.4005553

Sellier D, Fourcaud T, Lac P (2006) A finite element model for investigating effects of aerial architecture on tree oscillations. Tree Physiol 26:799–806. https://doi.org/10.1093/treephys/26.6.799

Strauß J, Stritih-Peljhan N, Nieri R, et al (2021) Communication by substrate-borne mechanical waves in insects: From basic to applied biotremology. In: Jurenka R (ed) Sound Communication in Insects. Academic Press, pp 189–307

Takanashi T, Nishino H (2019) Exploitation of vibration sensing for pest management in Longicorn Beetles. In: Hill PSM, Lakes-Harlan R, Mazzoni V, et al. (eds) Biotremology: Physiology, Ecology, and EvolutionApplied Biotremology. Springer International Publishing, Cham

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

Vogel S (2013) The Life of a Leaf. University of Chicago Press, Chicago, IL

Yanagisawa R, Suwa R, Takanashi T, Tatsuta H (2021) Substrate-borne vibrations reduced the density of tobacco whitefly Bemisia tabaci (Hemiptera: Aleyrodidae) infestations on tomato, Solanum lycopersicum: an experimental assessment. Appl Entomol Zool 56:157–163. https://doi.org/10.1007/s13355-020-00711-9

Zhang X, Guo Q, Xu Y, et al (2016) Mechanical testing of tomato plant stem in relation to structural composition. Agric Res 5:236–245. https://doi.org/10.1007/s40003-016-0209-7