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# Coupling computational vibrational models and experimental biotremology to develop a green pest control strategy against the greenhouse whitefly *Trialeurodes vaporariorum*

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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 multi-disciplinary approach to develop a control technique against the greenhouse whitefly, Trialeurodes vaporariorum. First, 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 to investigate a growing 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 in terms of insect population of the vibrational treatment, which was administrated through vibrational plates. The green methodology applied in this study represents an innovative, environmentally sound alternative to the usage of synthetic pesticides.

## 1. 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 least studied [1]. In particular, insects' vibrational communication is studied in applied biotremology to find alternative and ecological strategies for controlling insect pests [2] that involve 'behavioural manipulation' of the target insect, which means to disrupt intraspecific communication and thus to affect its ability to reproduce and proliferate [3]. 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 [4]. This knowledge may allow us to manipulate the insect's behaviour by means of artificial stimuli, such as disruptive noises or simulated calling signals [2,3,5,6]. A vibrational device is needed for these purposes, such as vibrational traps that release attractive signals, disturb their

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biological activities (i.e. feeding, oviposition) or repel them from a crop [7]. For example, vibrational mating disruption (exploiting males' interference signals) has been successfully applied against the leafhopper *Scaphoideus titanus* in a commercial vineyard in northern Italy [8]. In another case, a vibration exciter has been developed using a magneto-restrictive material, capable of inducing a startle response in the insect target [9].

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 [10-16]. 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 [17,18]. 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 [3], and they change shape and structure during the growth and life cycle [19]. 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 [20,21]. The 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 [22-25], 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 a 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 [26]; however, when a more complex geometry is adopted, vibrational modes and the dynamic response to a vibrational perturbation are usually extracted by numerical methods such as FEM [22-25], as within this work. Many examples are also reported in a recent review by de Langre [27], 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 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 [28,29]. Another option, mainly adopted in integrated pest management (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* [30]. Successful control can be obtained if the parasite is established on plants when natural infestations are small. Therefore, the efficacy of these techniques depends upon different factors such as host plant quality, temperature, usage of fertilizer, dimension of the greenhouse and stage of infestation [31]. 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 five 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. [32] 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 [32]. Therefore, the best strategy would consist of 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 might the female 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 GW signal involved in this stage (Chirp, Pulse Train and Female Responding Song). The signal was tested for a two-month trial on tomato plants in the greenhouse after plant infestation. In parallel, a FEM 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 an extension of the proposed system to other greenhouse crops.

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**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*) Three-dimensional FEM of a tomato plant, on which the same points were checked and compared with experiments.

# 2. Materials and methods

## 2.1. Three-dimensional finite-element modelling and analysis

For the tomato plant dynamics, a three-dimensional model of a real plant was developed from a free three-dimensional model downloaded from Sketchfab, which was then adapted and finally imported in the numerical solver Abaqus Standard 2018 (Dassault Systemes Simulia Corp., Providence, RI, USA), 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 threedimensional 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 five 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) [27]. 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 [33-35], thus for the stem and leaves a density  $\rho$  equal to 800 kg m<sup>-3</sup> and 700 kg m<sup>-3</sup>, respectively, an elastic modulus E of 1 GPa and 0.8 GPa, and for both a Poisson coefficient v 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

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spectra in the other seven 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 only the 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 [36], due to their small masses, compared with the whole plant [27]. This means that they should not affect the global (trunk) or even semi-global (branch) modes. Moreover, modelling a plant considering the sole stem would strongly simplify the problem. For this reason, we also analysed a tomato plant modelled only by its stem (S plant), to compare the differences in terms of both natural frequencies and substrate velocities with the SL plant.

#### 2.2. 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  $22 \pm 1^{\circ}$ 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 cm × 0.5 cm were placed) on both stems (six points) and leaves (four points), and vibrations were simultaneously recorded by setting the laser sensitivity to  $5 \text{ mm}^{-1} \text{ s}^{-1} \text{ V}^{-1}$ . 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.

#### 2.3. Fast Fourier transform and data analysis

Recordings were post-processed with Matlab 2020 userdeveloped script (1994–2021, The MathWorks, Inc.) to compute the fast Fourier transform 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.

#### 2.4. 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  $25 \pm 2^{\circ}$ C,  $70 \pm 5\%$  RH and 16: 8 (L:D), in mesh cages (Bugdorm-6620,  $60 \times 60 \times 120 \text{ cm}^3$ , Mega-View 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 laboratory of Fondazione Edmund Mach from August to October 2020.

#### 2.5. Plant rearing

All the seedlings used for the experiment were grown in 1 l pots in the greenhouse at  $25 \pm 2^{\circ}$ C,  $70 \pm 5\%$  RH and 16:8 (L:D).

When they reached an average height of  $43 \pm 10$  cm, we proceeded with introducing the whiteflies in the cages.

#### 2.6. Test products application

We applied three different treatments: water as a negative control, the disturbing signal and a pesticide (Decis Jet,  $2.5 \text{ ml } l^{-1}$ ) as a positive control. Before starting the infestation, all plants were placed in four 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 third or fourth 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  $\times$  $D24.5 \times H63.0$  cm); for each treatment there were four 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 four iron legs (height: 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 five peaks of amplitude corresponding with the fundamental frequency of the signals used by insects to communicate: 150, 200, 250, 300 and 350 Hz [32] (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 s and then was played back in loop 24/7. Plants' weight and signal propagation through the plants were assessed at the beginning and at the end of the trial.

#### 2.7. 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. 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 × 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).

### 3. Results

In figures 3 and 4, comparisons 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.



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

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

#### 3.1. Stem-leaves plant versus 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<sup>-1</sup> as a precautionary lower bound [37]. For this reason, we decided to fully analyse the SL plant model also in other configurations.

#### 3.2. Influence of plant growth on vibration 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 [26]. The density and the material stiffness of the tissue are not expected to vary much across 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  $D^4$ , and f should vary as  $D/h^2$ . Moreover,  $D \ll h$  and its range of variation is more limited while h appears with a power of 2, so that the most influential parameter is the second one, 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 7*a*, where the natural frequency variation is clearly evident. In particular it increased 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, there was 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.

#### 3.3. Whiteflies infestation

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 × 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 larger than both vibrations and insecticide (water versus vibrations: p = 0.008; water versus Decis Jet: p < 0.001; vibrations versus 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 versus 2nd: p = 0.04; 1st versus 3rd: p = 0.001; 2nd versus 3rd: p = 0.32).



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

### 4. 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 FEM 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. 6



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



Figure 5. Average and s.d. of measures on leaves and comparison with simulated results with respect to the different signal peaks. (a) 150 Hz, (b) 200 Hz, (c) 250 Hz, (d) 300 Hz and (e) 350 Hz.

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

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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



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 or a S plant model.



**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. (*b*) The comparison of the total velocity path through the plant by adopting one of the previous models.

the plant response to vibrational stimuli. For these reasons, we can state that the simulated response is a good approximation of 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 figures 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



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

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  $(0.01 \text{ mm s}^{-1})$ , with the only exception for 350 Hz, however, suggesting that the signal coverage on the plant was strong enough in amplitude to impair insect 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 [38]. 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 fifth 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 a subject 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 [39]. As far as the disruptive noise consists of a few selected harmonics that perfectly cover the whitefly mating signal, this does not exclude that some plant regulatory mechanisms might be affected by 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 three-dimensional 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, characterized by a small amplitude (of the order of tens of micrometres) and which results in infinitesimal displacements. Even the involved stresses on the plant are extremely small (a few kilopascals), 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 [40]. The adopted disruptive noise can be improved in spectral characteristics, evaluating which of the different frequency picks impairs insect 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 with disruptive vibrations. From this it follows 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 IPM and replacement of controversial tools such as broadspectrum insecticides [41-43]. Additionally, an increasing number of studies are being conducted on the effect of sound and vibrations on plant physiology showing in particular how these stimuli can have the ability to increase plant defence efficacy against a number of pathogens [44].

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 multi-disciplinary 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). Whether 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, it 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 [43], 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.

# Data accessibility. Data are available from the Dryad Repository at 10.5061/dryad.j0zpc86j0 [45].

Authors' contributions. A.B.: conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, writing—original draft and writing—review and editing; V.F.: conceptualization, data curation, formal analysis, investigation, methodology, validation, visualization, writing—original draft and writing—review and editing; V.M.: conceptualization, data curation, funding acquisition, methodology, project administration, supervision and writing—review and editing; N.M.P.: funding acquisition, project administration, resources, supervision and writing—review and editing.

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