

RESEARCH ARTICLE

Pollen release during buzz pollination depends on vibration amplitude and floral vibration transmission, but not on spectral composition

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ABSTRACT

Bees use vibrations across behavioural contexts, including a specialised foraging routine in which they vibrate flowers to release pollen (floral vibrations). Floral vibrations often have complex spectral properties, combining fundamental frequencies and their harmonics, yet their functional consequences remain unknown. We experimentally replicated bee buzzes with identical fundamental frequency (300 Hz) but different harmonic content (0, 2 or 4 harmonics) to determine their effect on pollen release, a key functional outcome for both the bee and the flower. We normalised signals to the same average power by holding root mean squared (RMS) acceleration constant and applied three RMS levels to test for interactions between power and spectral composition. We applied vibrations to flowers and quantified both the input signal (at the shaker) and the vibration transmitted at a distal anther, and measured pollen release. Harmonic content did not explain pollen release at any RMS level. In contrast, RMS acceleration strongly predicted pollen removal. The RMS measured at the distal anther was only weakly correlated with the input RMS, suggesting that the flower strongly modifies the vibration signal. Both input RMS and distal anther RMS explained pollen release, irrespective of spectral composition. Our results suggest that pollen release is determined by vibration amplitude (RMS acceleration) rather than spectral composition and is jointly governed by the bee-generated input and the mechanical filtering properties of the flower.

KEY WORDS: Bees, Biomechanics, Frequency spectrum, Functional analysis, Flowers, Harmonics

INTRODUCTION

Bees (*Anthophora*) use oscillatory movement of their indirect flight muscles to communicate, to choose mates, to thermoregulate, during nest construction, as well as while foraging for pollen in some kinds of flowers (reviewed in Vallejo-Marin, 2022). When harvesting pollen through vibrations, bees rapidly (100–400 times per second) contract pairs of antagonistic muscles that deform their exoskeleton and generate vibrations (Buchmann, 1983). The bee applies these vibrations to flowers while biting and curling its body around pollen-carrying structures, usually anthers, causing pollen to be ejected (De Luca and Vallejo-Marin, 2013; Woodrow et al., 2024). The use of vibrations to harvest pollen has evolved repeatedly across bee groups

and may occur in as many as half of all bee species (Cardinal et al., 2018). Many species of flowering plants have independently evolved flowers that require bee vibrations to release pollen (Russell et al., 2026), i.e. they are buzz pollinated.

Bee vibrations applied to flowers, called floral buzzes, are characterised by a fundamental frequency that presumably maps to the rate of muscle contraction of the thorax. However, in addition to this fundamental frequency, floral buzzes contain harmonics that describe frequency components that are multiples of the fundamental frequency (Buchmann et al., 1977; Cane, 2014; King and Buchmann, 2003) (Fig. 1). Floral buzzes tend to have a rich spectral composition, with more harmonics than other types of bee buzzes, including those generated during flight (Arvelos et al., 2025; Gonzalez-Almansa Laredo et al., 2026). The harmonics contained in floral buzzes rapidly decay in amplitude (Bochorny et al., 2021; Buchmann et al., 1977; De Luca et al., 2019; King and Buchmann, 2003), which suggests that they contain less energy compared with the fundamental frequency. Whether the spectral composition of floral buzzes has a functional effect, for instance in pollen release, is unknown.

Theoretically, it is possible that buzzes with harmonics might release more pollen compared with similar buzzes without harmonics. For example, adding harmonics to the fundamental frequency could allow the bee to explore multiple frequencies simultaneously during a single buzz. It has been suggested that if the frequency of a bee's buzz approximates closely the natural frequency of a flower, then the larger motion induced through resonance might increase the rate of pollen release (Buchmann and Jankauski, 2024). Studies of individual anthers suggest that the resonance of the anthers of some species should be higher than even the highest harmonics in a bee's buzz (King and Lengoc, 1993). Yet, other studies have shown that the first modes of vibration might occur at frequencies that would overlap with the harmonics range of floral buzzes (Pereira Nunes et al., 2021) and that resonant frequencies of individual anthers might be further lowered and brought within the bee's range as the bee weight loads onto the anther (Jankauski et al., 2022). However, even the simplest buzz-pollinated flowers possess multiple anthers attached to other floral structures such as petals that vibrate simultaneously, and the resonance of the whole floral structure should be considered (DeTar et al., 1968). Furthermore, including harmonics in buzzes might come at a cost. If the bee has a fixed amount of energy to deploy during floral buzzing, the redistribution of power across a wider range of frequencies might decrease the energy available at any one frequency. Connecting vibration properties including spectral composition, amplitude and buzzing tempo to their effects on pollen release can help us link bee behaviour with its functional outcomes.

Previous work has shown that some of the vibration properties of floral buzzes map to their functional effects, specifically to the

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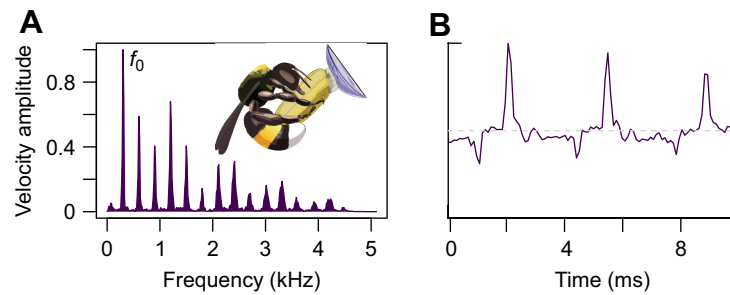


Fig. 1. The buzzes produced by bees while buzz-pollinating flowers (floral buzzes) are not a simple sinusoidal wave with a single frequency but consist of a variable and complex set of a fundamental frequency (f_0) and harmonics. The figure shows a 0.277 ms floral buzz by *Bombus terrestris* pollinating *Solanum citrullifolium* measured with a laser vibrometer (velocity, m s^{-1}) aimed directly to the dorsal side of the thorax. (A) Floral buzz in the frequency domain showing the fundamental frequency ($f_0=300$ Hz) and several harmonics. (B) Shape of the floral buzz in the time domain expanded over a period of 10 ms. Amplitude is shown on a unitless relative scale. Dashed line in B represents the zero reference. Additional examples are available in Dryad (<https://doi.org/10.5061/dryad.wstqjq328>). Data from Pritchard and Vallejo-Marín (2020).

amount of pollen released from flowers (De Luca et al., 2013; Harder and Barclay, 1994; Kemp and Vallejo-Marín, 2021; King and Buchmann, 1996; Rosi-Denadai et al., 2020). Experiments using artificial vibrations have demonstrated that vibration amplitude strongly determines rates of pollen release. For example, pollen release scales with the amplitude of acceleration or velocity and buzz duration across several plant species (De Luca et al., 2013; Kemp and Vallejo-Marín, 2021; Rosi-Denadai et al., 2020). In contrast, the frequency of the applied vibrations has a weaker and variable effect on pollen release (De Luca et al., 2013; Rosi-Denadai et al., 2020). Importantly, most experiments to date have used single-frequency floral buzzes, i.e. buzzes that are simple sinusoidal waves without any harmonics. These single-frequency floral buzzes do not capture the complex spectral composition of real bee buzzes, potentially introducing a bias in our inferences that spectral properties have small or negligible effects on pollen release.

Pollen release in buzz-pollinated flowers should depend on how the pattern of mechanical vibrations driven by the bee is experienced by the anthers (Boucher-Bergstedt et al., 2025; Buchmann and Hurley, 1978; Hansen et al., 2021; Shi et al., 2025). Buzz-pollinating bees can apply vibrations to parts of the flower, e.g. biting a focal anther while vibrating (Buchmann, 1983; Woodrow et al., 2024), but these vibrations then need to be transmitted across all the anthers in the flower. Thus, vibrations transmitted via both direct (the focal anther being vibrated) and indirect pathways (other non-focal anthers that experience the bee's vibrations) should contribute to pollen release (Vallejo-Marín, 2019). Bees can, in principle, modulate the vibrations directly applied to the focal anther, but floral properties might be important in determining how these vibrations are transmitted across the flower (Arroyo-Correa et al., 2019; King, 1993). The expectation that floral characteristics affect vibration transmission during buzz pollination is supported by experimental data. For example, *Solanum* flowers with experimentally fused anther cones (where the anthers are physically linked into a single structure) experience higher transmission of vibrations across the anthers and release more pollen than flowers in which anthers are held loosely and not directly connected to each other (Vallejo-Marín et al., 2022). Using high-speed videography across species in four plant families, Nevard et al. (2021) showed that flowers with tightly adpressed anther cones uniformly transmit vibrations across all anthers, while flowers with loosely arranged anthers or where morphologically distinct anthers occur in the same flower can amplify the amplitude displacement exhibited by non-focal anthers while the frequency of the vibration remains unchanged. Bees might also have behavioural

mechanisms to modulate vibration transmission in the flower. For instance, King (1993) suggests that the higher transmission of vibrations in flowers of *Symphytum officinale* achieved by *Bombus hortorum* compared with *Bombus terrestris* is due to the former inserting its head deeper into the corolla tube. Pollen release should thus consider not only the properties of the vibrations that the bee applies and its behaviour but also how these vibrations are transmitted within the flower to other pollen-carrying structures.

Here, we conducted an experimental study using a mechanical shaker and a buzz-pollinated plant (*Solanum pyracanthos*, Solanaceae) to address two main questions: (1) does the spectral composition (defined here as the presence of harmonics) of a floral buzz affect pollen release?; and (2) to what extent do the input vibrations on a focal anther versus the vibration experienced by non-focal anthers in the same flower independently contribute to pollen release? To address these questions, we used simulated floral buzzes with the same fundamental frequency (300 Hz) but different numbers of harmonics (0, 2 or 4 harmonics). Because we know that vibration amplitude affects pollen release (Vallejo-Marín, 2019; Vallejo-Marín and Russell, 2024), we standardised floral buzzes to have the same average acceleration amplitude. We chose to standardise the root mean squared (RMS) acceleration because we expected it to scale directly with the power and (for a fixed length of buzzing time) with the energy of the floral buzz. Thus, our experiment generated buzzes with the same power but varying spectral composition. Furthermore, we tested an additional buzz property in our experiment by varying the RMS acceleration of buzzes across three levels. This enabled us to investigate the combined effects of spectral composition and vibration amplitude on pollen release. In addition, we simultaneously measured both the input vibrations applied with a mechanical shaker to a focal anther and the response vibrations measured at the tip of a different anther of the same flower. Including both input and response vibrations in our statistical models of pollen release enabled us to estimate the relative contribution of the input (shaker) and response (distal anther) vibrations to the release of pollen grains. Finally, we employed frequency–response analyses using logarithmic chirps (120–4500 Hz) on buzz-pollinated flowers, to analyse how input vibrations are transferred to distal anthers across a range of frequencies.

MATERIALS AND METHODS

Study system

We used flowers from *Solanum pyracanthos* Lam., a buzz-pollinated species endemic to Madagascar but widely cultivated

around the world. Like most other *Solanum* species, *S. pyracanthos* has pentamerous flowers with five equally sized bright yellow anthers arranged centrally and forming a cone. The anther cone in *S. pyracanthos* is formed by closely adpressed, but unfused anthers that are free to move from one another (Vallejo-Marin et al., 2022). We chose this species because it possesses the canonical flower morphology of *Solanum*, a genus with approximately 1200 buzz-pollinated plants, and more generally a solanoid morphology, which has evolved repeatedly throughout the evolutionary history of angiosperms (De Luca and Vallejo-Marin, 2013; Faegri, 1986; Vogel, 1978).

We used plants from two commercially obtained accessions, 1179F (Chiltern seeds, Wallingford, UK) and Y5SSOPY (Rare Palm Seeds, München, Germany). The exact provenance of these seeds is unknown. Seeds were germinated as described in Vallejo-Marin et al. (2014), transplanted to 3 l pots with soil, and fertilised once a week. Plants were grown at the Uppsala University glasshouses with supplemental lights under a 16 h light:8 h dark cycle at 21°C:16°C temperature.

Signal (buzz) design

To determine the contribution of harmonics to pollen release, we generated signals (buzzes) with different numbers of harmonics using a standard harmonic series function:

$$s(t) = \sum_{k=1}^K A_k \sin(2\pi f_0 k t + \phi_k), \quad (1)$$

where $s(t)$ is the signal sent to the shaker, K is the number of frequency components, A_k is the amplitude of the k th harmonic component, f_0 is the fundamental frequency and ϕ_k is the phase of the k th harmonic. We created three harmonics treatments: (i) a pure tone consisting only of a single component $f_0=300$ Hz (1 h treatment), (ii) a fundamental plus two harmonics (300, 600 and 900 Hz; 3 h treatment) and (iii) a fundamental plus four harmonics (300, 600, 900, 1200 and 1500 Hz; 5 h treatment).

The amplitude of the harmonics within each signal was modelled with an exponential decay function:

$$A_k = e^{-\alpha(k-1)}, \quad (2)$$

where α is the exponential decay parameter. We set $\alpha=0.7$ so that the amplitude of each successive harmonic decreased by about 50%. The exponential decay parameter of pollination buzzes has not been estimated before, but the amplitude of harmonics often follows a rapidly decreasing pattern (King and Lengoc, 1993).

The phases of the harmonic components (ϕ_k) were drawn at random. In a signal composed of multiple frequencies, the phase of each component determines constructive and destructive interference among harmonics and thus affects the peaks and symmetry of the signal. Each random combination of phases is a stochastic realisation of a signal with a given spectral composition. We therefore created 10 independent signals using randomly generated phase combinations for each of the three harmonics treatments (30 signals in total). The signals used are provided in Dryad (<https://doi.org/10.5061/dryad.wstqjq328>) and examples of two signals for each treatment are shown in Fig. 2. Using 10 stochastic realisations per treatment allowed us to account for variation due to phase alignments, thus yielding a more robust sample of treatment-level differences in harmonic (spectral) composition.

To simulate bee buzzes with similar magnitude but a different number of harmonics, we standardised all signals to have the same RMS acceleration. By keeping the RMS of the input signal constant regardless of harmonic content, we aimed to mimic a situation where a bee has a fixed amount of power that can be distributed into a fundamental frequency and different numbers of harmonics. We note that while we kept RMS acceleration constant across different harmonic treatments, RMS velocity should necessarily decrease slightly (by about 10%) in signals with harmonics relative to a pure tone signal (see [Supplementary Materials and Methods](#) for the derivation of RMS velocity).

The final signal for playback was composed of four individual buzzes of 500 ms in duration separated by 200 ms gaps. The length

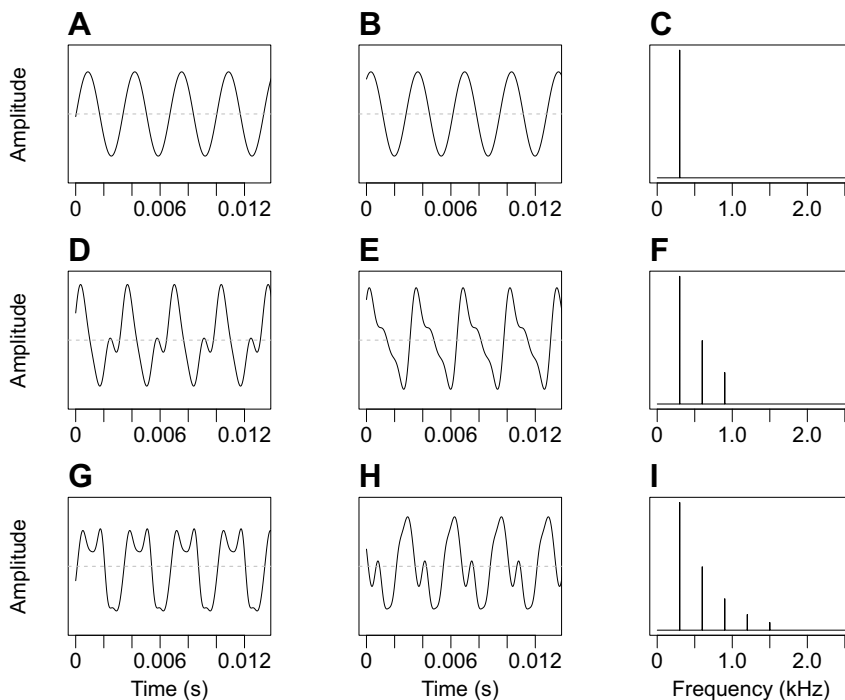


Fig. 2. Examples of the experimental signals. Signals contained only the fundamental frequency (300 Hz; A–C), the fundamental plus two harmonics (D–F), and the fundamental and four harmonics (G–I). The first two columns show the time domain of 2 of the 10 randomly generated signals per treatment. The third column shows the first signal in the frequency domain. Amplitude is shown on a unitless relative scale. The plots depict the signal outside the ramp envelope.

of individual buzzes is based on our earlier work with *Bombus terrestris* visiting other *Solanum* species (Arroyo-Correa et al., 2019; Gonzalez-Almansa Laredo et al., 2026; Vallejo-Marín and Lundgren, 2026). Each buzz had a half-raised-cosine ramp up and down of 50 ms to suppress spectral leakage and to avoid sudden spikes in voltage when playing back the signal (Kemp and Vallejo-Marín, 2021). The signals were exported as an uncompressed WAV object with a sampling rate of 96,000 Hz and 24 bits per sample. Signals were created in MATLAB 2026a (The MathWorks Inc, Natick, MA, USA).

Playback system and RMS calibration

To determine how different vibrations applied to flowers translate to pollen release, we built a playback system that allowed us to simultaneously vibrate the flowers, measure both input and response vibrations, and collect released pollen grains (Fig. 3). The system consisted of a computer that played back the signal connected to a linear power amplifier (LDS-LPA100, Brüel & Kjær, Nærum, Denmark). The amplifier powered a permanent magnetic shaker (LDS-V210, Brüel & Kjær) mounted on trunnions and the voltage output to the shaker was controlled via both the computer volume and a gain dial in the amplifier. To connect the shaker to the flower, we attached a 100 mm M4 metal setscrew (wand) to the vibrating plate of the shaker (Fig. 3). At the opposite end of the wand, we mounted a 10 mm long entomological pin size '0' using a 3D printed PLA base and cyanoacrylate glue. We measured the vibrations generated by the shaker (input excitation or stimulus) using a 0.2 g miniature accelerometer (352C23, PCB Piezotronics, Hüchelhoven, Germany) glued with cyanoacrylate to the vibrating plate. In addition, we measured the vibrations at the tip of the wand (response) using a laser vibrometer (VGO-200, Polytec, Waldbronn, Germany). The laser vibrometer was set to acquire data in acceleration units (m s^{-2}), making it directly comparable to the accelerometer units. The analog signal of both the accelerometer and laser vibrometer was acquired and digitised using a C-Series Sound and Vibration module (9250; National Instruments, Stockholm, Sweden) on a Compact DAQ chassis (cDAQ-9171, National Instruments) and a custom-written program

in LabVIEW 2026 (National Instruments). The sampling rate of the acquired signals was set to 20,480 samples per second, which allowed us to resolve frequencies up to approximately 10.1 kHz. The data were acquired as TDMS files and then converted to TXT files using a custom LabView program.

For each played back signal, we calibrated the shaker amplitude (measured with the accelerometer) to the desired RMS acceleration using a 30 s version of the uninterrupted experimental signal. The RMS of the uninterrupted signal was calculated in a live feed of the accelerometer readings. We slowly changed the gain of the amplifier until the shaker amplitude reached the desired RMS. Because during calibration acquisition, the signal is measured outside the ramps that characterise the experimental four-buzz signals, the raw RMS of the calibration will not match the RMS of the individual buzzes. We thus calculated the expected RMS of the individual buzzes accounting for the ramps up and down within each buzz and adjusted up the target RMS of the calibration signal. This enabled us to precisely adjust the shaker amplitude to target a specific RMS value within individual experimental buzzes.

We used three RMS acceleration values to investigate the relative contribution of average acceleration to pollen release. The target values we used for the RMS of individual buzzes were: 70.16, 102.90 and 149.67 m s^{-2} (corresponding to 75, 110 and 160 m s^{-2} in the calibration signal). The chosen values approximate the range of RMS acceleration in floral buzzes applied by *B. terrestris* to other *Solanum* flowers (Arroyo-Correa et al., 2019). The three RMS treatments were combined with the three harmonic treatments (9 total experimental combinations) and randomly paired with a different stochastic realisation of the harmonic treatment (9×10 stochastic realisations=90).

Flower attachment and pollen collection

During an experimental trial, we mounted a flower to the pin at the tip of the wand. We chose recently opened flowers (within 1–2 days) and cut them from the plant, leaving the pedicel as long as possible. We transported flowers to the laboratory in water-soaked flower foam (Oasis Floral Products, Houthalen-Helchteren, Belgium) to prevent wilting. All flowers were tested within 3 h of

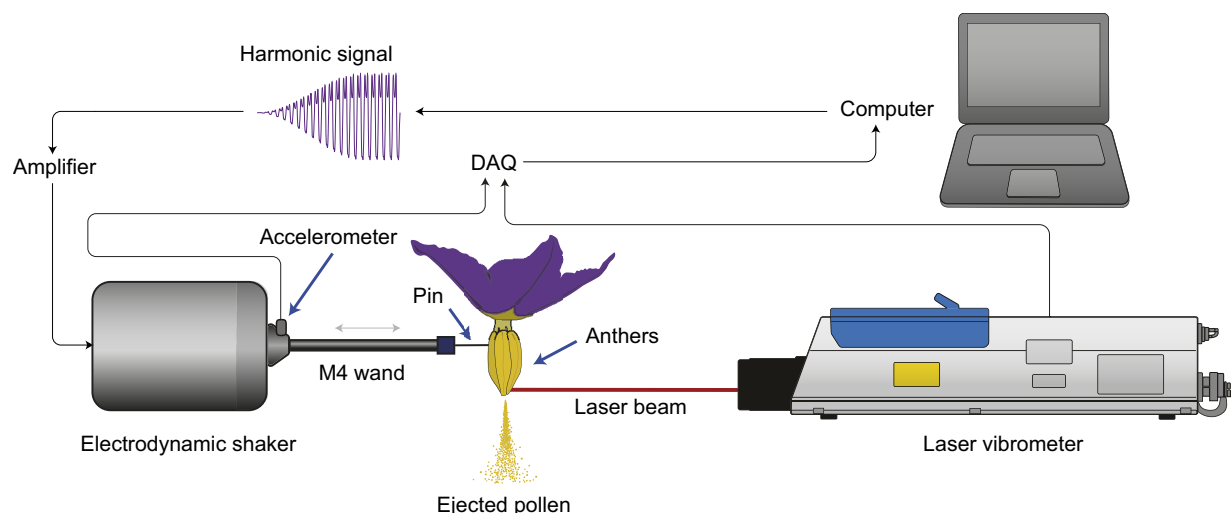


Fig. 3. Experimental design. The synthesised signal was sent to the electrodynamic shaker via a linear amplifier. An accelerometer at the base plate of the shaker was used to measure the input acceleration for calibration. The shaker then transmitted the vibrations to a focal anther through a metal pin inserted near the base of the anther. The laser vibrometer (acceleration units) measured the vibrations at the tip of an anther different from the focal anther (distal anther). Both laser and accelerometer signals were digitised with a DAQ system and stored on the computer. The ejected pollen grains were collected in a microcentrifuge tube and suspended in ethanol for subsequent counting in a Coulter Counter.

being excised. The pin was inserted into a single randomly chosen anther about 1/5 the distance from the base of the anther (Fig. 3), at 90 deg relative to the long axis of the anther. The flower was suspended by holding it at the base of the pedicel using a stainless-steel micro-V clamp (VK250/M; Thorlabs Sweden, Mölndal, Sweden) attached to a micromanipulator (M3301R, World Precision Instruments, Friedberg, Germany) to enable fine tuning of the flower's position. To measure the transmission of vibrations from the vibrated (focal) anther to other anthers in the flower, we aimed the laser at the tip of an anther opposite (distal) to the one being vibrated. In preliminary trials, we established that the low reflectivity of the anther tissue introduced a large amount of noise into the laser measurements. For this reason, we added a small (<1 mm²) piece of self-adhesive retro-reflective tape to the tip of the anther, taking care not to block the pore. This modification significantly improved the signal to noise ratio (SNR) and has been previously used in similar studies (Vallejo-Marin et al., 2022).

After attaching the flower to the system, we placed a microcentrifuge tube immediately below the anther pores to capture the released pollen. At the end of the buzzes, the anthers containing the unreleased pollen were collected in a separate tube. Collecting both released and unreleased pollen allowed us to estimate total initial pollen. Both pollen and anthers were stored in 1000 µl of 70% ethanol at 4°C until pollen counting.

Pollen counting

Pollen was counted using a Multisizer 4e Coulter Counter (Beckman Coulter Life Sciences, Indianapolis, IN, USA) using the methods described in Vallejo-Marin and Lundgren (2026). Tubes containing anthers were placed in an ultrasonic bath (Elmasonic S40H, Elma Schmidbauer GmbH, Singen, Germany) for 6 min to remove remaining pollen from inside the anther. After this, an aliquot, chosen to keep the concentration of particles below 10% as recommended by the instrument's manual, was taken from the tube and resuspended in 10 ml of Isotone II buffer. A 2 ml sample was analysed in the Coulter Counter and particles between 15 and 30 µm were counted and sized using 400 bins. The range of particle size used here was chosen based on our preliminary analyses of pollen grain size in *S. pyracanthos*. The number of particles counted was then extrapolated to the total volume in the microcentrifuge tube.

Transmission of vibrations of different frequencies within flowers

Vibrations applied to a single anther can cause other anthers to shake and release pollen as the vibrations travel through the flower tissue as well as by anther–anther collisions. The characteristics of this complex pathway for the transmission of vibrations across the anthers of a buzz-pollinated flower are very little understood. As a first step to understand how vibrations at different frequencies are transmitted across the flower, we estimated the frequency–response function (FRF) of flowers of *S. pyracanthos* in a separate experiment. We applied a frequency sweep to the flower using the same experimental system as described above. As before, the vibrations were applied to the base of a focal anther and measured with the laser vibrometer at the tip of a distal anther. For the frequency sweep, we generated a 6 s logarithmic chirp covering a frequency range of 120–4500 Hz in MATLAB (The-Math-Works-Inc., 2026). We used a logarithmic chirp to increase the resolution at lower frequencies where previous work has detected some of the natural frequencies of stamens occur (Jankauski et al., 2022; Pereira Nunes et al., 2021). We applied a raised cosine ramp at each of the start and end of the signal (50 ms of the signal at each end). The chirp had a frequency floor of 120 Hz as

preliminary tests suggested that vibrations below this value elicited resonance of the shaker–wand system. The chirp was exported as an uncompressed WAV file at 16-bit, 96 kHz sampling rate. Based on preliminary tests, we chose an amplitude equivalent of approximately 110 m s². This amplitude gave a SNR across even the highest frequencies without introducing any appreciable artifacts. For each experimental flower, we applied 3–4 replicated, manually triggered, chirps. In total, we analysed 59 chirps from 16 different flowers.

Data analysis

Characterisation of vibration properties

Vibration data were imported into R v.4.5.3 (<https://www.r-project.org/>) using RStudio v.2026.01.1 (<https://posit.co/>), and analysed with the package *seewave* (Sueur et al., 2008). After importing the data, we removed the offset of each signal and applied a high-pass filter at 80 Hz to remove background noise. To determine the start and end of each buzz, we used the accelerometer (excitation) channel because it had the highest SNR. We used the function *timer* in *seewave* and a threshold of 5% of the amplitude envelope of the accelerometer data. To retain the initial ramp of the vibration, the detected onset was extended by 9 ms. The resulting start and end times of each buzz were stored and used for subsequent analysis. For each buzz, we calculated the fundamental frequency, dominant frequency, RMS acceleration and peak acceleration as described in Vallejo-Marin et al. (2024). These variables were estimated for both excitation (accelerometer) and response (laser) signals.

Statistical analysis of pollen release

To analyse the relationship between pollen release and RMS acceleration and spectral properties (number of harmonics), we used generalised linear mixed effects models fitted with the package *glmmTMB*. We modelled pollen release as an overdispersed binomial process with a combined response variable of pollen released and pollen remaining. This effectively estimates the probability of pollen release after accounting for total pollen production in the flower. Accounting for total pollen production in the flower is important because previous work has shown that even flowers within the same individual vary strongly in primary pollen production (Gonzalez-Almansa Laredo et al., 2026). As explanatory variables, we used the RMS acceleration of the excitation (shaker) as well as the RMS acceleration of the response (anther tip). We included both the excitation and response RMS as explanatory variables because it enabled us to determine how both the applied vibrations (what the bee would force on a focal anther) and the vibration experienced by other anthers in the same flower (modulated by the floral properties) influence pollen release. Our results showed that these two variables are poorly correlated ($\rho=0.3$) indicating considerable variation in the response RMS even at a given excitation RMS level. As a random effect, we included plant identity and accession identity. Competing models (excitation RMS only, response RMS only, both excitation and response RMS, interaction between excitation and response RMS, and full model plus harmonic number; Table 1) were evaluated using AIC. The significance of individual variables in the final model was tested using likelihood ratio tests. The assumptions of the model were tested using the package *DHARMA* (<https://CRAN.R-project.org/package=DHARMA>).

FRF of flowers

For the analysis of the frequency sweeps, we removed the offset of both signals and identified the chirps using an amplitude envelope and a threshold of 5% the maximum amplitude envelope of the accelerometer channel. The start and end of each chirp was zero

Table 1. Model comparison

Model (fixed effects)	d.f.	logLikelihood	AIC	ΔAIC	AIC weight
RMS_{shaker}+RMS_{anther}+harmonics	7	−986.60	1988.6	0	0.667
RMS _{shaker} ×RMS _{anther} +harmonics	8	−986.28	1990.3	1.77	0.275
RMS _{shaker} +harmonics	6	−990.48	1994.0	5.40	0.045
RMS _{anther} +harmonics	6	−991.72	1996.4	7.88	0.013
RMS _{shaker} ×RMS _{anther} ×harmonics	14	−985.16	2003.9	15.36	0

All the compared models included plant ID as a random intercept. Bold indicates selected model.

padding to reduce edge effects. The FRF between the excitation signal was measured at the base of the shaker plate with the accelerometer, and the response signal was measured at the tip of the non-focal anther with the laser. The FRF can be used to infer the frequency-dependent gain of the signal and reveal resonant frequencies at which even small accelerations at the point of contact cause large accelerations at the anther tip. For the FRF, we used the Welch’s averaged periodogram method implemented in the MATLAB function *tfestimate* from the Signal Processing Toolbox (The MathWorks Inc.). Spectral estimates were calculated with a Hann window with size of 2048 samples. Coherence was calculated with the function *mscohere*. Coherence measures, as a function of frequency, the proportion of the response signal that is linearly predicted from the excitation signal. Individual chirp FRFs and coherence were averaged for each file. The MatLab code is provided in Dryad (<https://doi.org/10.5061/dryad.wstqjq328>).

AI use

ChatGPT 5.3 was used to assist with MATLAB and R coding including code development, annotation and plotting. We used Adobe Illustrator 2026 generative fill function to colour the bee in Fig. 1. The authors subsequently reviewed and edited the content as necessary and take full responsibility for the publication’s final content.

RESULTS

The *S. pyracanthos* flowers produced an average of 336,870 ±95,014 pollen grains (mean±s.d., *n*=90 flowers). Flowers produced both fully developed pollen grains (81.37±23.41%), and smaller, shrivelled, presumably inviable pollen grains. The average size of viable pollen grains was 26.46±0.26 μm (range 22.5–30 μm), and the size of inviable grains was 18.51±0.93 μm (15–22.5 μm) (*n*=349,660 pollen grains measured).

The shaker and tip of the wand produced nearly identical RMS acceleration across all harmonics treatments, indicating that at the tested fundamental frequency (300 Hz), the system behaves as expected (Fig. S1). In 90 trials, each with a different flower, the synthetic buzzes (each of 2 s total buzzing time, partitioned into

four 0.5 s buzzes) removed an average of 23,526±17,440 pollen grains or 8.3±8.5% of the total pollen in the flower (<1% to 56% of pollen per flower).

Model selection supported the additive model including both excitation RMS acceleration and anther response RMS acceleration (AIC weight=0.67; Table 1). Although these explanatory variables are moderately correlated (Pearson’s *ρ*=0.323), both were significant when included in the same model, indicating that each explains unique variation in pollen release. See Fig. 4 for an example comparing excitation and anther response vibrations. Adding a two-way interaction between excitation and response RMS acceleration did not significantly improve model fit. Harmonic treatment had no effect on pollen release either as a main effect or through interactions with RMS acceleration (Tables 1 and 2).

In the selected model, both excitation and anther RMS acceleration had a positive effect on pollen release (Table 2, Fig. 5). The effect of increased excitation RMS acceleration on pollen release can be illustrated using model predictions (*emmeans*) under different scenarios. At an average anther RMS acceleration and no harmonics, the proportion of released pollen at 65.48 m s^{−2} excitation RMS acceleration (low RMS treatment) would be 4.93%, while at 93.54 m s^{−2} (medium RMS treatment), the proportion of released pollen would be 6.39% (a 30% relative increase). The model predicts that further increasing excitation RMS acceleration to 121.6 m s^{−2} (high RMS treatment) would result in 8.25% of the available pollen being released, a 67% increase relative to the pollen released at medium RMS. A similar comparison of the effect of increasing anther RMS acceleration (at average excitation RMS and no harmonics) would result in pollen release of 5.25%, 6.11% and 7.50% for values of anther RMS acceleration corresponding to the 25% (116.83 m s^{−2}), 50% (243.52 m s^{−2}) and 75% quantiles (418.41 m s^{−2}) of the values observed in the data. Overall, increasing RMS acceleration resulted in more pollen released, with excitation RMS acceleration producing stronger changes in pollen release than anther RMS acceleration of a distal anther.

The FRF and coherence analysis of the shaker–wand system (no flower attached) showed that at the tested frequency of 300 Hz, the

Table 2. Effect of vibration properties on pollen release in buzz-pollinated *Solanum pyracanthos* flowers

Predictor	Estimate	s.e.	z	P (coeff)	χ ² (LRT)	d.f.	P (LRT)
Fixed effects							
Excitation RMS	0.00981	0.00345	2.84	0.004	7.75	1	0.005
Anther RMS	0.00126	0.00036	3.51	<0.001	10.24	1	0.001
Harmonics	—	—	—	—	3.26	2	0.196
Other coefficients							
Intercept	−3.971	0.370	−10.74	<0.001	—	—	—
Harmonic (3 h)	−0.0117	0.189	−0.06	0.951	—	—	—
Harmonic (5 h)	0.291	0.185	1.57	0.116	—	—	—

The excitation RMS variable represents the root mean square (RMS) acceleration (m s^{−2}) of the shaker applying vibrations to the focal anther. The anther RMS variable represents the RMS acceleration measured at the tip of a different (distal) anther in the flower. The harmonics variable represents the three applied harmonic treatments (1 h, 3 h and 5 h, corresponding to a 300 Hz signal plus 0, 2 or 4 additional harmonics, respectively). The model was fitted using a beta-binomial generalised linear mixed effects model with a logit link. Plant identity was included as a random effect. LRT, likelihood-ratio test. Bold indicates significance.

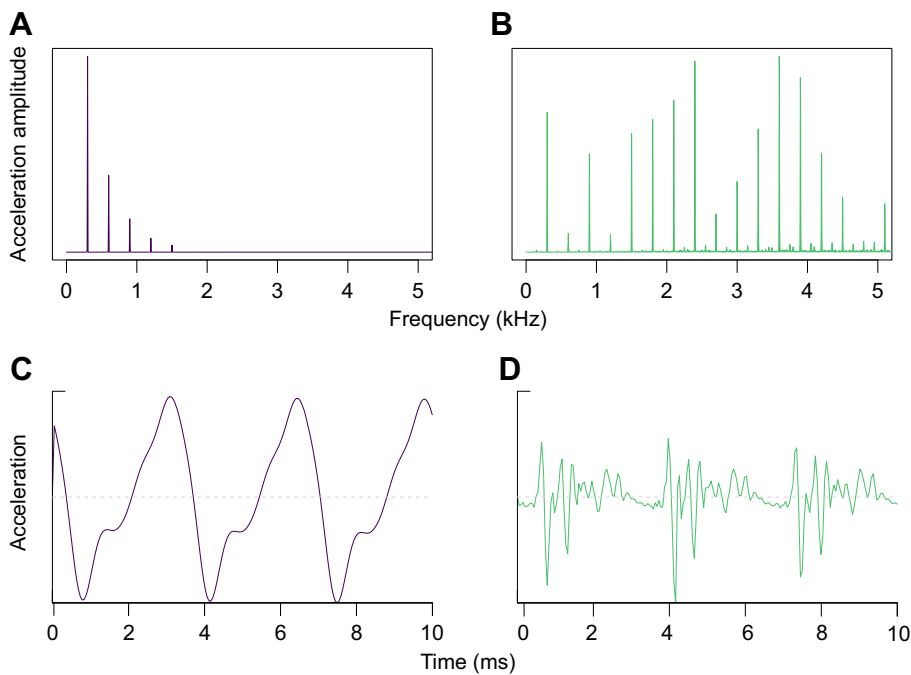


Fig. 4. Example of an experimental vibration (H177) containing one fundamental frequency at 300 Hz and four harmonics (5 h signal) applied to a *Solanum pyracanthos* flower and measured either at the shaker or at the tip of a distal anther. (A,C) Frequency spectrum (A) and time domain (C) of the excitation signal measured with an accelerometer on the shaker. (B,D) Frequency spectrum (B) and time domain (D) of the response signal measured with a laser vibrometer aimed at the tip of a distal anther opposite to the anther being vibrated by the shaker (Fig. 3). Amplitude is shown on a unitless relative scale. Notice that the number and amplitude of harmonic peaks in the response signal at the anther tip do not mirror the excitation spectral properties.

RMS acceleration measured at the shaker base accurately reflects the RMS acceleration at the tip of the wand (Fig. S2). Outside this frequency, we detected both amplification and attenuation of the excitation RMS in the shaker–wand system at certain frequencies (e.g. 1500 Hz; Fig. S2), but the FRF coefficients were relatively small (<1.2). For the FRF of the shaker–flower system, we analysed 16 flowers belonging to 10 plants (four from accession Y5SSSOPY, and six from accession 1179F). The average excitation RMS for these chirps was $110.4 \pm 11.9 \text{ m s}^{-2}$. The shaker–flower FRF showed amplification of the anther acceleration below 300 Hz and at higher

frequencies, e.g. 1500–2000 Hz (Fig. 6). However, the coherence decayed rapidly above 250–300 Hz (Fig. 6), suggesting that only vibrations applied at low frequency can linearly predict the acceleration experienced at the anther tip.

DISCUSSION

Here, we have shown that the presence of harmonics in simulated pollination buzzes has no measurable effect on pollen release. Our experimental results support the hypothesis that pollen release in buzz-pollinated species is correlated mostly with the averaged RMS

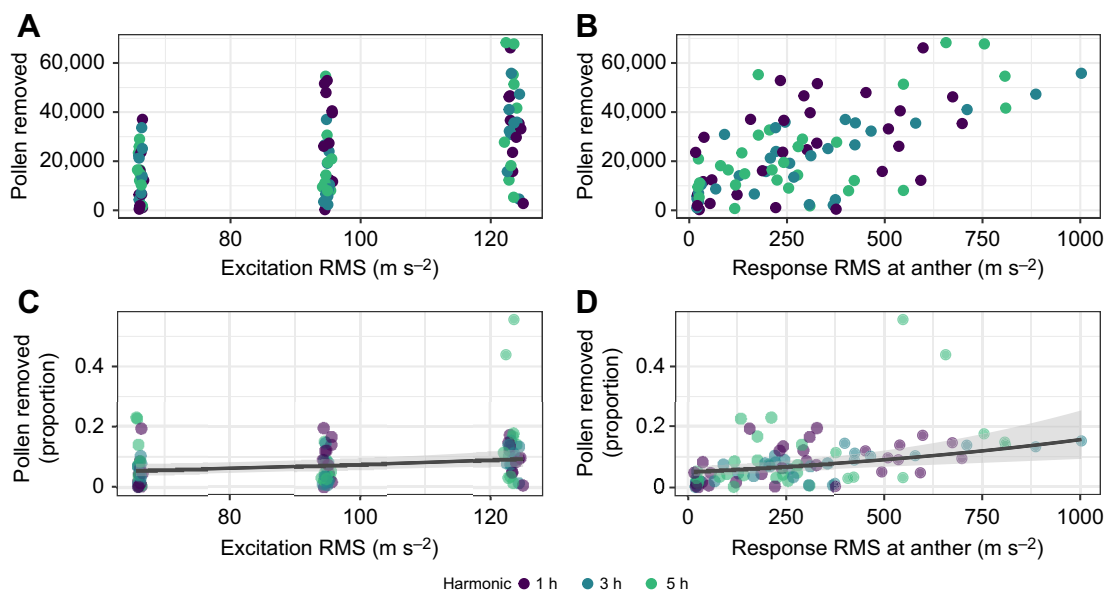


Fig. 5. Pollen release as a function of both the excitation root mean square (RMS) acceleration input by the shaker (left) and the response RMS acceleration measured at the tip of a distal anther (right). (A,B) The response in terms of pollen number. (C,D) The response as a proportion of pollen removed (relative to the observed total number of pollen grains in the flower). The total number of pollen grains in the flower was obtained by summing the number of grains removed and the number of grains remaining in the flower. The lines in C and D represent the model prediction of the predicted proportion of pollen released, and the shaded region represents the 95% confidence interval obtained from the beta-binomial model shown in Table 2. Sample size=90 flowers.

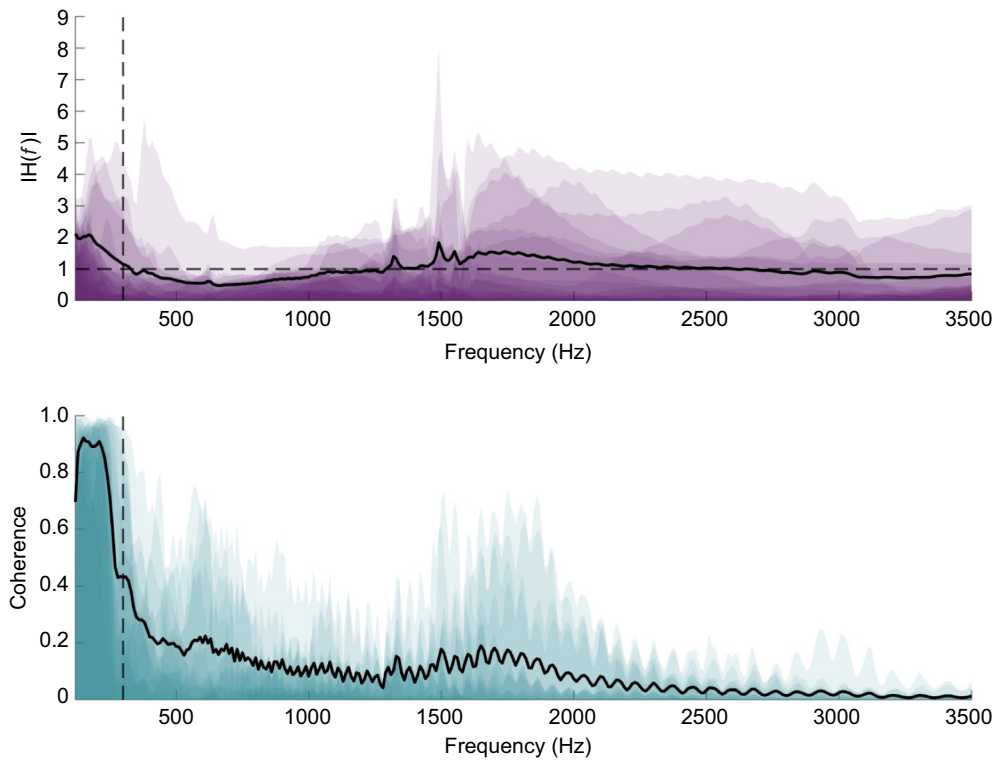


Fig. 6. Frequency–response function (FRF; top) and coherence (bottom) in *S. pyracanthos* flowers exposed to a 6 s, 120–4500 Hz logarithmic chirp.

The response was measured at the anther tip of a distal anther not directly attached to the shaker (excitation signal). The solid line shows the average response across all flowers. The vertical dashed lines indicate 300 Hz. The horizontal dashed line in the FRF panel (top) shows equal excitation and response. $|H(f)|$, is the absolute value of the transfer function. Sample size=59 chirps, 16 flowers.

acceleration (a measure of average power) of the vibration delivered to the flower, rather than by its spectral properties. Empirical studies have shown that amplitude acceleration is positively correlated with pollen released across different frequencies and *Solanum* species (De Luca et al., 2013; Rosi-Denadai et al., 2020). In theoretical models of pollen release (Boucher-Bergstedt et al., 2025; Buchmann and Hurley, 1978), vibration amplitude is thought to be correlated with pollen release by means of the increased velocity, acceleration and jerk (changes in acceleration) imparted to the anther. Acceleration might be a good predictor of pollen release because it directly relates to the force imparted to the pollen grains inside the anther. For the pollen grains to be mobilised in the anther and eventually ejected through the anther pore, it might be necessary to apply sufficient force to break adhesive forces causing the pollen grains to stick to each other and to the anther walls (King and Lengoc, 1993). Even when displacement is relatively small, vibrations at relatively high frequencies can achieve substantial acceleration amplitude (Vallejo-Marin, 2019) as, in sinusoidal motion, peak acceleration scales with the square of frequency. Thus, although frequency has been found to influence pollen release in other empirical studies (e.g. Harder and Barclay, 1994), this effect might come mostly from the effect of increasing frequency on acceleration amplitude, rather than from frequency effects per se.

So, if harmonic structure has no detectable effect on pollen release, why do bee buzzes contain multiple frequency components in the form of harmonics (Arvelos et al., 2025; Gonzalez-Almansa Laredo et al., 2026; King and Buchmann, 2003)? Harmonics naturally arise because the oscillatory movement of the bee's thorax does not generate a perfect sine wave. A consistent distortion of the oscillation of the bee's thorax will decompose, through the Fourier series, into a fundamental frequency plus integer multiples (French, 1971). If the distorted sinusoid wave remains locally stable (such as in short pollination buzzes), the result will be a harmonic series. Therefore, we would expect that pollination buzzes and other

buzzes produced in behaviours involving the rapid contraction of the bee's thorax should generally contain harmonics even if they do not have a specific functional advantage in terms of pollen release.

Transmission pathways for pollination buzzes

Our study also highlights that averaged RMS acceleration contributes to pollen release through two partially independent pathways: direct and indirect.

Direct pathway

The first pathway is through the excitation RMS acceleration applied at the point of contact with the flower. It captures the input vibrations that a bee would apply through direct contact of its mandibles or body with a focal anther or anthers. While the bee holds the anther during the application of vibrations (for example, through biting with its mandibles), this pathway can be thought of as a forced oscillator where the flower's oscillations are directly driven by the bee. The applied vibrations cause the anther or anthers that are in contact with the bee to shake, potentially putting in motion the pollen grains inside the anther and causing them to be expelled through the anther pores. If the coupling between bee and flower is tight, the motion of the vibrated anther at the point of contact should track closely the motion of the oscillation of the bee's body (e.g. its thorax or the mandibles). This pathway of transmission is probably mostly influenced by the bee's coupling with the flower (e.g. how tightly the bee holds the anther while buzzing) and by local properties of the anther being vibrated, such as the anther's length and stiffness.

Indirect pathway

The second pathway occurs through the vibrations transmitted to anthers that are not being directly touched by the bee. Our analyses indicate that the RMS acceleration experienced by other anthers different from the one being vibrated contributes independently to

pollen release. This second pathway reflects in part how the applied vibrations are transmitted across the flower and should be influenced by both bee–flower coupling and the characteristics of the flower’s material properties and architecture.

Our experimental results show that pollen release during simulated bee buzzes is explained better while considering the independent contributions of both direct and indirect pathways for the transmission of vibrations from the bee to the pollen-carrying anthers. Given the complexity in the geometry and material properties of the flowers and the multiple interacting parts, as well as the variation in how a bee holds a flower and potentially contacts multiple structures in the flower (King and Buchmann, 2003), it might be difficult to predict or model *a priori* how bee vibrations are transmitted across the flower across these two pathways. Previous biophysical models of the vibrational properties of buzz-pollinated flowers have focused on individual anthers or stamens (Jankauski et al., 2022; King and Lengoc, 1993), and only rarely have entire flowers being considered (Xu et al., 2025). Our second experiment approached this issue through a comparison of the transmission of vibrations across the flower over a range of frequencies.

The flower as a mechanical filter

Analysis of the transfer function of vibrations via the indirect pathway across a range of frequencies suggests that buzz-pollinated flowers behave as mechanical filters. At low frequencies (<250–300 Hz for *S. pyracanthos*), excitation vibrations linearly predict the motion at the anther tip of distal anthers (high coherence). In contrast, vibrations at higher frequencies are associated with low coherence. The pattern of transmission of vibrations thus depends on the frequency at which they are vibrated. Plant tissues generally behave as mechanical filters that differentially transmit vibrations depending on their frequency.

In insects that communicate using substrate-borne sounds (often using plants as the substrate; Cocroft and Rodríguez, 2005), substrate properties can differentially affect signals depending on their frequency. The properties of the plant thus impose a frequency filter (Michelsen et al., 1982), and insect’s substrate-borne signals are usually composed of lower frequencies and pure tones (Cocroft and Rodríguez, 2005). In buzz-pollinated flowers, the vibrations applied by the bee act on pollen grains through the substrate. Our results suggest that a bee producing lower frequency vibrations might be able to accelerate the tip of the anther in a linearly predictable manner, while vibrations at higher frequencies may result in unpredictable changes in anther tip acceleration. Mechanistically, the low coherence at the anther tip at high frequencies probably arises from the activation of higher modes of vibration as well as from stochastic interactions between the flower parts (for example, as different anthers come into contact as the flower shakes).

At higher frequencies, the variable vibration properties of a complex, highly articulated flower composed of multiple parts might reduce the predictability of its natural frequencies. Because of within-species variation in flower architecture (Cresswell, 1998; Herrera, 2009), as well as stochasticity in the interaction among flower parts and bee coupling during the buzz (Vallejo-Marin and Russell, 2024), exploiting natural frequencies to induce particular resonance modes might be difficult. It is tempting to speculate that bees might be able to better predict the outcome of their buzzes (in terms of anther tip motion and pollen release) when using lower frequency vibrations than when vibrating at higher fundamental frequencies or using higher harmonics. This would favour allocating more energy (or power) to the fundamental frequency of a bee’s buzz rather than spreading out the vibrational energy across a broad frequency band using harmonics.

Further work is needed to determine whether the low coherence at higher frequencies observed here with *S. pyracanthos* is a general feature of other buzz-pollinated plants, especially given the high level of morphological diversity seen in their flowers (Kopper et al., 2024). Similar empirical studies across other plant species with different floral morphologies such as in Melastomataceae, Orobanchaceae or Ericaceae, would be desirable. In addition, modelling approaches might help to determine the mechanism by which coherence changes across the frequency range. Recent work using finite element (FE) analysis has provided key insights into the motion of individual stamens; for example, by showing that geometry and mass loading affect the modes of vibration of 3D reconstructions of stamen geometry (Jankauski et al., 2022). Similar models have also been used to simulate the effect of vibration properties on the ejection of pollen grains using the entire flower geometry in *Solanum* (Shi et al., 2025) and *Pedicularis* (Orobanchaceae) (Xu et al., 2025). Buzz-pollinated species of *Pedicularis* have anthers enclosed inside a tube formed by the corolla. Xu et al. (2025) showed that in *Pedicularis*, the location at which the bee applies vibrations to a flower affects the motion of anthers inside the corolla tube (Xu et al., 2025). These FE analyses and other modelling approaches, including multibody mechanical models, have the potential to provide mechanistic predictions of how different types of vibrations affect anther vibrations and this is currently an area of active research.

Conclusions

Our results support the hypothesis that pollen release in buzz-pollinated flowers is mostly driven by vibration amplitude (RMS acceleration) and that under the conditions tested here, spectral composition has a negligible effect. Thus, the rich harmonic structure of floral bee buzzes does not increase pollen release. Instead, harmonics may arise as the incidental by-product of the mechanical properties of the bee’s exoskeleton during the production of vibrations. Our study also highlights how pollen release depends both on the vibration acceleration at the point of contact with the bee (direct pathway) and on how these vibrations are transmitted to distal anthers (indirect pathway). Flower architecture and tissue properties will determine how vibrations are transmitted via the indirect pathway. Selection on plants to regulate pollen release might thus favour traits that either increase or decrease vibration transmission through this indirect pathway. In addition, bees could modulate how vibrations are transmitted primarily via the direct pathway. For example, instead of grabbing and vibrating one anther only, a bee might first gather all anthers together and then buzz, effectively converting all vibrations into directly forced oscillations. Altogether, our findings suggest that the extent to which vibrations are converted into pollen release depends both on the mechanical properties of flowers and on how vibrations are applied by bees.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: M.V.-M., N.J.; Data curation: M.V.-M., A.M.; Formal analysis: M.V.-M.; Funding acquisition: M.V.-M., N.J.; Investigation: M.V.-M., A.M.; Methodology: M.V.-M.; Project administration: M.V.-M.; Software: M.V.-M.; Supervision: M.V.-M., N.J.; Visualization: M.V.-M.; Writing – original draft: M.V.-M.; Writing – review & editing: M.V.-M., A.M., N.J.

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Data and resource availability

Data are publicly available in Dryad (Vallejo-Marín et al., 2006): <https://doi.org/10.5061/dryad.wstqjq328>. All other relevant data and details of resources can be found within the article and its [supplementary information](#).

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