Studies for the development of pest management of tobacco whitefly Bemisia tabaci using substrate-borne vibrations on tomato, Solanum lycopersicum in facility horticulture

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Studies for the development of pest management of tobacco whitefly *Bemisia tabaci* using substrate-borne vibrations on tomato, *Solanum lycopersicum* in facility horticulture

Ryuhei YANAGISAWA

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Graduate School of Systems Life Sciences
Kyushu University
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Contents

Chapter1

Substrate-borne vibrations reduced the density of tobacco whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) infestations on tomato, *Solanum lycopersicum*: an experimental assessment

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Abstract

Managing pests with insecticides is probably the most conventional available control method. However, insecticide overuse often results in resistance and subsequent pest resurgence, and often adversely affects the ecosystem. The physical management of insect pests by utilizing substrate-borne vibrations, sounds, or both is increasingly attracting attention as an alternative, as it has modest ecosystem impacts. This method exploits vibroacoustic insect communication used for mating and the perception of approaching enemies, provoking behavioral responses in an ingenious manner. I aimed to examine whether substrate-borne vibrations effectively drive away tobacco whiteflies [*Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae)], which are serious agricultural pests. To do so, *B. tabaci* individuals were artificially introduced into greenhouses where tomato (*Solanum lycopersicum* L.) plants were reared. A substantial reduction in the average density of *B. tabaci* nymphs and adults was achieved by transmitting vibrational stimuli to the plants. At the same time, no obvious reduction was found in the number of tomato plant flowers. Although the performance of the vibrational device and transmission procedures requires further improvement, the present results shed light on the potential of substrate-borne vibrations as a promising alternative for pest management.

Introduction

The tobacco whitefly *Bemisia tabaci* Gennadius (Hemiptera:Aleyrodidae) is a significant global agricultural pest that causes serious damage to vegetable and ornamental crops: directly by consuming phloem tissue and indirectly by causing sooty molds due to honeydew secretion (Mound and Halsey 1978). Whiteflies act as a vector for disease through the transmission of plant pathogenic viruses such as those in the genera *Begomovirus*, *Crinivirus*, *Ipomovirus*, *Carlavirus*, and *Torradovirus* (Jones 2003; Navas-Castillo et al. 2011). In particular, *B. tabaci* serves as a vector for the tomato yellow leaf curl virus (TYLCV; *Begomovirus* in Geminiviridae). This virus has a wide host range and causes serious symptoms such as leaf curling and yellowing, which subsequently lead to yield reduction (Kil et al. 2014). Moreover, *B. tabaci* comprises more than 40 cryptic species or "biotypes" among which the most invasive are the Middle East-Asia Minor 1 (MEAM1 or B biotype) and Mediterranean (MED or Q biotype) (De Barro et al. 2011; Vyskočilová et al. 2018). In addition to the habit of infesting a wide range of plant species (Bradshaw et al. 2019), *B. tabaci* resistance against pesticides is a serious problem because it may allow the rapid spread of invasive biotypes that express strong resistance to a variety of insecticides (Luo et al. 2010; Wang et al. 2020). To overcome the problem of resistance, it is necessary to combine approaches to prevent the spread of potentially harmful *B. tabaci* biotypes (Horowitz et al. 2011; Riley and Srinivasan 2019).

Intra- and interspecific insect communication, mediated by mechano-receptive information such as substrate-borne vibrations, airborne sounds, or both, has recently attracted considerable attention for pest management (Mankin et al. 2013; Polajnar et al. 2015; Takanashi et al. 2019; Uechi and Takanashi 2021). Vibrations or sounds similar to those produced by insects can be used to provoke disturbances in communication between individuals and behaviors in various ways, leading to fitness reduction (Eriksson et al. 2012; Lee et al. 2012; Lujo et al. 2016; Takanashi et al. 2019).

Unlike chemical pesticides, the exploitation of vibrations or sounds does not release harmful compounds into the environment, although a lowered sensitivity to temporary successive stimuli, habituation, should be taken into account (Kishi and Takanashi 2019; Loxdale 2018; Rohde et al. 2019; Takanashi et al. 2019).

Here, for the first time, I aimed to test whether substrate-borne vibrations resulted in significant overall disturbance to the reproduction and settlement of *B. tabaci* on tomato leaves, using the observed number of individuals as an index. I additionally examined whether the reproduction of tomato plants was affected by vibrational stimuli, using the number of flowers as a fitness indicator.

Materials and methods

Bemisia tabaci cultures

Bemisia tabaci (B biotype) individuals were collected from green peppers (*Capsicum annuum* L.) grown in Yaese, Okinawa, Japan on June 2, 2017. Prior to the experiment, they were reared for six

generations on kidney beans (*Phaseolus vulgaris* L.) in an acrylic case [0.3 m (height, H) ×0.29 m (width, W) ×0.24 m (length, L)] at 25±2 °C with a photoperiod of 14:10 (L:D) h.

Experimental design

This study was conducted in a greenhouse [5 m (H)×9 m (W)×19 m (L)] at the experimental farm of the University of the Ryukyus, Okinawa, Japan. Two vinyl houses [2 m (H)×1.8 m (W)×8 m (L)] were constructed parallel to each other and 2 m apart in the greenhouse. The northernmost structure is referred to as 'house 1' and the other as 'house 2' (Fig. 1b). A steel rack [1.72 m (H)×0.96 m (W)×0.45 m (L)] was placed in the center of each house. A control driver (SMT-KN-DR-001; Shonan Metaltec, Kanagawa, Japan) of a vibrational exciter, made using giant magnetostrictive materials (L: 0.203 m, diameter: 46 mm; SIP-100/10-MB wp; Shonan Metaltec; Fig. 1a), was placed on the rack. The vibrational exciter was controlled by an outlet timer (534–02; Sogo Laboratory Glass Work, Kyoto, Japan).

To grow 12 tomato plants in each house, props (L: 2.1 m, diameter: 11 mm) were buried at depths of 0.3–0.4 m, 0.5 m apart (Fig. 1c). A tomato seedling (Momotaro®; Takii Seed, Kyoto, Japan) was planted in a pot that was filled with mixed soil (depth: 0.26 m, diameter: 0.26 m) on October 30, 2017. The stem of each plant was tied to a prop using a soft wire (#4907052726697; Takagi Co. Ltd., Niigata, Japan). In total, 24 seedlings that had been cultivated in liquid fertilizer (Ohtsuka House 1+2®, "A" prescription; OAT Agrio, Tokyo, Japan) were planted in the 2 houses. In the vibration treatment area, the vibrational exciter was fixed between props at a height of 1.5 m above the ground (Fig. 1c). Three pieces of plastic rod (L: 1 m, diameter: 11 mm) that transferred vibrational stimuli to the props were connected serially to the exciter and were attached to the props using supporting clips (props joint clip, 11×11 mm; Takagi Co. Ltd., Niigata, Japan; Fig. 1). I did not set the vibrational exciter or plastic rods in the non-vibration-treatment area (Fig. 1b).

The vibrational exciter was operated for 1 min (a cycle of 1 s pulse and 9 s pause) every 30 min between 7:00 and 18:00, under the control of the outlet timer described above. The output vibrational frequency was 100 Hz. The vibrational frequency was determined based on the findings of previous studies on other insects (Kishi and Takanashi 2019; Takanashi et al. 2016) that reported startle responses (see "Discussion" for further details) and the number of abdominal movements of *B. tabaci* counted in courtship behavior (Yanagisawa, unpublished data). The stimulation cycle and timing were configured to moderate habituation against continuous stimuli and were based on preliminary experiments and a previous study (Kishi and Takanashi 2019).

Vibrational intensity was measured using an accelerometer (Type 3052-A-030; Brüel & Kjær, Nærum, Denmark) connected to an input module (Type 4519-003; Brüel & Kjær), that was controlled by a laptop computer (CF-S9; Panasonic, Osaka, Japan) on December 26, 2017. The accelerometer was fixed to the target position using plastic tape. I measured vibrations at the

intersections of props and plastic rods, and on plant stems at a height of 0.4 m above the ground. Vibrations were recorded using the PULSE Data Time Recorder software (Type7708; Brüel & Kjær); zero-to peak values were used for acceleration measurements. Measurement of acceleration was conducted five times at each position on the same day. The output frequency (100 Hz) was detected in the harmonic series of the detected signals based on spectral analysis (fast Fourier transform: type Hamming, window size=512).

Field surveys

Two days before starting the experiment (on November 15, 2017), 30 adult (1- to 9-day-old) *B. tabaci* were released on each tomato plant. These individuals were not sexed before the release. Field surveys started on November 17, 2017, and ended on January 1, 2018, and were conducted every 5 days (ten surveys were conducted in total). Prior to conducting the surveys, I randomly selected three compound leaves from each tomato plant. Three leaflets were then randomly selected from the compound leaves, and the number of *B. tabaci* adults and nymphs on leaflets was counted by visual observation. The number of flowers was also recorded for each flower cluster in each tomato plant. Yellow sticky traps (New Insect Bang Bang®; Daikyo Giken Kogyo, Kanagawa, Japan) were suspended from plastic rods using polyethylene strings so that the traps were placed at almost equal intervals at a height of 1.5 m (Fig. 1b, c). Each trap was replaced every 5 days, and the number of *B. tabaci* adults trapped was recorded.

Statistical analysis

Prior to conducting my statistical analysis for insects and plants, I compared the acceleration between the measurement positions. I first used a generalized linear mixed model (GLMM) to examine how the acceleration decreased in proportion to the increase in the distance from the vibrational exciter. The intensity of the vibration varied depending on the height of the measurement position and the horizontal distance from the vibrational exciter, therefore, the effects of these variables were included as explanatory (fixed) variables. A Gaussian error distribution with an identity link function was applied to the error distribution. The order of repeated measurements and greenhouses (houses 1 and 2) were included as random effects.

I then examined whether the periodical vibrational stimuli generated by the vibrational exciter effectively decreased the population density of *B. tabaci* adults and nymphs on leaves. In the GLMM, the number of *B. tabaci* adults or nymphs was included as the response variable, with treatment type being included as an explanatory (fixed) effect. Plant location, nested within greenhouses, and the date of the survey were included in the models as random effects. In both cases, I first postulated Poisson errors, but detected greater overdispersion than expected based on the dispersion estimator $\hat{\phi} = D/(n-p)$ proposed by Wedderburn (1974), where D is the deviance of the

model, n is sample size, and p is the number of parameters for the model (adults: $\hat{\phi}$ = 2.046, nymphs: $\hat{\phi}$= 14.017). Therefore, I adopted the negative binomial GLMM with a log-link function for the above comparisons.

Additionally, I compared the average and total number of tomato plant flowers between the treatment and control groups. Because I detected overdispersion (average number: $\hat{\phi}$ = 1.877, total number: $\hat{\phi}$= 8.919), a negative binomial GLMM with a log-link was used. The average number of flowers was included as the response variable, with the treatment type included as an explanatory variable. Plant location, nested within greenhouses, and the date of the survey were included in the model as random effects. Similarly, the total number of flowers per tomato plant was included as the response variable, the treatment 13 type was included as an explanatory variable, and greenhouse location was included as a random effect.

Finally, I examined the number of *B. tabaci* adults caught on yellow sticky traps set in the houses (Fig. 1). A negative binomial GLMM with a log-link was adopted because overdispersion was detected ($\hat{\phi}$ = 6.477). The number of *B. tabaci* was included as the response variable, and the vibrational condition (with or without vibrations) of the installation site was included as an explanatory variable. Traps, greenhouses, and survey dates were included as random effects.

All statistical analyses were conducted using the R statistical software ver. 4.0.0 (R Core Team 2020).

Results

Significantly smaller values for vibrational accelerations were detected at the lower height (0.4 m) positions compared with higher (1.5 m) positions (GLMM: $\beta = 34.02$, $t = 16.56$, $p < 0.001$). As such, in the comparison, we examined how the acceleration decreased as the horizontal distance between the vibrational exciter and measurement positions increased (Table 1). At the higher positions, the acceleration tended to decrease as the distance increased (GLMM: $\beta = -0.16$, $t = -3.33$, $p=0.002$); however, such a relationship was not detected at the lower positions (GLMM: $\beta = 0.02$, $t = 1.00$, $p = 0.322$).

On average, the number of *B. tabaci* nymphs was significantly lower in the vibrational treatment than in the control treatment (GLMM: $\beta=-0.62$, $z = -2.271$, $p = 0.023$; Table 2; Fig. 2, 3). Similarly, the average number of *B. tabaci* adults on the tomato leaves was significantly lower in the vibrational treatment than in the control treatment (GLMM: $\beta = -0.33$, $z = -2.147$, $p = 0.032$; Table 2).

However, neither the average nor the total number of tomato plant flowers differed significantly between the control and treatment groups (GLMM: $\beta = 0.266$, $z = 0.551$, $p = 0.582$ and $\beta = 0.162$, $z = 0.357$, $p = 0.721$, respectively; Table 2).

The number of *B. tabaci* adults caught on the yellow sticky traps was compared between installation

sites (the area treated with vibrations versus the area without vibrations), but no significant differences were detected (GLMM: $\beta = 0.217$, $z = 1.038$, $p = 0.299$; Table 2).

Discussion

The present study revealed that the application of vibrations to tomato plants using a vibrational exciter reduced the overall number of *B. tabaci* on these plants. It is notable that approximately 40% fewer nymphs were observed on the plants that were subjected to vibrational treatment compared to the control. Additionally, I conducted a secondary experiment under different conditions and confirmed a significant decrease in the number of *B. tabaci* nymphs and adults on tomato leaves that were subjected to vibration (Yanagisawa et al. unpublished data). The significant reduction in the nymphal density of *B. tabaci* implies that the reproduction and settlement of *B. tabaci* on the plants is disturbed by vibrations.

Male and female whiteflies, including *B. tabaci*, communicate via substrate-borne vibrations (Kanmiya 2006). Notably, the male vibrational signals and the subsequent female responses vary, even among closely related species (Kanmiya 2011). During the courtship stage, males emit calling and courtship sounds consisting of a burst separated by long irregular intervals. Females subsequently respond to males by emitting sounds comprising short and simple bursts (Kanmiya 2006). The temporal and spectral domain characteristics of male sounds differ even among closely related species, and females do not respond to the sounds emitted by males of different species (Kanmiya 2011). This results in strict premating reproductive isolation (Perring et al. 1993). Based on these facts, male vibrational signals should be critical for species discrimination by females, and for the decisions by females to accept or refuse mating. It is therefore possible that the vibrational signals generated by the exciter interrupt the courtship sequences between the sexes, consequently hindering mating opportunities as a disturbance effect. Substrate-borne vibrations could also cause "startle responses" in *B. tabaci*, such as fast jerky movements, freezing, and escaping (Bullock 1984; Friedel 1999). Startle responses are considered to have evolved for evasion from predation, and can be found in various insect species (e.g., Kishi and Takanashi 2019; Takanashi et al. 2016; Tsubaki et al. 2014; Uechi and Takanashi 2021). Marked reduction in nymphs might be due to not only a disturbance effect, but also due to startle responses induced by the signals irrelevant to courtship sounds. Future studies need to be carried out to carefully address how the transmitted vibrational signals manipulate behavior in *B. tabaci*.

In the adult stage, vibrations reduced the number of individuals observed by 26%, which was much smaller than the percentage in the nymphal stage. Moreover, the number of trapped *B. tabaci* did not differ between the vibration-treatment and non-vibration-treatment areas. Unlike the apterous nymphal stages, *B. tabaci* adults can fly for long distances and colonize various plant species (Bar et al. 2019). I did not place any shields between the experimental areas, which allowed *B. tabaci* adults

to move freely between them, and some of the emerged *B. tabaci* migrated due to the vibrational stimuli. It is therefore likely that an influx of *B. tabaci* adults emerged in the non-vibration-treatment area simultaneously. Analogous to the 'push–pull' approach to managing pests (Miller and Cowles 1990), vibrations are expected to 'push' *B. tabaci* adults out of the infested plants by exploiting their startle response. To enhance the effect of vibrations, future studies should develop a method to 'pull' escaping and migrating *B. tabaci* adults by utilizing traps attractive to this species.

Together with the assessment of pest management efficacy, I also investigated the impact of substrate-borne vibrations on plants. However, I did not find an overall difference in the blooming of tomato plant flowers between the vibration- and non-vibration-treatment areas. This might indicate that physiological conditions remained almost the same irrespective of the presence or absence of vibrational stimuli. However, attention should be paid to the indirect effect of *B. tabaci*, which is likely to negatively affect plant reproduction as well as the direct effect of vibration. The positive and negative effects exerted by vibration on the plants should be carefully evaluated under more targeted experimental designs in the future.

My results indicate that the vibrational stimuli decreased with increasing distance from the exciter. The maximum and minimum average accelerations were 76.75 m/s^2 and 6.96 m/s^2, respectively, suggesting more than a 10-fold difference between them (Table 1). Such a spatially heterogeneous vibrational transmission might drive whiteflies into areas in a greenhouse with lower accelerations, resulting in an unintended concentration of crop damage. As the size of farming fields managed by the use of vibrations increases, more exciters are required, which consequently increases costs. To optimize the efficiency of exciters, it is necessary to use assembling materials that are suitable for transmitting vibrations while suppressing vibrational attenuation and increasing the accelerations from the exciter.

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Table 1

Averaged acceleration at measuring positions.

Horizontal distance (m) from vibration actuator	Mean ± SD (m/s²)	
	1.5m High from the ground	0.4m high from the ground
House 1		
0.5	67.1 ± 0.7	11.9 ± 0.2
1	48.5 ± 0.3	21.2 ± 0.6
1.5	48.6 ± 0.8	7.0 ± 0.4
2	73.2 ± 0.5	18.2 ± 0.8
2.5	21.3 ± 0.2	13.2 ± 1.5
3	46.6 ± 0.3	16.8 ± 0.8
House 2		
0.5	76.8 ± 0.3	20.2 ± 0.3
1	53.9 ± 1.0	23.4 ± 1.0
1.5	38.8 ± 0.2	13.3 ± 0.2
2	29.4 ± 0.4	7.2 ± 0.3
2.5	57.7 ± 0.2	19.8 ± 0.3
3	53.8 ± 0.3	28.3 ± 0.1

Measurements were repeated five times at each position.

Table 2

Numbers of larval and adult whiteflies and flowers on plants.

Variable	Mean ± SD	
	With excitation	Without excitation
Insects		
Nymphs (/observation/plant)	5.9 ± 11.5	10.5 ± 16.9
Adults (/observation/plant)	0.5 ± 0.9	0.6 ± 1.0
Adults trapped (/trap)	8.9 ± 6.2	7.6 ± 6.8
Plants		
Flowers (/observation/plant)	0.6 ± 0.9	0.5 ± 0.9
Accumulated number of flowers (/plant)	5.6 ± 4.9	4.8 ± 5.5

Figures

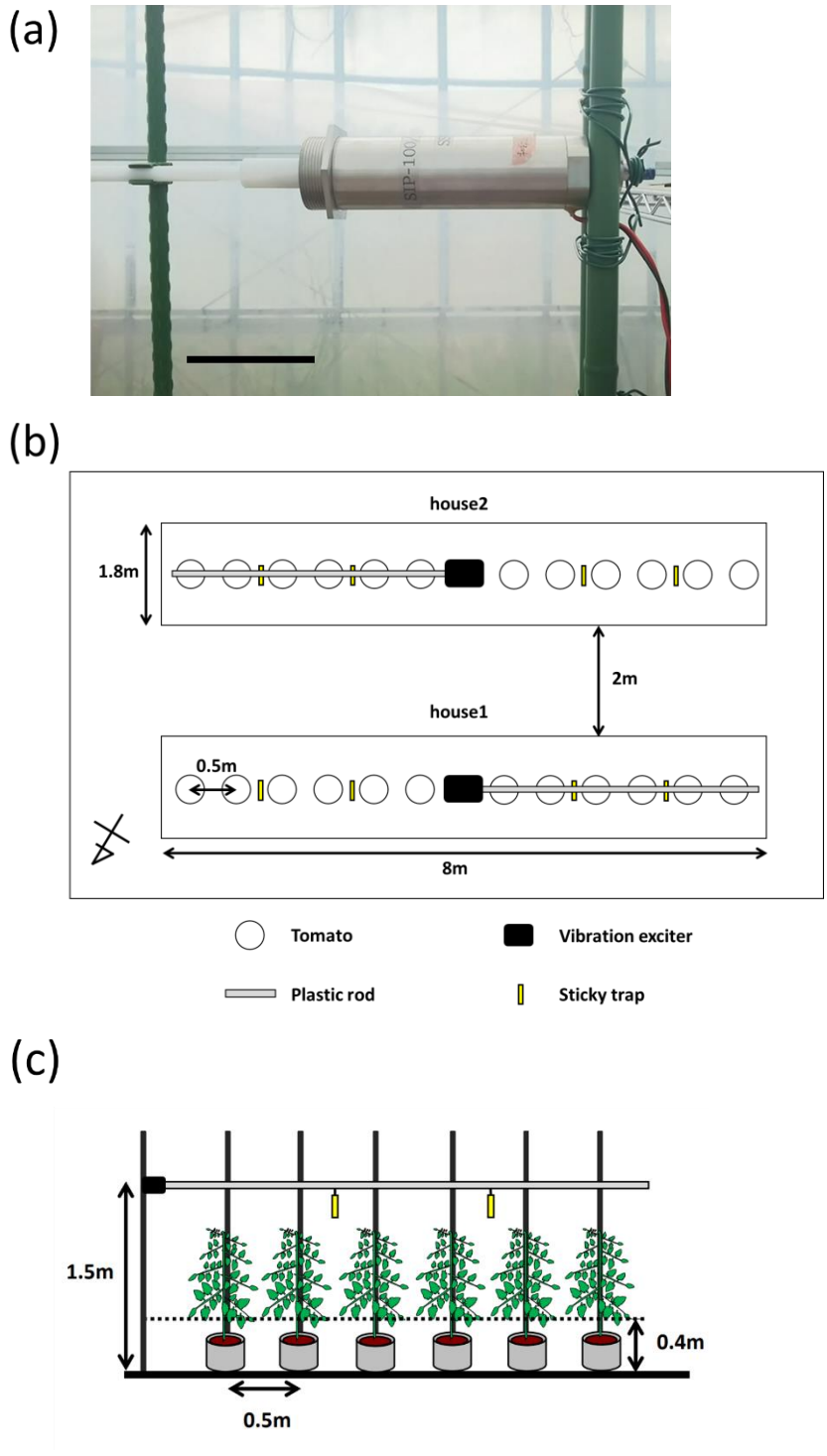

Fig. 1

Schematic diagram of tomato plant positions and associated equipment for providing vibrations: (a) vibrational exciter (scale bar: 0.10 m), (b) horizontal view of experimental setting, (c) vertical view.

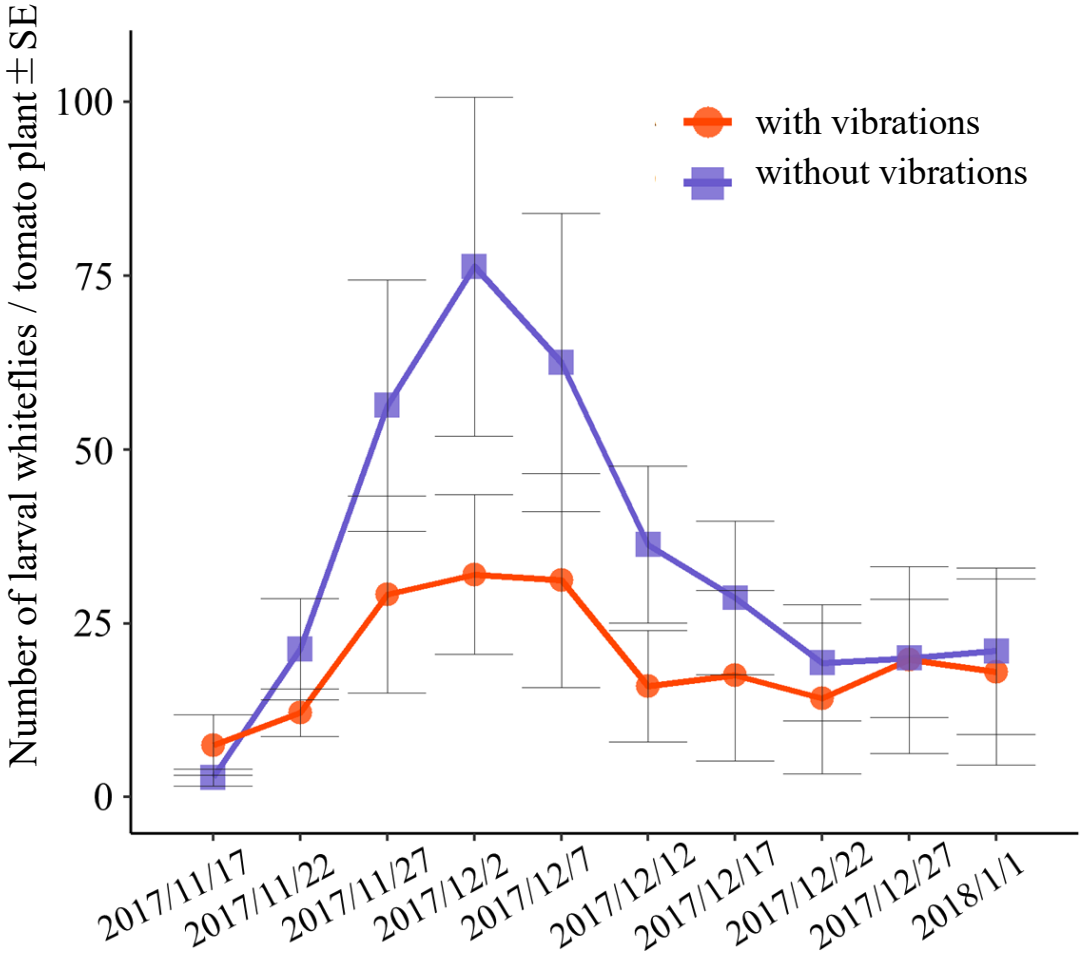

Fig. 2

Temporal change in the number of nymphal *Bemisia tabaci* per tomato plant in house 1. Error bar indicates the standard error.

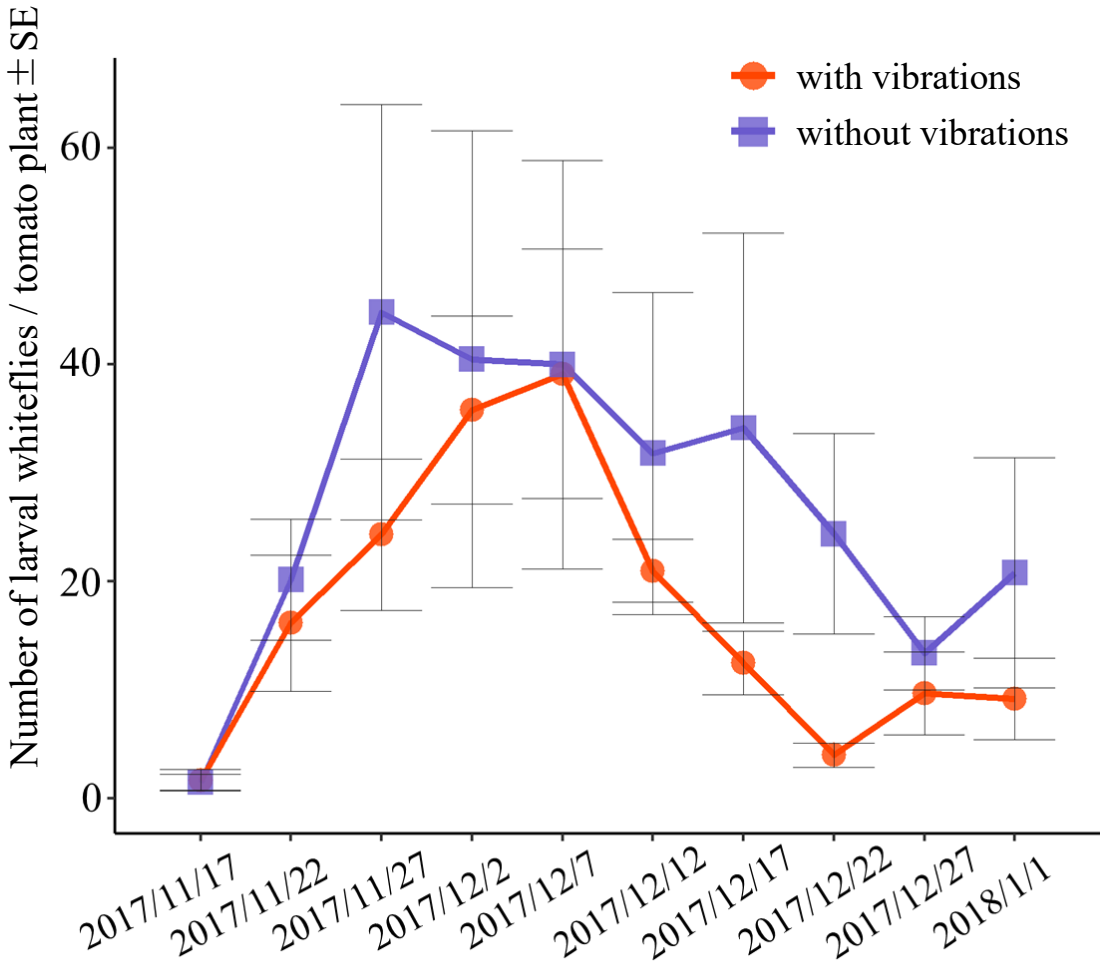

Fig. 3

Temporal change in the number of nymphal *Bemisia tabaci* per tomato plant in house 2. Error bar indicates the standard error.

Chapter2

Dual effect of substrate-borne vibrations on the pest density and fruit production in tomato plants

Abstract

Substrate-borne vibration is a significant physical stimulus that determines insect behavior, induces various behaviors, and is used as a means of conspecific or interspecific communication. Vibrations have the potential to be used for pest control. The previous study revealed that the vibration of tomatoes infested with *Bemisia tabaci* indeed reduced the density of *B. tabaci*. Here, I installed partitions to inhibit the movement of *B. tabaci* between the treatments and examined the effect of substrate-borne vibration on whiteflies. The number of *B. tabaci* adults and nymphs counted in the vibration treatment was significantly about 40% lower than those in the non-vibration treatment, suggesting inhibition of reproduction and settlement on the plant by providing vibrations. I also examined whether vibrations modify plant height, the mean number of blooming flowers and fruit sets, yield, size, and fruit quality (sugar content, acidity, and sensory test) of tomatoes. No significant differences were found in the amount of height growth and the number of blooming flowers and fruit sets plants between the treatments. The number of fruits harvested in the vibration treatment increased by approximately 30% compared to the non-vibration treatment, suggesting that vibration might have promoted pollination, reduction of pest damage, and enhanced physiological activity by improving plant health. The quality of fruits including taste was maintained in the treatment. The direct and indirect mechanisms that cause the reduction of *B. tabaci* density need to be clarified in the future.

Introduction

Substrate-borne vibration is one of the cues important for determining the behavior of many insects and is used for escape from predators, foraging, and communication between conspecifics (Cocroft and Rodríguez 2005; Virant-Doberlet et al. 2019; Takanashi et al. 2019). Prey exhibits anti-predator behaviors such as startle response, death-feigning, freezing, and dropping behavior by perceiving vibrations generated by the predator. (Bullock 1984; Friedel 1999; Miyatake et al. 2004; Gish et al. 2012; Takanashi et al. 2016). In the beetle *Trypoxylus dichotoma* L. (Coleoptera: Scarabaeidae), the pupae emit vibratory signals by rotating their abdomen through internode elongation and by beating their pronotum against the wall of the pupal cell, inducing larvae freezing to prevent the destruction of the pupal cell. The freeze response of the larvae to the vibrations generated by the pupae is regarded as an avoidance behavior entailed by vibrations from predators such as moles (Kojima et al. 2012). Thus, vibrational signals can also play a role in the social signaling of populations (Hartbauer 2010; Kojima et al. 2012). In addition, some species of Hemiptera, are known to use substrate-borne vibrations in their mating behavior between sexes (e.g. Ichikawa and Ishii 1974; Čokl and Virant-Doberlet 2003; Wenninger et al. 2009). Therefore, in many insects, substrate-borne vibration plays various roles and is closely related to innate behavior.

There are many studies available that attempt to use behaviors related to vibration for pest control. Representative studies are the mating disruption caused by artificial substrate vibration in pests whose mating behavior is mediated by vibrational signals (e.g. Eriksson et al. 2012; Lujo et al. 2016; Mazzoni et al. 2017; Krugner and Gordon 2018). These studies aimed to reduce male-female encounters and mating and to reduce reproduction by either inducing mating disturbance by artificial signals that temporally and frequency mask pest species-specific mating signals or by attracting males to a fake vibration source by attractive artificial signals that mimic the female's response signals. Alternatively, pest behaviors could be manipulated by inducing their behaviors in response to incidental vibrations (Polajnar et al. 2015; Takanashi et al. 2019). The pine pest *Monochamus alternatus* (Hope) (Coleoptera: Cerambycidae), exhibited freeze and startle response at vibrations below 1 kHz, and preliminary experiments have shown that low-frequency vibration inhibits feeding and other behaviors (Takanashi et al. 2019). Furthermore, nonspecific vibrations caused aphids, which are not known to vibrational communicators, to leave the plant (Parent et al. 2022). Chronic vibration stress can also cause physiological disturbances apart from behaviorally relevant stimuli (Polajnar et al. 2015). For example, when *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) larvae were vibrated at 100 Hz for 3 days at a rated power of 0.5 W, the levels of neuroactive biogenic amines altered, the developmental period of the larvae was delayed, and their body weight decreased. In addition, all larvae died in the tests at the rated power of 8 and 10 W (Hirashima et al. 1993). Thus, vibration has enough potential to be used as a tool for pest control.

The tobacco whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is a significant

tropical and sub-tropical agricultural pest, a species complex composed of genetic groups or biotypes with different host preferences, geographic ranges, and genetic backgrounds (Byrne and Bellows 1991; Boykin et al. 2007; De Barro et al. 2011). *B. tabaci* feeding causes physiological disorders such as the silvering of squash and irregular ripening of tomatoes (Maynard and Cantliffe 1989), and the honeydew produced by whiteflies results in sooty mold growth and suppress photosynthetic potential (Perkins 1983). In addition, *B. tabaci* vectors more than 200 species of plant pathogenic viruses such as *Begomovirus* (Geminiviridae), *Crinivirus* (Closteroviridae), *Ipomovirus* (Potyviridae), *Carlavirus* (Betaflexiviridae), *Torradovirus* (Secoviridae) (Polston et al. 2014), in especially, tomato yellow leaf curl virus (TYLCV) significantly reduces the yield of tomatoes (Picó et al. 1996). Two cryptic species, Middle East-Asia Minor 1 lineage (MEAM1, formerly biotype B) and Mediterranean lineage (MED, formerly biotype Q) have strong insecticide resistance and are replacing native populations (Liu 2007; Wang et al. 2010; Chen et al. 2016; Hopkinson et al. 2020; Park et al. 2021). To prevent the development of insecticide resistance, it is necessary to combine several effective approaches to control the spread of harmful *B. tabaci* cryptic species (Horowitz et al. 2011; Riley and Srinivasan 2019).

In a previous study, in greenhouse plots of tomatoes infested with *B. tabaci*, tomato plants *Solanum lycopersicum* L. were vibrated intermittently at 100 Hz and the number of *B. tabaci* on the leaves was found to be lower than that in the non-vibrated treatments (Yanagisawa et al. 2021). However, since there was no partition between the vibratory and non-vibratory treatments that could restrict the movement of *B. tabaci* in the study, the adults were able to fly freely between the two treatments. Vibrations may have overestimated the effectiveness of vibration control by causing adult whiteflies in the vibration area to temporarily move into the control area. Therefore, the first objective was the assessment of the effect of substrate-borne vibration on *B. tabaci* settlement and reproduction in a greenhouse with a partition between treatments with and without vibration, which inhibit the movement of the pest.

Plant responses to vibration are varied, including root elongation (Takahashi et al. 1991), increased germination rate (Uchida et al. 2002), increased biomass allocation to the root system, decreased dry weight of reproductive structures at maturity, delayed blooming and fruiting, and accelerated senescence (Niklas 1998). Control methods that apply substrate vibration to plants might affect the physiology of the plants and consequently the yield (Polajnar et al. 2015). Although determining whether the effects of the plants that appear when exposed to vibrations are negative or positive for the plant is difficult, it is important in agricultural production to evaluate the effects on the final fruit yield and quality. Sekine et al. (2022) found that the height and number of fruiting tomato plants exposed to 300 Hz vibration differed from those of the no vibrated plants, but the number of fruiting tomatoes vibrated at 30 Hz was significantly higher than that of the untreated plants, which had the effect of promoting pollination. In the previous study, no difference was found in the

accumulated number of tomato flowers, but plant height, fruit set, and yield were not investigated (Yanagisawa et al. 2021). Thus, I next aimed to test the effects of vibration on plant height, number of fruit sets, and yield.

In the taste and flavor of tomato fruits, Sugar content, acidity, and their ratio are important parameters (Stevens et al. 1977; Bucheli et al. 1999; Baldwin et al. 1991). The vibration of tomato fruit has been reported to reduce sugar content (Pathare and Al-Dairi 2021), titratable acidity (Al-Dairi et al. 2021), and degraded eating quality (Nakamura et al. 1977), and these studies have examined the effects of vibration during transportation. Therefore, finally, I aimed to examine whether substrate-borne vibration affects the quality of tomato fruits in terms of sugar content and acidity, and taste.

Material and methods

B. tabaci and tomato plant cultivation

Bemisia tabaci (MEAM1) individuals were collected from green peppers (*Capsicum annuum* L.) grown in Yaese, Okinawa Japan, in November 2008, and transferred from Okinawa Prefectural Agricultural Research Center, Itoman, Okinawa, Japan on June 2, 2017. They were reared for approximately 35–40 generations on kidney beans (*Phaseolus vulgaris* L.) in acrylic case (0.24-m long ×0.29-m wide×3.0-m high) at 25±2 °C with a photoperiod of 14:10 (L:D) h before the experiment.

The tomato cultivar was “Momotarou Hope” (Takii Seed, Kyoto, Japan) for the experiment. Tomato seeds were sown in cell trays on October 3, 2019, and were replanted in polyethylene pots (12-cm daimeter×10-cm depth). They were replanted in pots (26-cm diameter×26-cm depth) with soil mixture [mixing ratio 2:1:1 (Shimajiri mahji:Vermiculite:Peat moss)] between November 25 to 28 for the experiment. Liquid fertilizer (Ohtsuka House 1+2®, “A” prescription; OAT Agrio, Tokyo, Japan) was supplied in each pot. Since tomato russet mite *Aculops lycopersici* (Massee) (Arachnida: Eriophyidae) infestation occurred during cultivation, bifenazate (Danitarou, 1000 times diluted water; Sumitomo Chemical Garden Products Inc, Tokyo, Japan) was sprayed on February 4, 2020, due to control the mite.

Experimental design

All the experiment was conducted in an experimental field（26°25’37”N. 127°76’36”E.）at the Faculty of Agriculture, University of the Ryukyus. Two greenhouses (8.5-m long×1.8-m wide×2.7-m high) were constructed in parallel at 3 m interval. The southernmost one was designated as 'House A' and the other as 'House B' (Fig. 1a). Two metal pipes with a diameter of 19 mm (8-m long) were fixed at 2.4m high in each greenhouse in parallel at 60 cm interval (Fig. 1a). The two pipes were connected to a 1m metal pipe (19 mm diameter) at the center of the greenhouse. Vibrational exciter equipped with magnetostrictive Fe-Co clad steel (Prototype; Tohoku Steel, Miyagi, Japan) was set at the center of the 1m pipe (Fig. 1b). Four blocks were set in each greenhouse and were isolated using partitions

made of insect mesh (0.4 mm hole size) (Daio-sunshine-super-soft N-4700; Innovex, Tokyo, Japan) so as to interrupt the movement of *B. tabaci* between blocks (Fig. 1c). Note, however, that these partitions did not completely inhibit the movement of *B. tabaci* between blocks, due to the entry of adults during human movement and through gaps in the partitions. Six tomato pots were installed in each block at intervals of 0.6 m. Treatment (with vibrations) and control (without vibrations) were assigned alternately to the blocks.

Tomato plants in vibrational treatment blocks were supported using soft wire (2.5 mm diameter) (VSF 0.5 mm; Onamba, Osaka, Japan) wrapped around the plants and were suspended from a metal pipe above the plants. The lower end of the soft wire was fixed using a U-shaped bolt (23-mm diameter×40-mm heigh) attached on a metal pipe (diameter: 19 mm) set horizontally at approximately 35-cm-high (Fig. 1c, d). The soft wire and the metal pipe were connected by a tension clamp (mini-tension clamp parallel 19.1 mm diameter, Malsa, Niigata, Japan) (Fig. 1d). Vibrations were transmitted from the vibration exciter through the metal pipes and soft wires to each plant. The tension of the soft wire was always adjusted to be strong enough to prevent vibration damping. In control blocks, tomato plants were supported by the props (2.4-m long×16-mm diameter) buried 0.3–0.4 m under the ground.

Vibration setup and acceleration measurement

The vibrational exciter was operated for 1 min (a cycle of 1 s pulse and 9 s pause) every 10 min between 6:30 and 18:30 under the control of the outlet timer (534–02; Sogo Laboratory Glass Work, Kyoto, Japan). The output vibrational frequency was 100 Hz. The vibrational frequency and cycle were determined based on previous studies to avoid habituation to continuous stimulation (Kishi and Takanashi 2019; Yanagisawa et al. 2021; Sekine et al. 2022). The timing of applying stimuli was also determined based on the time that is considered to be necessary for disrupting mating in *B. tabaci* (MEAM1) (Zang and Liu 2007).

The vibrational signals were measured using an accelerometer (Type NP-2106; Ono Sokki, Kanagawa, Japan / Type VP-32; IMV, Osaka, Japan) connected to a charge converter (Type SA-A1; RION, Tokyo, Japan), and the signals were fed into data acquisition hardware (Type SA-A1; RION) on January 9, 2020. The accelerometers were fixed with paper tape to a stem approximately 0.3 m above the surface of the pot soil. Frequencies (Hz) and accelerations (m/s^2, zero-to-peak) were measured at the second, fifth, and sixth plants away from the vibrational exciter in each experimental block on the same day. The output signal (100 Hz) and its harmonics were confirmed in the detected signals through spectral analysis (fast Fourier transform: Hanning window, 1 kHz bandwidth, 1600 lines).

Field investigations

Once every 2 weeks from December 2, 2019, to January 6, 2020 (four times in total), eight adult *B.*

tabaci male and female pairs (1–9 days old since eclosion) were released on each plant. The field investigations were conducted once every week from December 4, 2019, to April 3, 2020.

The number of adults and nymphs on three randomly selected compound leaves were counted by visual observation. The height from the surface of the pot soil to tip of a plant (cm) (hereafter defined as "plant height") and the number of flowers and fruits were also recorded. Two pieces of yellow sticky traps (New Insect Bang Bang®; Daikyo Giken, Kogyo, Kanagawa, Japan) were suspended from both ends of a block at a roof pipe 2.7-m-high in each block so that the traps were placed at intervals of 0.5 m (Fig. 1c). All the traps were exchanged in every investigation. The collected traps were transferred to the laboratory, and the number of trapped adults *B.tabaci* was counted.

Tomato fruit harvest and measurements

Tomato fruits were harvested every one to three days when they began to ripen to red, and the fruit ID was recorded on paper masking tape (Scotch Brand Tape 2899, 3M, Minnesota, US) and attached to each fruit. The harvested fruits were transferred to the laboratory. After removing the masking tape, the weight of the fruit (g) was measured using a digital scale (SF-400C, JiangYin SuoFei Electronic Technology, Jiangsu, China), and the weight rounded to the second decimal place was recorded. The width from the stem side to the opposite side of the tomato fruit was defined as the short diameter, and its vertical width was defined as the long diameter. The maximum values of fruit short and long diameters (mm) were measured using a digital caliper (GTDC-100; ARCLANS, Niigata, Japan). The fruits were then exposed to room temperature (22±4 °C) until full maturity. Some of the fruits were frozen at -18 °C to prevent fruit spoilage over time.

At least 30 g of each fully ripe fruit (n=263) was made into juice using a hand blender (THM322; Tescom, Tokyo, Japan), placed in a 15 mL tube in a centrifuge tube respectively, and centrifuged (Thermo Scientific Sorvall ST 8R; Thermo Fisher Scientific, Massachusetts, USA) at 25°C for 2 min under a relative centrifugal acceleration of 19613.3 m/s^2. The 0.2 mL supernatant solution was dropped onto the sensor of a sugar and acid meter (PAL-BX|ACID1; ATAGO, Tokyo, Japan), and the total soluble solids content (Brix: %) of each fruit was measured. The acidity (total citric acid equivalent) was measured after x50 dilution of the solution with distilled water. The supernatant of each juice was placed in 1.5 mL microtubes and stored at -18°C for the analysis of monosaccharides. Some of the fruits (n=29) were cut into pieces for organoleptic tests, and approximately one-third of the fruit was used for Brix and acidity determinations.

Quantitative analysis of monosaccharides using High Performance Liquid Chromatography (HPLC)

Total soluble solids content (Brix) in tomato fruit is about half of reducing sugars, but they also contain

organic acids, amino acids, and minerals (Davies and Hobson 1981; Young et al. 1993; Baxter et al. 2005). Brix reflects the sugar content of the fruit to some extent, but the exact concentration of reducing sugars cannot be known. Hence, I quantified soluble monosaccharides in tomatoes using HPLC.

Microtubes (1.5mL) containing the supernatant of each juice were thawed at room temperature (approximately 25±3 ℃) and centrifuged at a relative centrifugal acceleration of 19613.3 m/s^2 for 2 min using a centrifuge (MYSPIN 6, Thermo Fisher Scientific; Massachusetts, USA). The supernatant solution of 0.3 mL was transferred to a 2.0 mL microtube and diluted 5-fold by adding 1.2 mL of distilled water (because the original solution could not analyze in the preliminary test). After the content was gently stirred by tapping, it was passed through a nylon syringe filter (0.22-μm diameter) and injected into a 2.0 mL vial. The HPLC system used LC-20AD (SHIMADZU, Kyoto, Japan). The column was SCR-101N (300-mm long×7.9-mm×diameter×10-μm thickness) (SHIMADZU, Kyoto, Japan) with a guard column (SCR(N) 228-09619-92; SHIMADZU, Kyoto, Japan). The oven temperature was maintained at 40 ℃ for 15 min with ultrapure water at a flow rate of 0.8 mL/min.

For quantitative analysis, aqueous mixtures containing fructose, glucose, and sucrose (mixing ratio: 1:1:1) were prepared at concentrations of 0.25 %, 0.50 %, 0.75 %, and 1.00 % (w/v %), and standard samples at each concentration were placed in 2.0 mL vials. These mixed standard samples were analyzed twice under the same conditions as described above, and calibration curves were constructed from the averages of the areas of the peaks of the chromatograms obtained. The peak area values of each monosaccharide were converted to its concentration from the calibration curves, and these values were converted into the original concentration of each monosaccharide in the fruit.

Sensory test of tomato fruit

To examine whether there is a difference in fruit taste between treatments with and without vibrations, a questionnaire investigation was conducted four times (March 9, March 17, April 2, and April 7, 2020) at the University of the Ryukyus campus. Two to four ripe fruits of similar weight (on average; March 9: 196.2 g, March 17: 179.8 g, April 2: 156.3 g, April 7: 150.3 g) were used per investigation of each treatment. Fruit pieces from each treatment were placed in each of the two food preservation containers. The containers were randomly assigned a number. The order of supplied containers was rearranged to avoid the effect of the order of the containers on the judgment. After having three pieces of fruit in each container, the subjects chose the quality on a five-point scale in terms of “sweetness”, “sourness”, “rich taste” and “preference”. The number of respondents was 18, 25, 38, and 45 in the first, second, third, and fourth surveys, respectively.

Statistical analysis

All statistical analyses were performed using the R 4.2.1 (R Core Team 2022; RStudio 2022). The generalized linear mixed models (GLMMs) were constructed using the *lme4* library (version 1.1-30) and the *glmmTMB* library (version 1.1.4), and the significance of explanatory (fixed) effect was evaluated using a likelihood ratio test (LRT) in the R 4.0.5 *car* library (version 3.1-0). The cumulative link mixed models (CLMM) were constructed using the *ordinal* library (version 2019.12-10). Model selection for each model was determined based on the Akaike information criterion (AIC) (Johnson and Omland 2004).

I used GLMMs with an identity link function in gaussian distribution error (LMM) to examine how the acceleration decreased in proportion to the increase in the horizontal distance from the source of the vibrations. The acceleration at each measurement point (zero to peak and 100 Hz root mean squares [RMS] analyzed by FFT) was the response variable, and the horizontal distance from each measurement point to the vibrational exciter was the fixed variable. block ID (eight levels), nested within greenhouse ID (two levels) was included in the model as random effects.

The effect of treatments with vibrations on the number of *B. tabaci* adults and nymphs on leaves was evaluated using GLMMs with a log-link function in Poisson distribution error (Poisson-GLMM). However, based on the dispersion estimator $\varphi = D/(n - p)$ proposed by Wedderburn (1974), where D represents the deviance of the model, n the sample size, and p the number of parameters for the model, I found that overdispersion was greater than the expected (φ was estimated by *RVAideMemoire* library (version 0.9-81-2), $\hat{\phi}$ = 3.051 [adult], $\hat{\phi}$ = 39.082 [nymph]). Therefore, I adopted the negative binomial error GLMM (NB-GLMM) with a log-link function for the above comparisons. In the NB-GLMMs, the number of *B. tabaci* adults and nymphs counted on each compound leaf was included as a response variable, with treatment (with or without vibration) included as a fixed effect. Plant ID (48 levels), nested within block ID and greenhouse ID, and investigation dates (18 levels) were included in the model as random effects. Furthermore, I examined whether the number of *B. tabaci* adults and nymphs in the vibrational treatment increased at a constant rate with increasing horizontal distance from the vibrational exciter using NB-GLMMs with a log-link function. In NB-GLMMs, the response variable was the number of *B. tabaci* adults and nymphs on each compound leaf in the vibrational treatment, and the horizontal distance from each plant to the vibrational exciter was included as an explanatory (fixed) variable. Plant ID, nested within block ID and greenhouse ID, and investigation dates were included in the model as random effects.

Additionally, I compared the number of *B. tabaci* adults caught on yellow sticky traps set in each treatment. An NB-GLMM with a log-link function was adopted because overdispersion was detected ($\hat{\phi}$ = 13.837). The number of *B. tabaci* adults caught on one side of the trap was included as the response variable, and the vibrational condition (with or without vibrations) of the installation site was included as a fixed effect. Trap location (16 levels), nested within block ID and greenhouse ID, and investigate dates were included as random effects.

I examined whether periodic vibration stimuli generated by the vibrational exciter had any effect on the growth and reproduction in plants. The difference between the plant height on the first investigation date and the plant height on each investigation date was defined as the "Cumulative height difference (CHD)". In addition, the ratio of the number of fruit sets per number of flowers was defined as the fruit set rate. I compared CHD, the number of blooming flowers, fruit sets, fruit set rate per plant, and each fruit set rate per flower cluster from the first to the fourth cluster in each treatment. The cumulative height difference was applied LMM. In the LMM, the ln-transformed CHD in each plant was a response variable, and the treatment type was included as a fixed effect. Plant ID, nested within block ID and greenhouse ID, and investigation dates (17 levels) were included in the model as random effects. Since many data had zero blooming flower and fruiting numbers, the hurdle Poisson GLMM (HP-GLMM) with a log-link function proposed by Cragg (1971) was adopted. In the HP-GLMMs, the number of blooming flowers and fruit sets on each plant were included as response variables, and the treatment type was included as a fixed effect. Plant ID, nested within block ID and greenhouse ID, and investigation dates (15 levels [blooming flower], 14 levels [fruit set]) were included in the models as random effects. The fruit set rate per plant and each fruit set rate per flower cluster was applied GLMM in Binomial distribution error (Binomial-GLMM) with a logit-link function. In the Binomial-GLMM of the fruit set rate per plant, the number of flowers and fruits each plant was included as the response variable, and the treatment type as a fixed effect. Block ID, nested within greenhouse ID was included in the model as random effects. In the Binomial-GLMMs of each fruit set rate per flower cluster (first to the fourth cluster), the number of flowers and fruits per each plant cluster (first to the fourth cluster) were response variables, and the treatment type was included as a fixed effect. The models' random effects included block ID, nested within greenhouse ID.

I examined whether the vibration stimuli generated by vibrational exciter affected the yield of tomato plants. LMMs with an identity link function were fitted to the number of tomatoes harvested and the average weight of fruit. In the LMM of the number of harvested tomatoes, the number of harvested fruits from each plant was included as the response variable, the treatment type as a fixed effect. Block ID, nested within greenhouse ID was included in the model as random effects. In LMM of the average weight of fruit, the ln-transformed weight of each fruit was included in the model as the response variable, and the treatment type as a fixed effect. The model random effects included plant ID, nested within block ID and greenhouse ID. Furthermore, whether the vibrational stimuli affect the average size of the fruit was examined. LMMs with an identity link function were fitted to the average fruit short and long diameters. In the LMMs, the short and long diameters of each fruit were included as the response variables, and the treatment type was included as a fixed effect. Plant ID, nested within block ID and greenhouse ID, and included as random effects.

Moreover, the effect of treatments on fruit quality was evaluated using LMMs with an identity link function. LMMs were fitted to the total soluble solids content (Brix), acidity, sugar-acid

ratio, fructose concentration, and glucose concentration. Sucrose was excluded because it was not detected by HPLC. In LMMs, Brix, the ln-transformed acidity, Brix with offset as acidity, fructose concentration, and glucose concentration in each fruit were included as response variables, and treatment type as the fixed variable. The models' random effects included plant ID, nested within block ID and greenhouse ID.

In the sensory test, I evaluated whether there was a difference in fruit taste evaluation between treatments with and without vibrations using the CLMMs with a logit link function. Each ordinal scale response variable of CLMMs fitted to the number of respondents for each score of "sweetness," "sourness," "richness," and "preference", and the treatment type included as the fixed effect. Respondent ID (86 levels) and investigation dates (4 levels) were included as random effects.

In addition, the quality of the fruit used for sensory testing was evaluated for differences between treatments with and without vibration using LMMs. In LMMs, Brix, acidity, and Brix with offset as acidity in each fruit were included as the response variables, and the treatment type was included as the fixed effect. Plant ID, nested within block ID and greenhouse ID was fitted to random effects. Finally, principal component analysis (PCA) was conducted to determine how the Brix, glucose concentration, fructose concentration, and acidity of each fruit used in the sensory test corresponded to the mean value of each index score on each investigation date. The first two principal components were selected and shown in the biplot.

Results

The relationship between acceleration and horizontal distance from the vibration source at each measurement point is shown in Figs. 2 and 3. The tomato stems that were exposed to vibration showed accelerations ranging from 1.2 to 9.0 m/s^2. Although the acceleration at House A was seen as damping with increasing horizontal distance from the vibration source, the acceleration at each measurement point did not decrease significantly at a constant rate with increasing horizontal distance from the vibration source to each measurement point (LRT for the LMM: $\beta = -0.002$, df = 1, $\chi^2 = 0.052$, p = 0.8196 [zero to peak], LRT for the LMM: $\beta = 0.001$, df = 1, $\chi^2 = 0.091$, p = 0.763 [100 Hz RMS]).

Temporal change in the number of *B. tabaci* adults and nymphs is shown in Fig. 4 and 5. On average, the number of *B. tabaci* adults and nymphs on the compound leaves was significantly lower in the treatment with vibrations than in the control (LRT for the NB-GLMM: $\beta = -0.524$, df = 1, $\chi^2 = 7.411$, p = 0.0065 [adult], LRT for the NB-GLMM: $\beta = -0.537$, df = 1, $\chi^2 = 9.435$, p = 0.0021 [nymph]). Note that after March 20, 2020 in House B, most of the tomato plants died due to *A. lycopersici* damage, and the mite infested all the greenhouses (Fig. 5). The number of *B. tabaci* adults and nymphs at each plant in the vibrational treatment did not increase significantly at a constant rate with increasing horizontal distance from the vibrational exciter to each plant (Fig. 6; LRT for the NB-GLMM: $\beta = -0.0002$, df = 1, $\chi^2 = 0.037$, p = 0.8478 [adult], Fig. 7; LRT for the NB-GLMM: $\beta = -0.0008$, df = 1, χ^2

= 1.288, p = 0.2564 [nymph]).

In terms of the number of *B. tabaci* adults caught on the yellow sticky trap, there was no significant difference between the treatment with and without vibrations (Fig. 8; LRT for the NB-GLMM: $\beta = -0.108$, df = 1, $\chi^2 = 0.137$, p = 0.7115).

In addition, CHD, the number of blooming flowers, the number of fruit sets, fruit set rate per plant, and fruit set rate per each cluster (first to the fourth cluster) significantly were not significantly different between treatment and control (Fig. 9; LRT for the LMM: $\beta = -0.043$, df = 1, $\chi^2 = 1.724$, p = 0.1892 [CHD], Fig. 10; LRT for the HP-GLMM: $\beta = 0.093$, df = 1, $\chi^2 = 1.632$, p = 0.2014 [blooming flower], Fig. 11; LRT for the HP-GLMM: $\beta = 0.03$, df = 1, $\chi^2 = 0.049$, p = 0.8243 [fruit set], LRT for the Binomial-GLMM: $\beta = 0.1597$, df = 1, $\chi^2 = 0.822$, p = 0.3646 [fruit set rate per plant], Fig. 12; LRT for the Binomial-GLMM: $\beta = -0.1062$, df = 1, $\chi^2 = 0.2817$, p = 0.5956 [fruit set rate per the first cluster], LRT for the Binomial-GLMM: $\beta = 0.1038$, df = 1, $\chi^2 = 0.2205$, p = 0.6387 [fruit set rate per the second cluster], LRT for the Binomial-GLMM: $\beta = -0.1279$, df = 1, $\chi^2 = 0.155$, p = 0.6938 [fruit set rate per the third cluster], LRT for the Binomial-GLMM: $\beta = -0.5176$, df = 1, $\chi^2 = 1.5667$, p = 0.2107 [fruit set rate per the fourth cluster]).

In the vibrational treatment, the number of harvested tomato fruits was significantly higher than that in the control (Fig. 13; LRT for the LMM: $\beta = 1.792$, df = 1, $\chi^2 = 10.27$, p = 0.0014). However, there was no significant difference in the average fruit weight between the treatment with and without vibrations (Fig. 14; LRT for the LMM: $\beta = 0.0165$, df = 1, $\chi^2 = 0.027$, p = 0.8691). Similarly, the average fruit short and long diameters were not significantly different between the treatment types (Fig. 15; LRT for the LMM: $\beta = 0.616$, df = 1, $\chi^2 = 0.179$, p = 0.6719 [short diameter], LRT for the LMM: $\beta = 0.447$, df = 1, $\chi^2 = 0.065$, p = 0.7994 [long diameter]).

On average, fruit total soluble solids content (Brix), acidity, sugar-acid ratio, fructose concentration and glucose concentration were not significantly different between the treatment with and without vibrations (Fig. 16 and 17; LRT for the LMM: $\beta = -0.083$, df = 1, $\chi^2 = 0.20$, p = 0.6548 [Brix], LRT for the LMM: $\beta = -0.038$, df = 1, $\chi^2 = 1.523$, p = 0.2172 [acidity], LRT for the LMM: $\beta = -0.031$, df = 1, $\chi^2 = 0.029$, p = 0.8645 [sugar-acid ratio], LRT for the LMM: $\beta = -0.0023$, df = 1, $\chi^2 < 0.0001$, p = 0.9835 [fructose], LRT for the LMM: $\beta = -0.064$, df = 1, $\chi^2 = 0.350$, p = 0.5542 [glucose]).

The results of judgement from the sensory test of the fruit are shown in Fig. 18. Regarding the fruit richness and preference, there was no difference between the treatment with and without vibrations (Wald test for the CLMM: $\beta = -0.245$, z = -1.045, p = 0.296 [richness], Wald test for the CLMM: $\beta = 0.082$, z = 0.341, p = 0.733 [preference]). However, in the vibrational treatment, fruit sweetness was significantly higher than that of the non-vibration treatment (Wald test for the CLMM: $\beta = 0.568$, z = 2.361, p = 0.0182). Moreover, the fruit sourness in the treatment with vibrations was significantly lower than that in the treatment without (Wald test for the CLMM: $\beta = -1.185$, z = -4.833, $p < 0.0001$).

In the fruits used for the sensory test, there was no significant difference in fruit total soluble solids content (Brix) and the sugar-acid ratio between the treatment with and without vibrations (Fig. 19; LRT for the LMM: $\beta = -0.033$, df = 1, $\chi^2 = 0.016$, p = 0.8987 [Brix], LRT for the LMM: $\beta = 0.142$, df = 1, $\chi^2 = 0.313$, p = 0.5756 [sugar-acid ratio]). Meanwhile, the acidity of fruit in the vibrational treatment was significantly lower than that in the non-vibration treatment (Fig. 18; LRT for the LMM: $\beta = -0.185$, df = 1, $\chi^2 = 8.674$, p = 0.0032).

In the analysis of PCA, PC1 (33.7%) and PC2 (30.5%) explained 64.2% of the total variation (Fig. 20). The principal component loadings of PC1 for Brix and each monosaccharide were positive, while the principal component loadings of PC2 for acidity and acidity were negative.

Discussion

Decrease in the density of *B. tabaci* by vibrations

In the previous study, because no shields were placed between the vibratory and non-vibratory treatment to restrict the movement of *B. tabaci*, the adults of *B. tabaci* could move freely between the two treatments which may have induced some bias in the estimation of the density of *B. tabaci* (Yanagisawa et al. 2021). This might have inflated individuals in the non-vibratory treatment. In the present study, partitions were settled between the area of the treatment with and without vibrations to inhibit the movement of *B. tabaci*. A decreased proportion in the number of both adults and nymphs counted on leaves in the vibrational treatment accounted for approximately 40% compared to the number in the non-vibratory treatment, suggesting that the density of *B. tabaci* decreased as a result of the inhibition of its reproduction induced by the excitation of plants. Especially at densities of less than 10 average nymphs per compound leaf in the vibration treatments, *B. tabaci* nymph increase appeared to be more suppressed than in the non-vibratory treatment (Fig.4 and 5). The effect of vibrations might be different depending on the density of the pest.

Hypotheses of reduced density of *B. tabaci* by the vibrations

Vibrational signals in whiteflies are responsible for species (including biotype or genotype) recognition during courtship (Kanmiya 2006). Species-specific substrate-borne vibrations as courtship stimuli and their mimetic signals tend to cause the disturbance of male-female communication and disrupt mating (e.g. Eriksson et al. 2012; Lujo et al. 2016; Nieri and Mazzoni 2019). In acoustic recordings by Kanmiya (2006), the power spectral density of burst signals (short and strong signal) during courtship in *B. tabaci* males (MEAM1) has a trapezoidal peak at approximately 270 to 450 Hz with ca. 230 Hz peak frequency of the female response signal. The vibrations at 100 Hz output from the vibrational exciters also covered harmonics in the bandwidth between 200 and 500 Hz, suggesting that the applied vibrations could have masked the mating signal of *B. tabaci*, thereby disturbing the

male-female communication and decreasing their density. Females of *B. tabaci* are arrhenotoky (haplodiploidy), in which males hatch from unfertilized eggs (Byrne and Devonshire 1996). If mating is disrupted by vibrations, the sex ratio of the population could become male-biased due to the uncopulated females, which might eventually lead to population collapse due to female depletion.

In some species, such as beetles (Coleoptera) and aphids (Hemiptera: Aphididae), vibrations induce irregular behaviors such as short freezes, startle response, and dropping behaviors (Takanashi et al. 2016; Gish et al. 2012). The number of *B. tabaci* adults on the compound leaves in the vibratory treatment was significantly lower than that in the non-vibratory treatment (Fig. 4 and 5). The rate of increase in *B. tabaci* was highly likely reduced by the vibrations. In this case, depending on the density of the pest, it would be expected that the number of insects captured by the sticky traps in the vibratory treatment would be lower than in the control treatment; however, there was no significant difference in the number of insects captured between the treatments. This may be because of the fact that the capture rate for the number of adults in the vibration treatment was higher than that in the control treatment. In other words, the vibrations could have induced such behaviors as a startle response and avoidance in *B. tabaci*, which caused inhibition of its settlement on the plants.

Green peach aphids, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae), exposed to sounds with specific frequencies (1000, 2000 Hz) showed a decrease in progeny, a delay in the development period from larva to adult, and an increased sensitivity to imidacloprid (Seok et al. 2010). Similarly, when exposed to 100, 500, 1000, 5000, and 10000 Hz frequency sounds, marked reductions in honeydew production and feeding frequency were observed (Lee et al. 2012). Although both are sound reactions, the large amplitude of airborne sound at close range may also exert vibration-mediated stimulus (Parent et al. 2022). The vibration could induce physiological stress on *B. tabaci*, which might cause behavioral disruption such as feeding and oviposition and adverse effects on the development.

The application of vibration to pest control technology

The application of nonspecific vibrations for pest control possibly leads to negative effects on the surrounding environment. There is a report that increased sound pressure of anthropogenic noise has a negative effect (and a positive effect for some taxa) on taxon-level abundance (Bunkley et al. 2017). Arthropods locate their foods by sensing vibrations generated by their captive targets (reviewed in Virant-Doberlet et al. 2019; Brownell and van Hemmen 2001). Parasitic wasps also search their hosts based on incidental or specific vibrations or sounds emitted by the movement of the host (review in Virant-Doberlet et al. 2019; Meyhöfer and Casas 1999; Broad and Quicke 2000; Laumann et al. 2007, 2011). In generalists as spiders and specialist arthropods as parasitic wasps, both of which are natural enemies of *B. tabaci*, searching behaviors for a host might be more or less disturbed by vibratory stimuli, which should be evaluated in future studies.

The acceleration seemed to decrease with horizontal distance from the excitation source to

each measurement point but did not vary significantly by a constant proportion (Fig. 2 and 3). Vibrations were transmitted from the vibrational exciter to the tomatoes through a complex of different substrates such as plants and props. In particular, large variations in the accelerations are expected to be due to the tension of the softwire and the dimensions of the plants. The number of *B. tabaci* adults and nymphs counted did not show a constant decrease with the distance from the vibrational exciter to each plant (Fig. 6 and 7). Under the present condition of the power output of the exciter and the transmission procedure employed in the experiment showed that *B. tabaci* density reduction is expected up to a distance of approximately 4 m from the exciter. In the future, the intensity of vibration and the density of *B. tabaci* should be investigated in a wider range of facilities to determine the effective amplitude (Sekine et al. 2022).

Effects of vibration on plant growth

Plants respond adaptively to various mechanical stimuli such as wind, sound, vibration, and touch stimulus (Chehab 2009). Positive and negative plant responses to vibration have been reported, for example, delayed reproduction (Akers and Mitchell 1984), reduction in height (Mitchell 1992), changes in biomass allocation patterns such as roots and stems (Niklas 1998), root elongation (Takahashi 1991), and increased germination rate (Uchida et al. 2002). In a previous study, there was no significant reduction in tomato height in 300 Hz vibration treatment (Sekine et al. 2022). Similarly, the present results show that there is no significant difference in the CHD, the number of blooming flowers and fruit sets per investigation between treatments with and without vibrations, and no significant growth and reproductive delay were observed (Fig. 9–11).

Effects of vibrations on plant reproduction

In the present study, the number of harvested fruits in the vibration treatment increased significantly by about 30% and production increased more than non-vibration (Fig. 13). A previous study reported that 30 Hz vibrational treatment increased the number of fruit sets and promoted pollination compared with 300 Hz treatment and non-vibration (Sekine et al. 2022). However, there was no difference in fruit sets number and fruit set rate between the treatments (Fig. 11, 12). Pollination enhancement by 100 Hz vibration could be less effective. The increase in the number of harvested fruits in the vibratory treatment may have been due to greater fruit loss in the control. *B. tabaci* causes physiological disorders such as abnormal fruit formation (Maynard and Cantliffe 1989), and reduced leaf area and biomass (Farina et al. 2022). Differences in the pest density among treatments might have contributed to the proportion of lost fruits. However, I did not investigate in this study the factors responsible for the decrease in the number of fruits during the period between fruiting and harvest. When the assimilate supply of tomato plants was the same, fruit size increased as the number of fruits decreased (Heuvelink 1997), but there was no significant difference in the average fruit weight (Fig. 14). This

may have been due to differences in the density of *B. tabaci* between treatments, which may have affected the assimilative supply of the plants.

Possibility of increasing fruit production via vibration-induced physiological change in plants

In addition, the increased fruit production might be due to improved assimilative supply or reduced pest damage via physiological change in the tomato plant caused by the vibration. Sound vibrations increased yields of cotton, strawberries, lettuce, and tomatoes, suggesting that optimal sound stimulation enhances physiological activity in plant cells (Hou et al. 2010; Qi et al. 2010; Hassanien et al. 2014; Mishra et al. 2016; Hassanien et al. 2020). This might be a result of cells being receptive to sound vibration stimuli and changes in hormones such as indole-2-acetic acid (IAA) and gibberellin (GA) levels and gene expression related to growth and development, which promote cell division (Hassanien et al. 2014). If the vibration-induced growth promotion had been, it could have improved the assimilative supply of the plants, leading to increased fruit production. In the future, there should add another treatment without pests to clarify whether the increased fruit production in the vibrational treatment was due to reduced pest damage or the physiological response of the plant.

The later, vibration may have altered signaling circuits related to phytohormones such as jasmonic acid (JA) and salicylic acid (SA) involved in defense responses to damage by pests, increasing defense levels (Appel and Cocroft 2014; Body et al. 2019). In *Arabidopsis thaliana* L., artificial stimuli mimicking *Pieris rapae* L. caterpillar chewing induced chemical defense (Appel and Cocroft 2014). In addition, the vibration stimuli generated by the herbivores alter gene expression patterns and secondary metabolites related to the signaling transduction of several injury and stress response signaling hormones (Body et al. 2019). The plant against the vibrations response could have altered their external morphology and internal physiological activity, resulting in an indirect negative effect on the reproduction and settlement of the plant of *B. tabaci*. While the above described the potential direct effects of pest vibration, indirect effects via the plant should also be considered in the future.

Effect of vibration on fruit quality

The exposure of fruit to vibrations during transport has been reported that reduced total soluble solids (Pathare and Al-Dairi 2021), decreased titratable acidity, and increased the ratio of total soluble solids to titratable acidity (Al-Dairi et al. 2021), and increased lycopene, vitamin C, and total phenolic content (Altuntas and Ozkurt 2019). In this study, there was no effect of vibrations on the sugar and acidity of the fruit (Fig. 16 and 17). Nakamura et al. (1977) found that vibrated tomato fruits at the stage of turning fruit colour at 29.4 m/s^2, had significantly lower organoleptic evaluation than control fruits and tended to have a lighter taste and more mealy texture. In this study, there were no significant differences in taste preference between treatments, but the taste could be the possibility depending on

the intensity of the vibration. The fruits in the vibration treatment were sweeter and less sour than those in the non-vibration treatment (Fig. 18). The acidity of the fruits in the excitation treatment used in the sensory test was significantly lower than those in the non-excitation treatment, and the sugar-acid ratio also appeared to be higher than that in the non-vibration treatment, although not significantly (Fig. 19). The sugar and acidity content of tomatoes and their ratio are important parameters for flavor and sweetness (Stevens et al. 1977; Bucheli et al. 1999; Baldwin et al. 1991). Since the parameters related to sugar and the principal component loadings of sweetness were different (Fig. 20), acidity may influence the sweetness. Fruit richness showed a vector component in the same direction as preference (Fig. 20), indicating that richness is related to preference. Glutamic acid, a factor related to umami, is found in higher amounts in tomatoes than in other vegetables (Yamaguchi and Ninomiya 2000). Fruit richness could be related to glutamic acid content. Since there were no differences in the acidity and sugar-acid ratio of the harvested fruits between the treatments with and without vibrations (Fig. 16 and 17), it remains possible that the fruits in the non-vibrational treatment used in the sensory test did not reflect the taste of the harvested fruits due to the selection of fruits with a biased acidity (Fig. 19). In addition, vibrations may affect coloration anomalies and fruit firmness (Nakamura et al. 1977; Pathare and Al-Dairi 2021). Further detailed investigation with a larger number of fruits is needed to add data on glutamic acid content as an index of flavor, and on fruit flavor, appearance, and texture in the sensory test.

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Figures

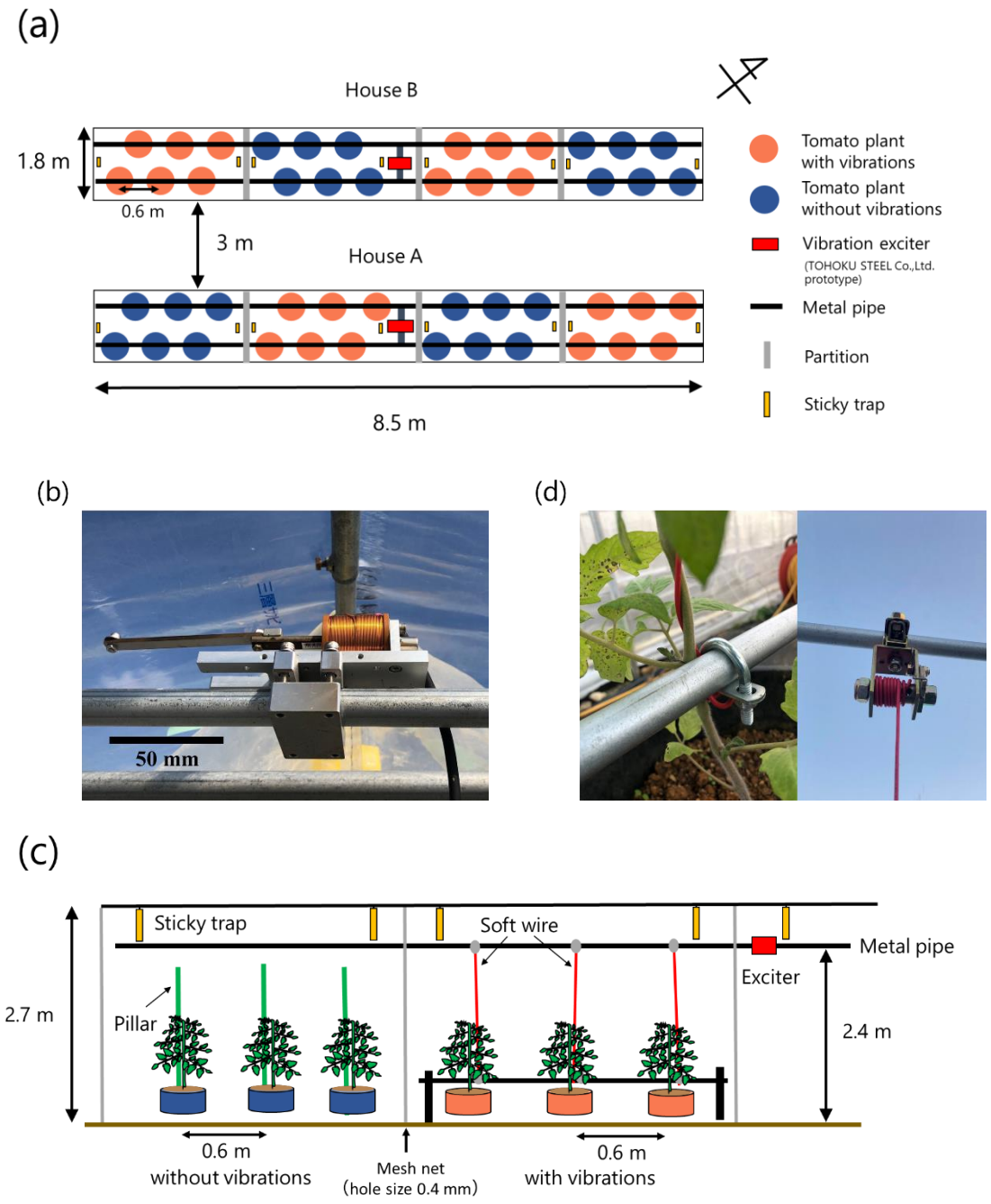

Fig. 1

Schematic diagram of tomato plant positions and associated equipment for providing vibrations: (a) horizontal view of the experimental setting, b vibrational exciter (scale bar: 50 mm), (c) vertical view, (d) left: lower soft wire to lower metal pipe connection, right: upper soft wire to upper metal pipe connection.

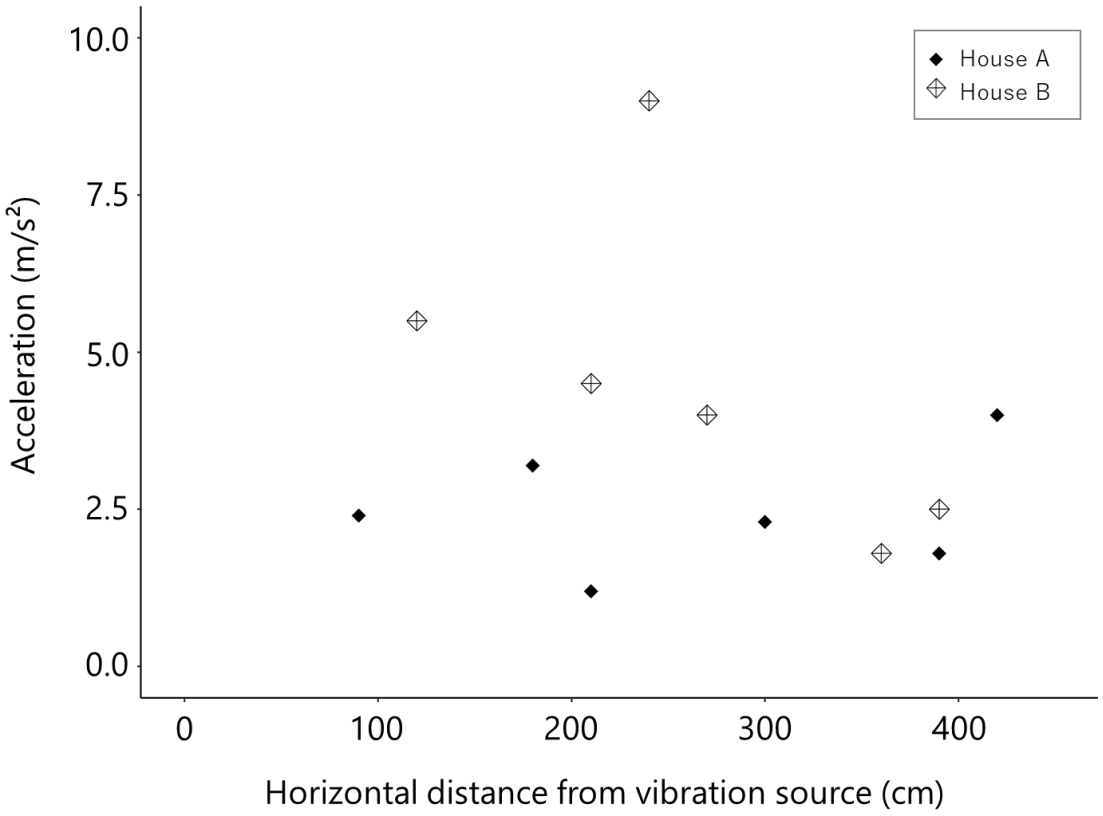

Fig. 2

The relationship between the acceleration (m/s^2, zero-to-peak) of each measuring point and the horizontal distance from the vibration source to each point. Each measuring point at the tomato stem is 0.4 m high from the ground. Black and white diamonds represent measuring accelerations in the experimental houses A and B.

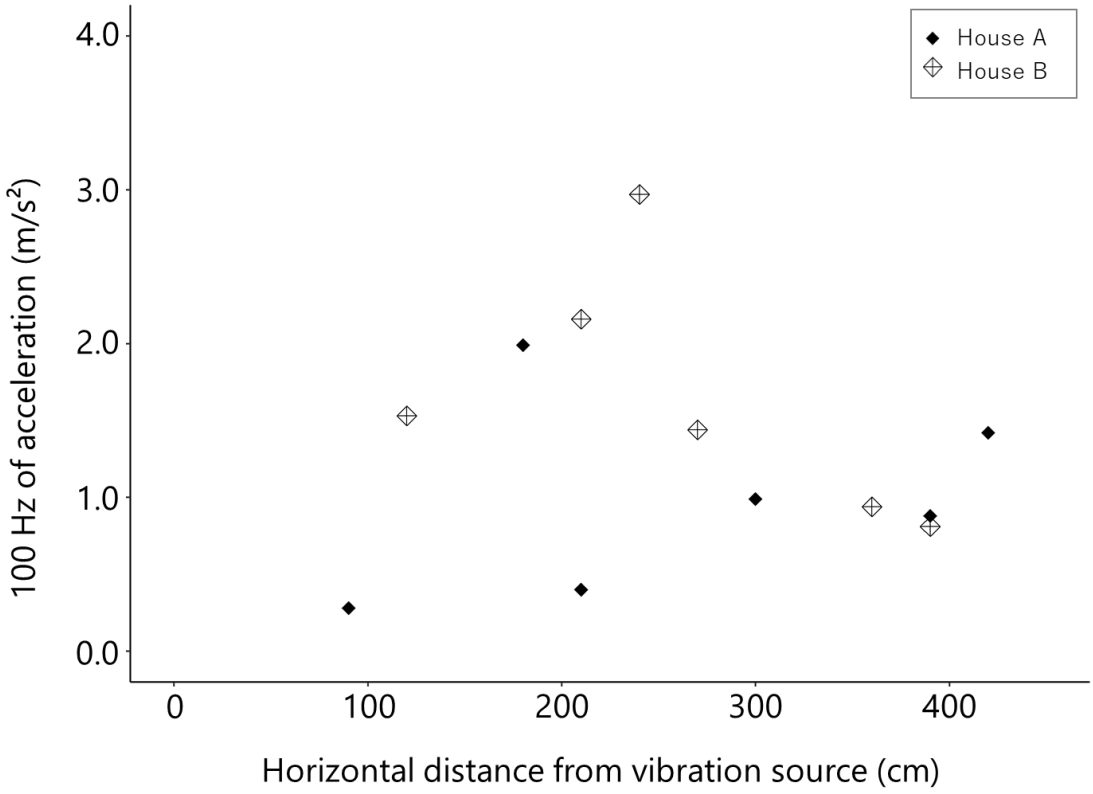

Fig. 3

The relationship between 100 Hz of acceleration (m/s^2, RMS) of each measuring point was analyzed by FFT and the horizontal distance from the vibration source to each point. Each measuring point at the tomato stem is 0.4 m high from the ground. Black and white diamonds represent measuring accelerations in the experimental houses A and B.

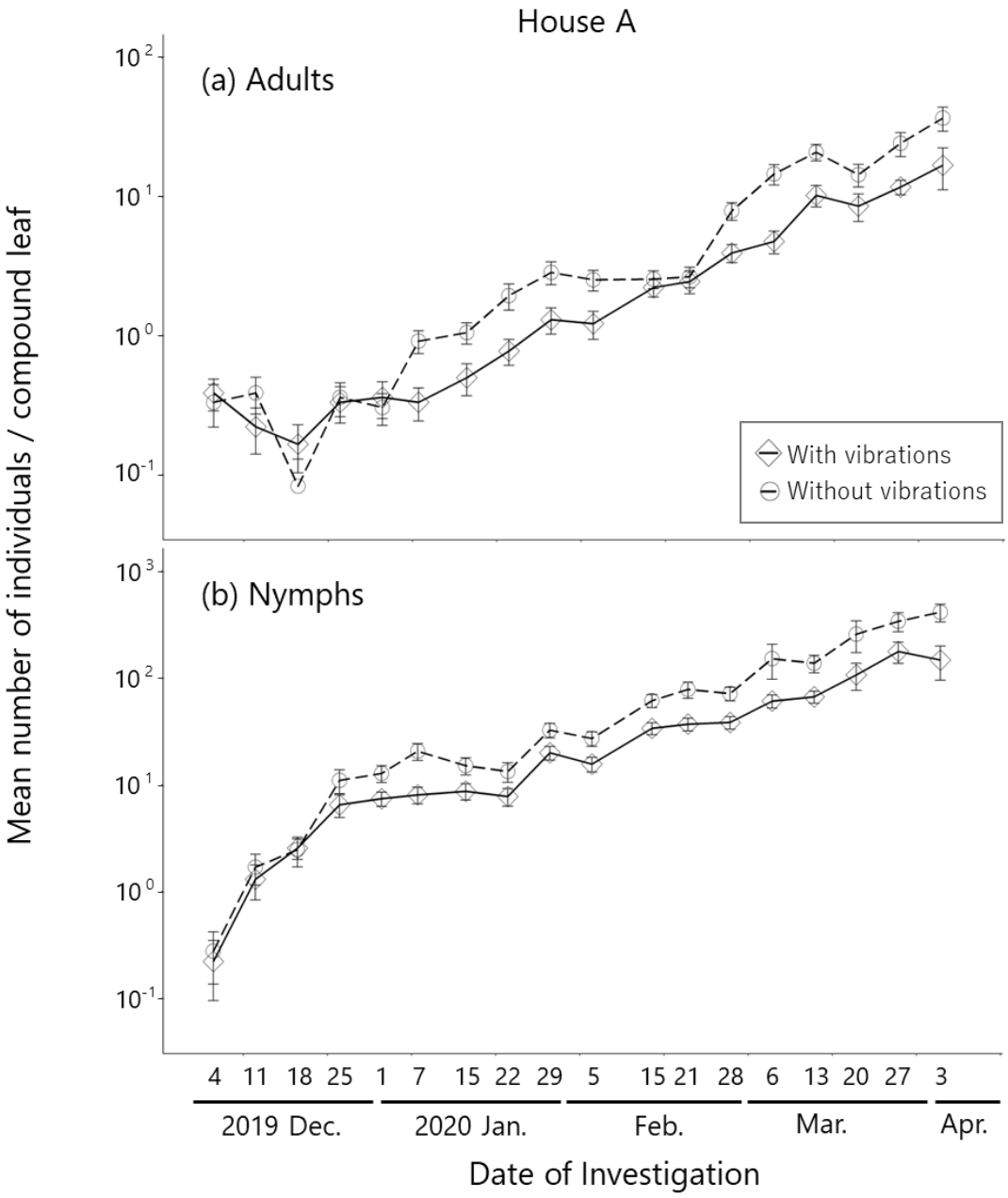

Fig. 4

Temporal change in the number of each (a) *Bemisia tabaci* adult and (b) nymph on the tomato plants in experimental house A. Diamonds and Circles represent the mean number of *B. tabaci* in the treatment with and without vibrations. The error bars represent the standard error of the means. The y-axis is a common logarithm.

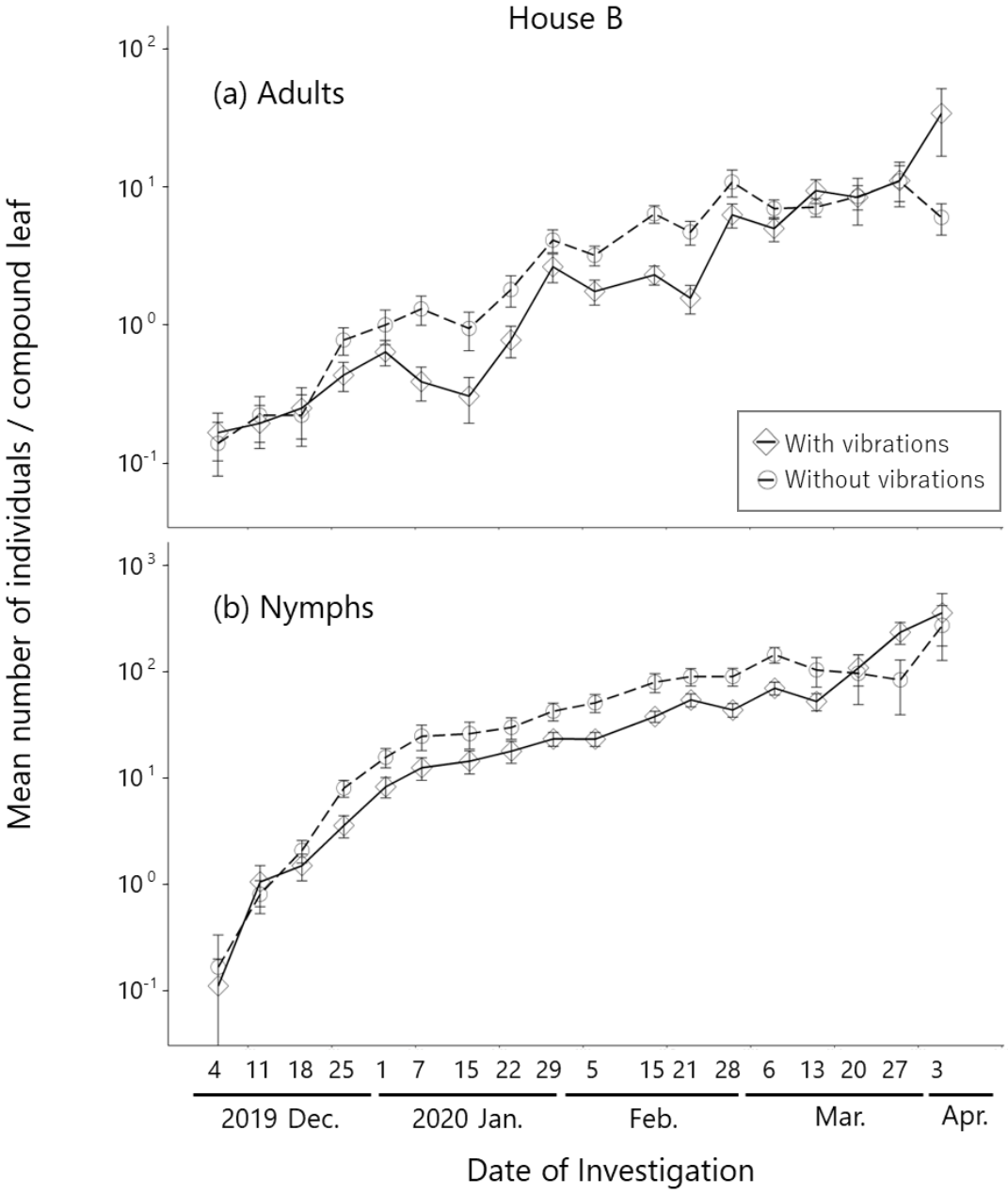

Fig. 5

Temporal change in the number of each (a) *Bemisia tabaci* adult and (b) nymph on the tomato plants in experimental house B. Diamonds and Circles represent the mean number of *B. tabaci* in the treatment with and without vibrations. The error bars represent the standard error of the means. The y-axis is a common logarithm. Note that most of the plant died after March 20, 2020.

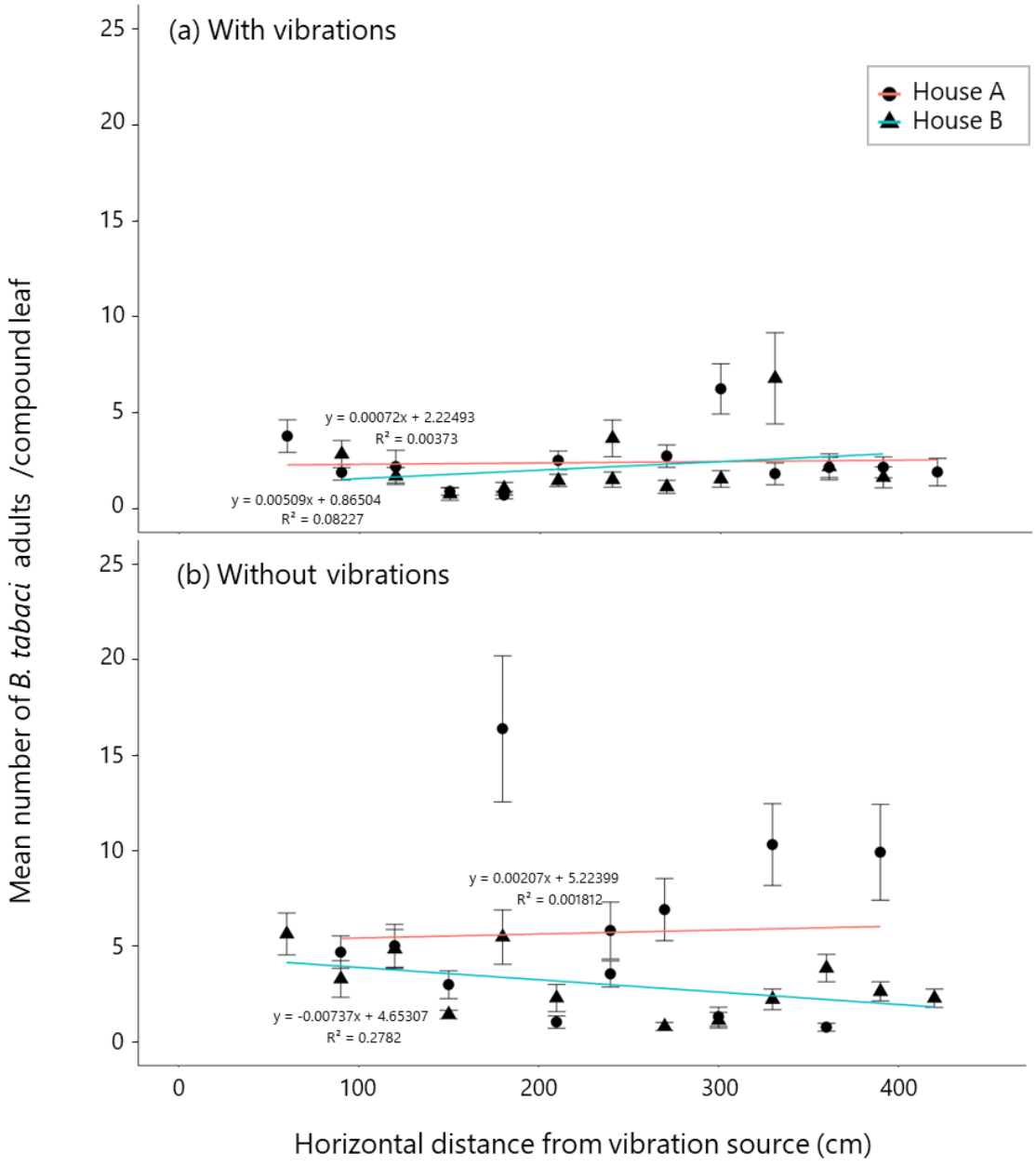

Fig. 6

The relationship between the number of *Bemisia tabaci* adults on the tomato plants and horizontal distance from the vibration source to each plant in the treatment (a) with and (b) without vibrations. Circles and triangles represent the average in 18 investigations of the number of *B. tabaci* adults on each plant's compound leaf in experimental houses A and B. The error bars represent the standard error of the means. The red and blue lines are the regression line of houses A and B. There was no significant relationship between treatments with vibrations and distance from the vibration source (likelihood ratio test, p= 0.85).

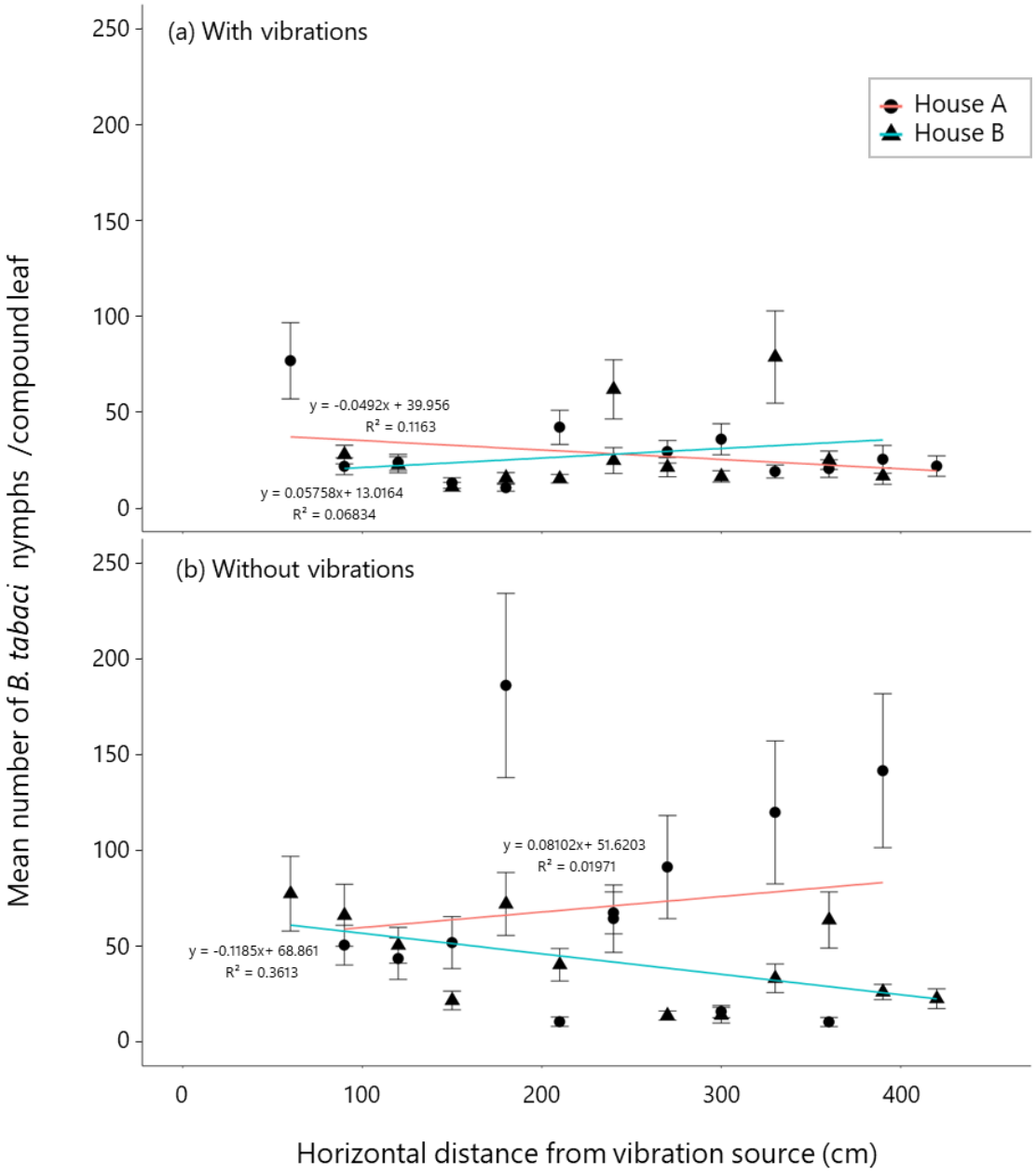

Fig. 7

The relationship between the number of *Bemisia tabaci* nymphs on the tomato plants and horizontal distance from the vibration source to each plant in the treatment (a) with and (b) without vibrations. Circles and triangles represent the average in 18 investigations of the number of *B. tabaci* nymphs in experimental houses A and B. The error bars represent the standard error of the means. The red and blue lines are the regression line of houses A and B. There was no significant relationship between treatments with vibrations and distance from the vibration source (likelihood ratio test, p= 0.26).

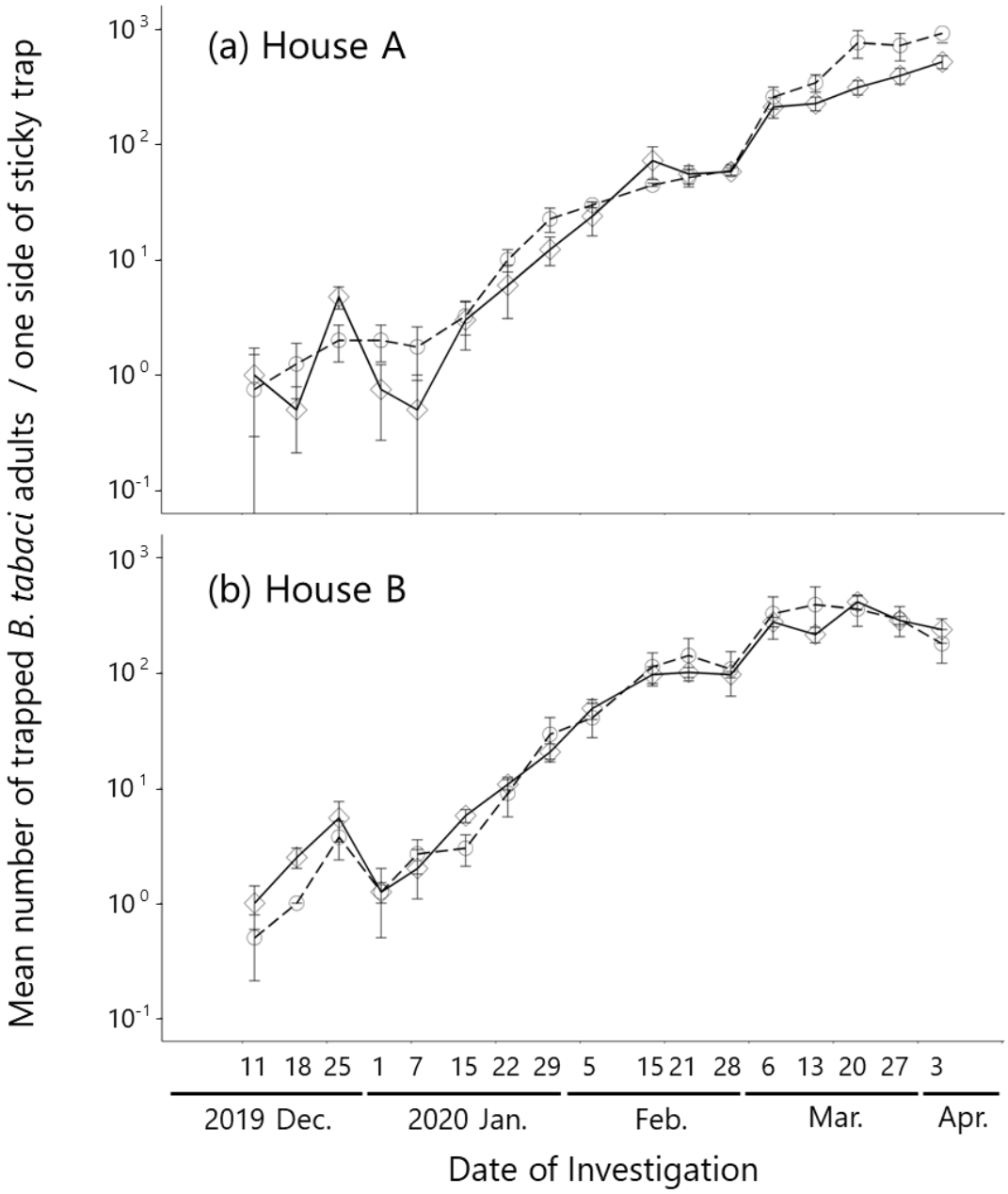

Fig. 8

Temporal change in the number of trapped *Bemisia tabaci* adults on the one side of yellow sticky traps in the experimental (a) house A and (b) house B. Diamonds and Circles represent the mean number of *B. tabaci* adults in the treatment with and without vibrations. The error bars represent the standard error of the means. The y-axis is a common logarithm.

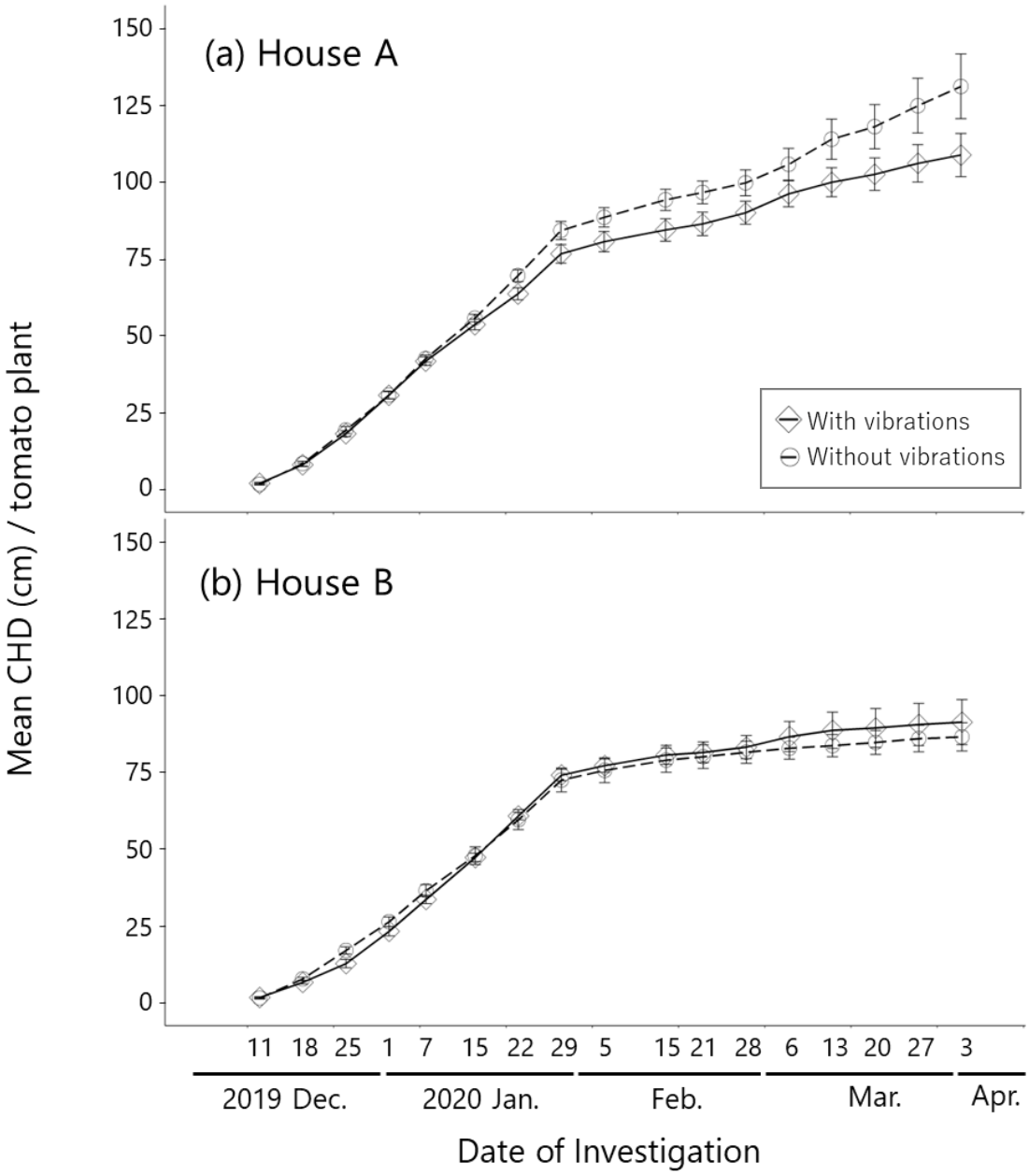

Fig. 9

Temporal change in each cumulative height difference (CHD) of tomato plant in the experimental (a) house A and (b) house B. Diamonds and Circles represent the mean CHD in the treatment with and without vibrations, which is the difference in the first height of each plant. The error bars represent the standard error of the means.

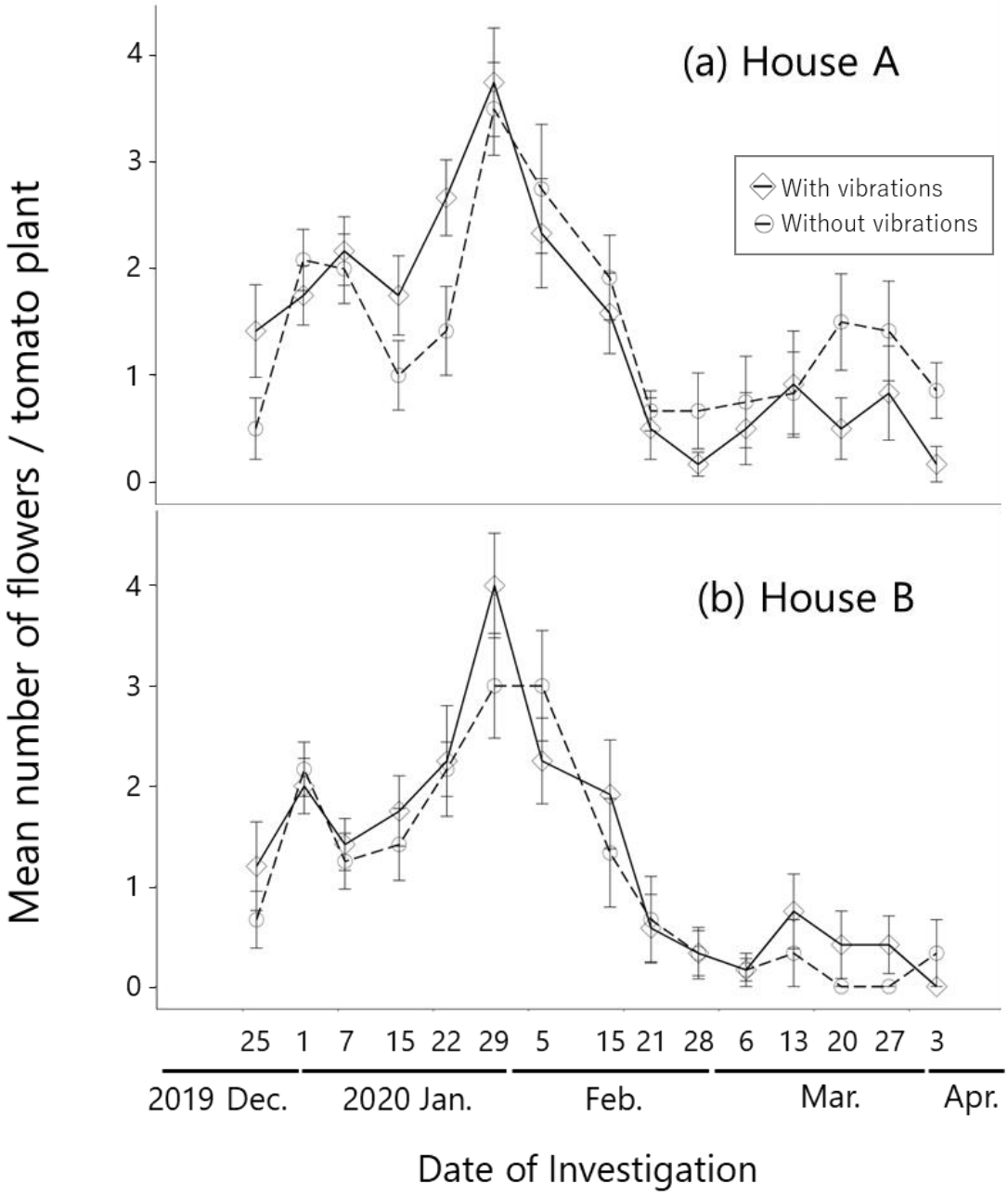

Fig. 10

Temporal change in the number of blooming flowers on tomato plants in the experimental (a) house A and (b) house B. Diamonds and Circles represent the mean number of blooming flowers in the treatment with and without vibrations. The error bars represent the standard error of the means.

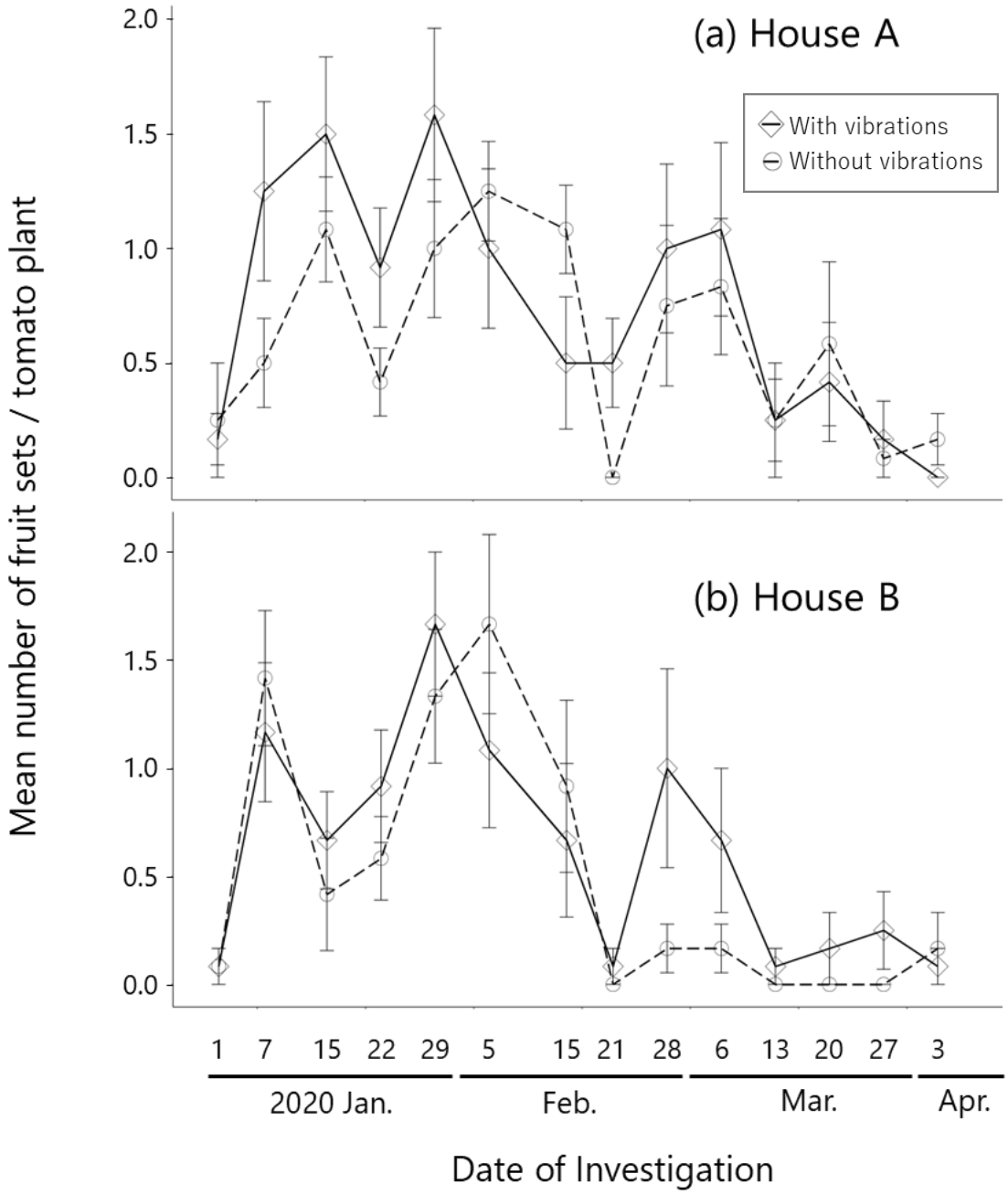

Fig. 11

Temporal change in the number of fruit sets on tomato plants in the experimental (a) house A and (b) house B. Diamonds and Circles represent the mean number of fruit sets in the treatment with and without vibrations. The error bars represent the standard error of the means.

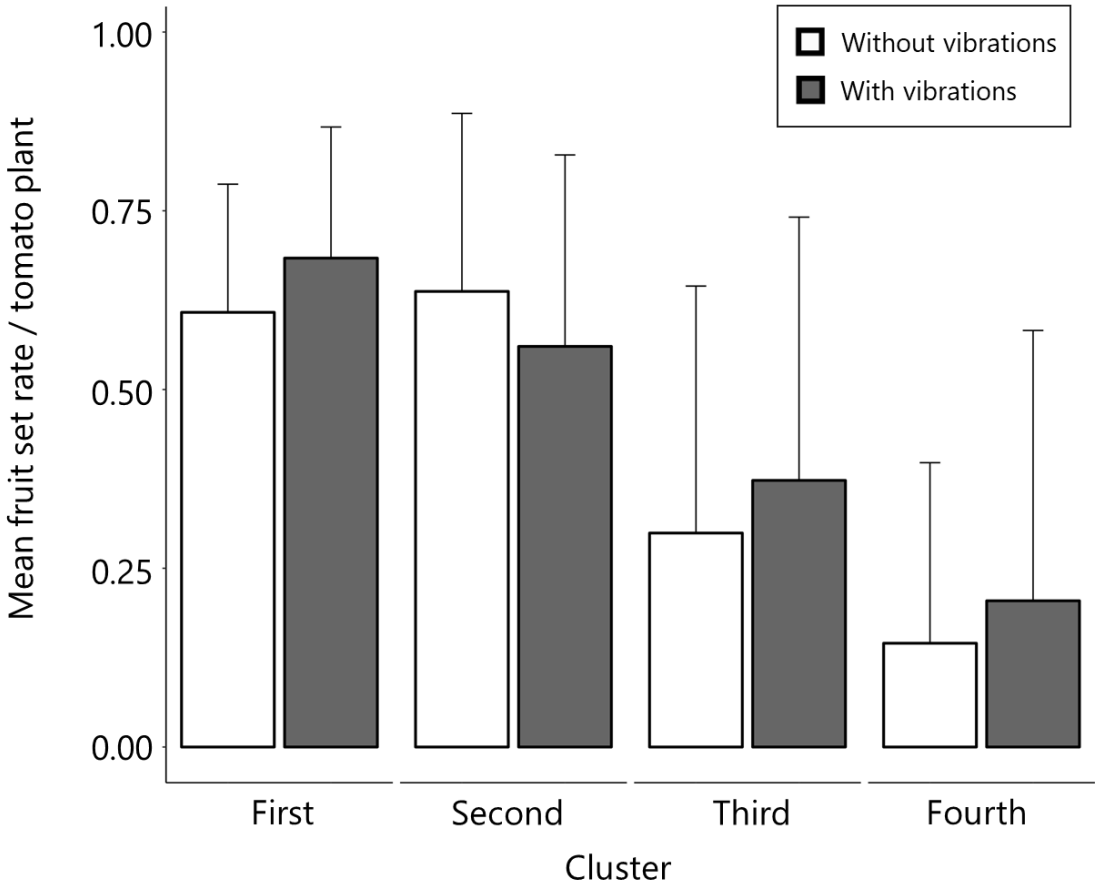

Fig. 12

Fruit set rate for each cluster from the first to fourth cluster. White and grey bars represent the mean ratio of the number of fruit sets per number of flowers on each cluster in the treatment without and with vibrations. The error bars represent standard deviations.

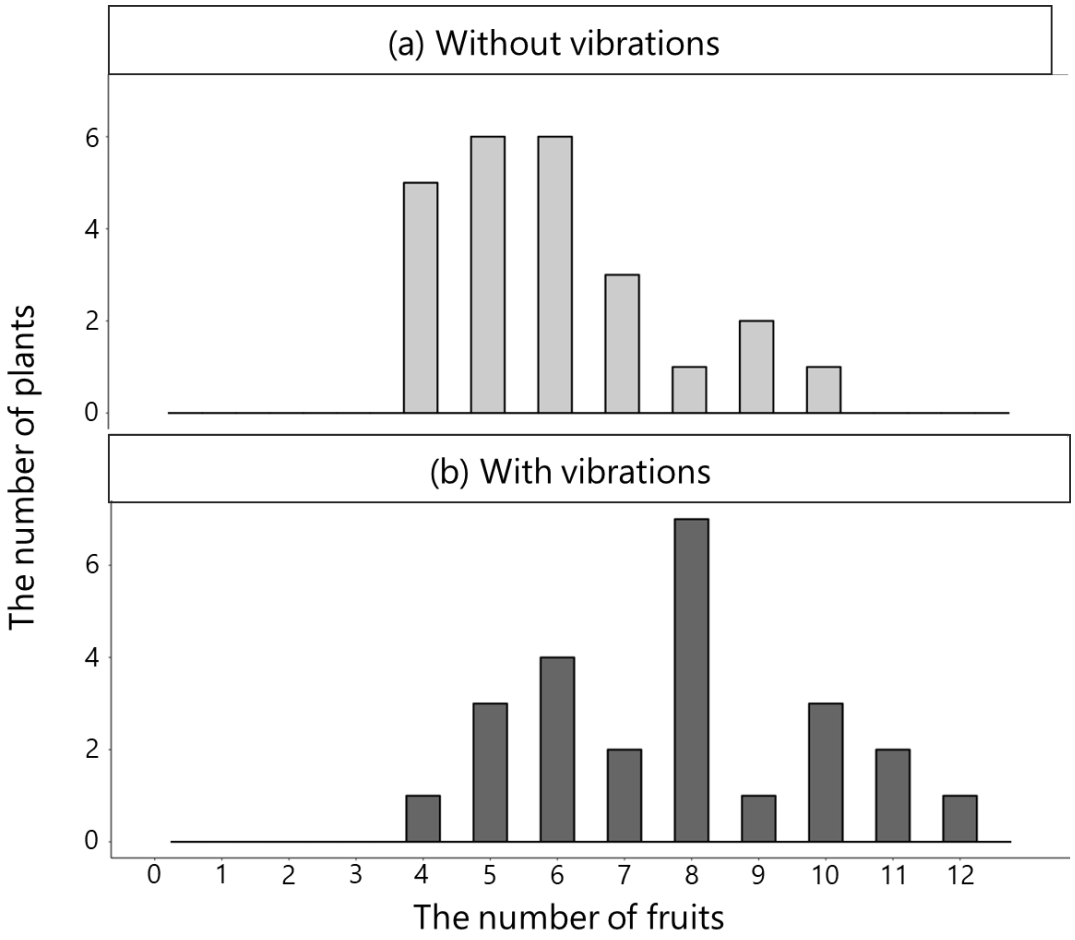

Fig. 13

The histograms of the number of fruits on tomato plants in treatment (a) without and (b) with vibrations.

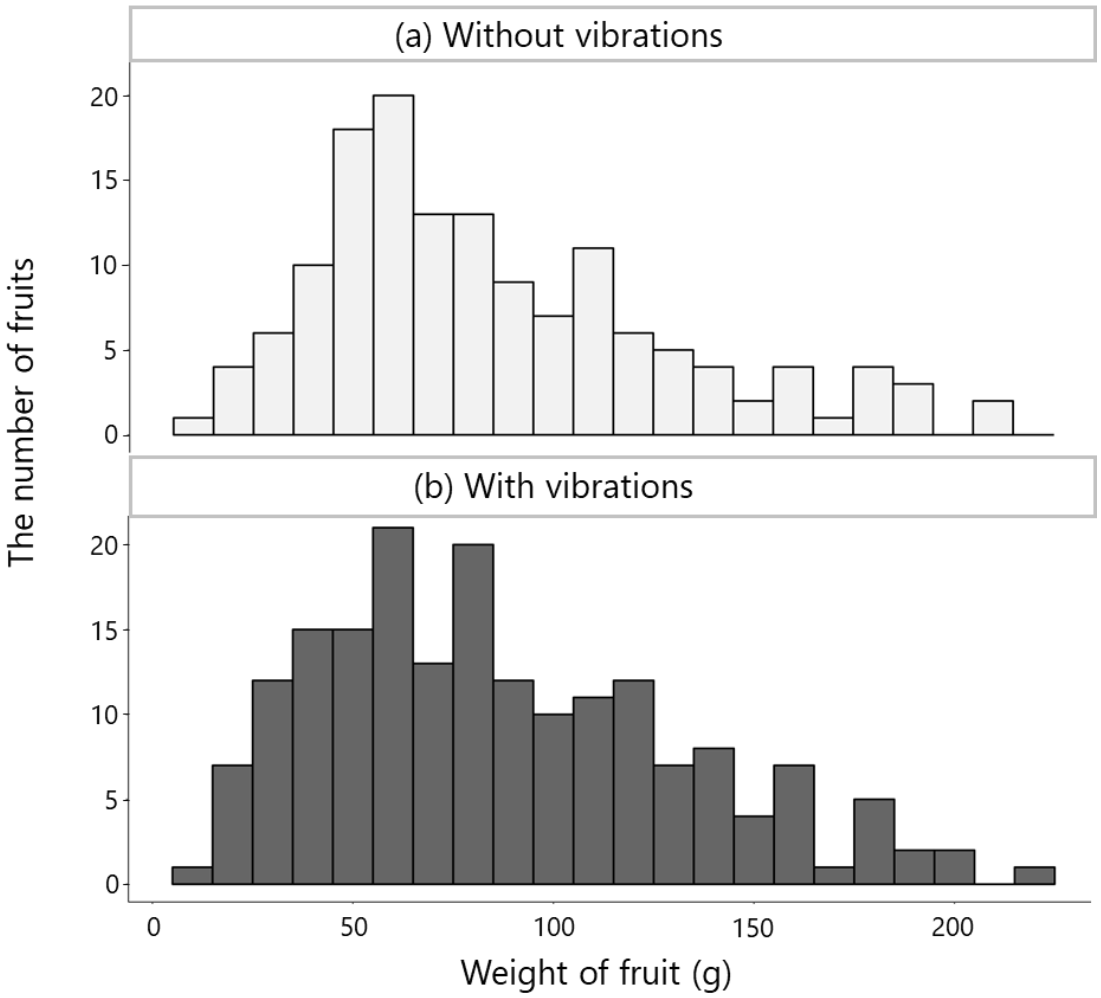

Fig. 14

The histograms of the weight of fruit in the treatment (a) without and (b) with vibrations.

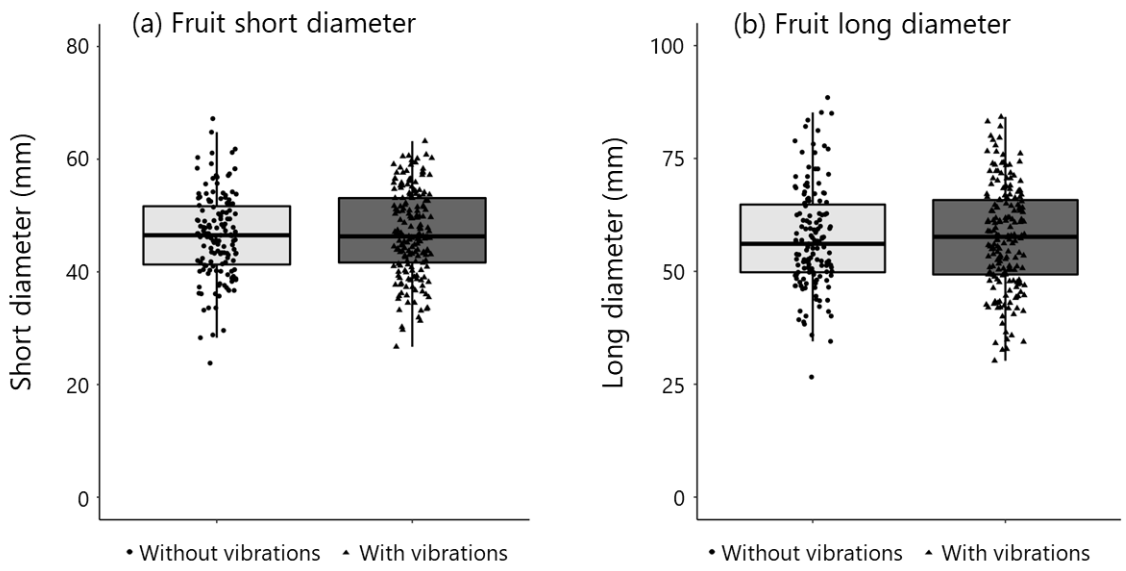

Fig. 15

The box and whisker diagrams of (a) fruit short and (b) long diameter. Light and dark gray boxes represent treatment without and with vibrations. The horizontal lines in each box are medians. The lower and upper hinges represent the first and third quartiles. The dots represent observations for each fruit. The upper whiskers extend from the hinge to the largest value no further than 1.5 x third quartiles from the hinge. The lower whiskers extend from the hinge to the smallest value at most 1.5 x first quartiles of the hinge.

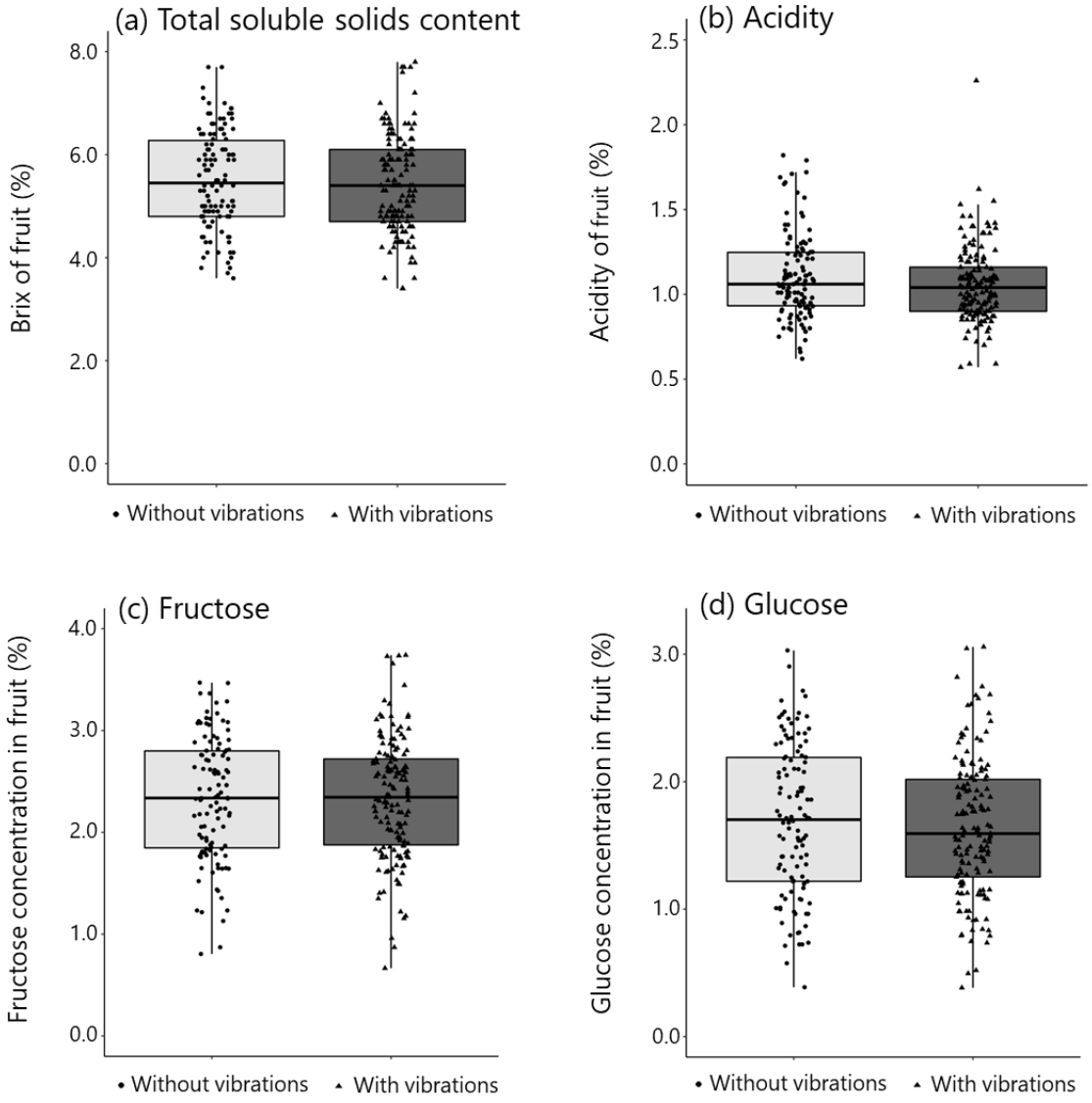

Fig. 16

The box and whisker diagrams of (a) total soluble solids content (Brix), (b) acidity, (c) fructose concentration, and (d) glucose concentration in the fruit. Light and dark gray boxes represent treatment without and with vibrations. The horizontal lines in each box are medians. The lower and upper hinges represent the first and third quartiles. The dots represent observations for each fruit. The upper whiskers extend from the hinge to the largest value no further than 1.5 x third quartiles from the hinge. The lower whiskers extend from the hinge to the smallest value at most 1.5 x first quartiles of the hinge.

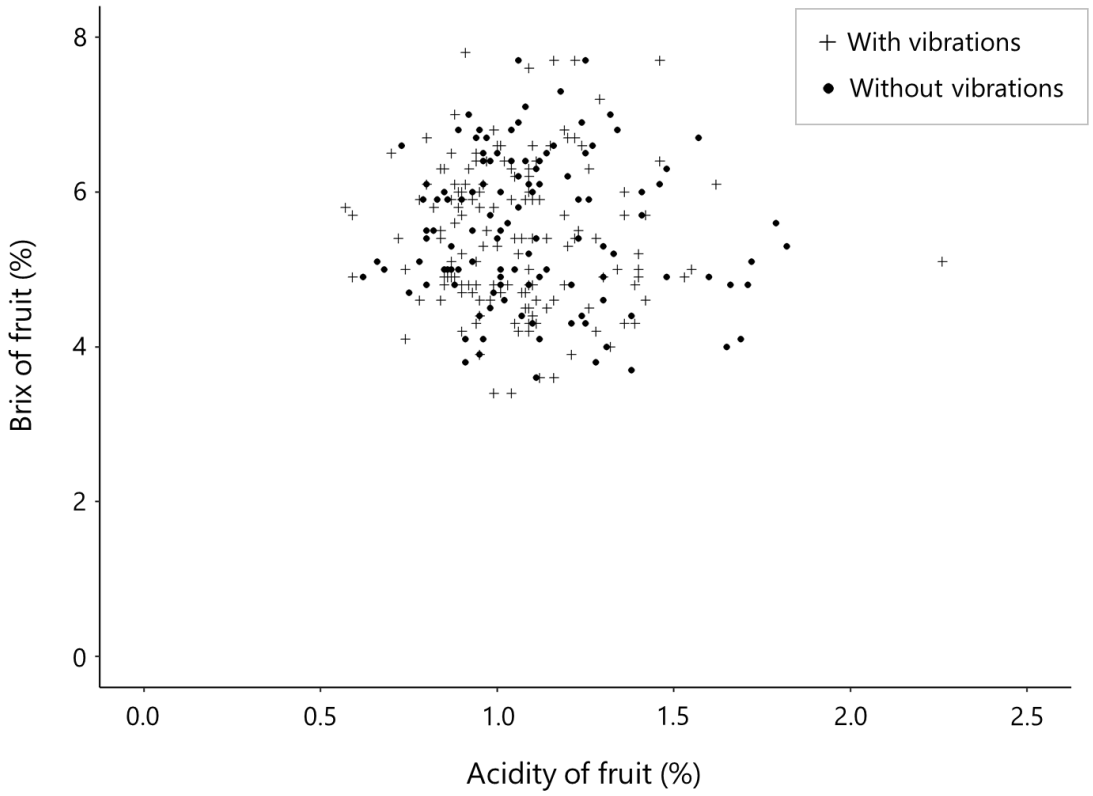

Fig. 17

The relationship between the total soluble solids content (Brix) and acidity of the fruit. Crosses and circles represent each acidity and Brix of fruit in the treatment with and without vibrations.

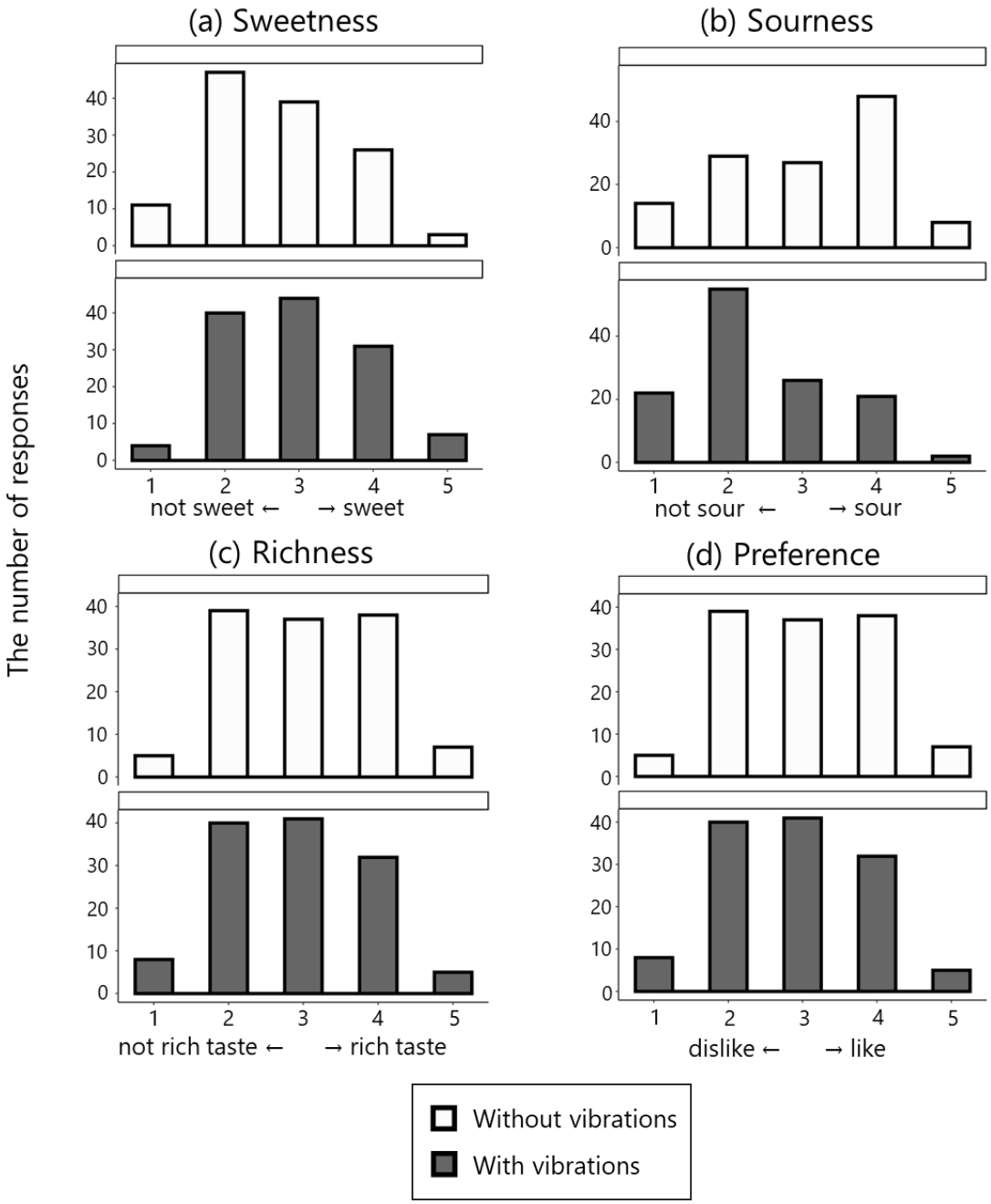

Fig. 18

The histograms of the number of responses to sensory tests in (a) sweetness, (b) acidity, (c) richness, and (d) preference of tomato fruit. White and dark gray boxes represent treatment without and with vibrations.

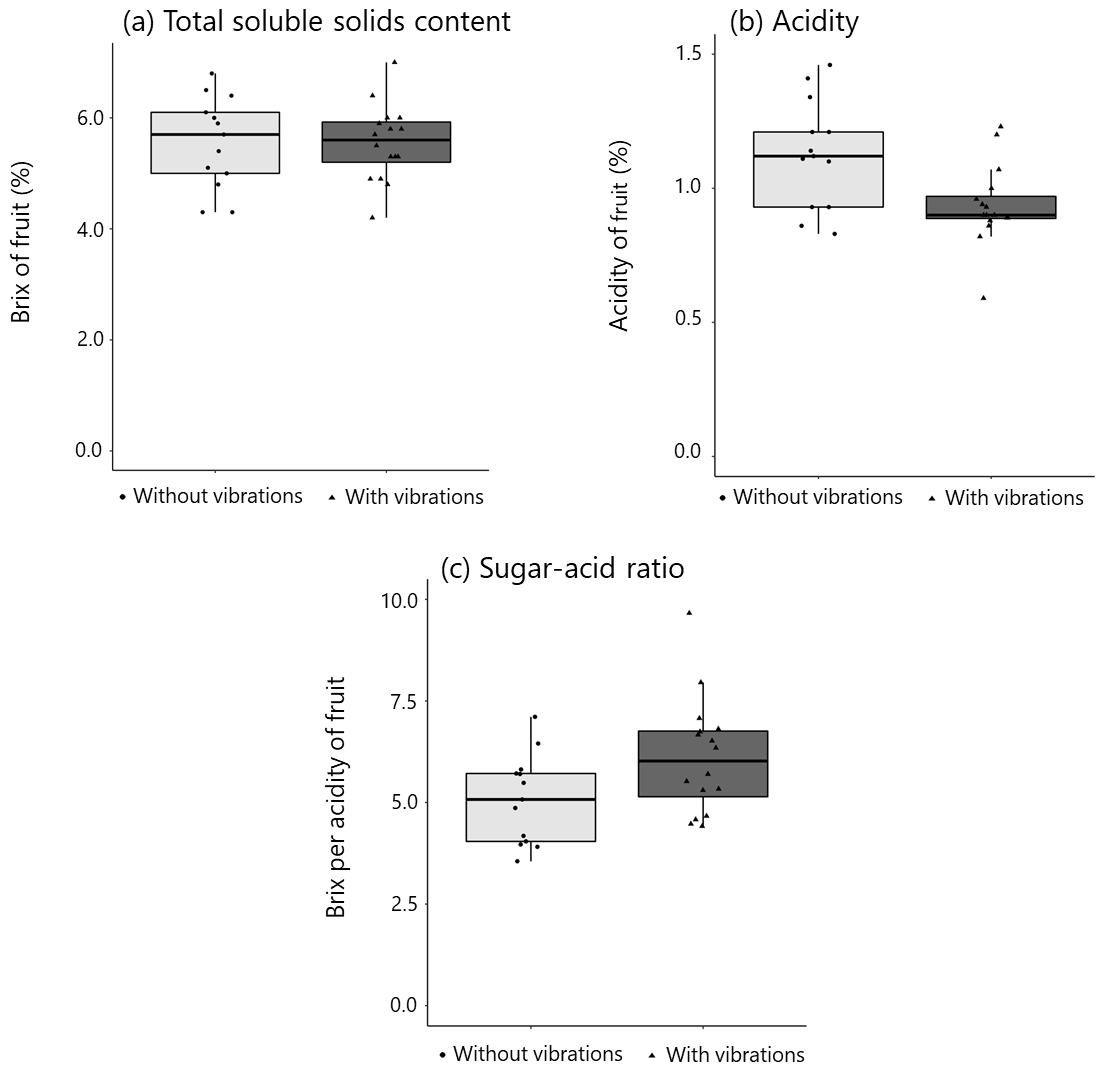

Fig. 19

The box and whisker diagrams of (a) total soluble solids content (Brix), (b) acidity, and (c) sugar-acid ratio (Brix per acidity) of fruit in the sensory tests. Light and dark gray boxes represent treatment without and with vibrations. The horizontal lines in each box are medians. The lower and upper hinges represent the first and third quartiles. The dots represent observations for each fruit. The upper whiskers extend from the hinge to the largest value no further than 1.5 x third quartiles from the hinge. The lower whiskers extend from the hinge to the smallest value at most 1.5 x first quartiles of the hinge.

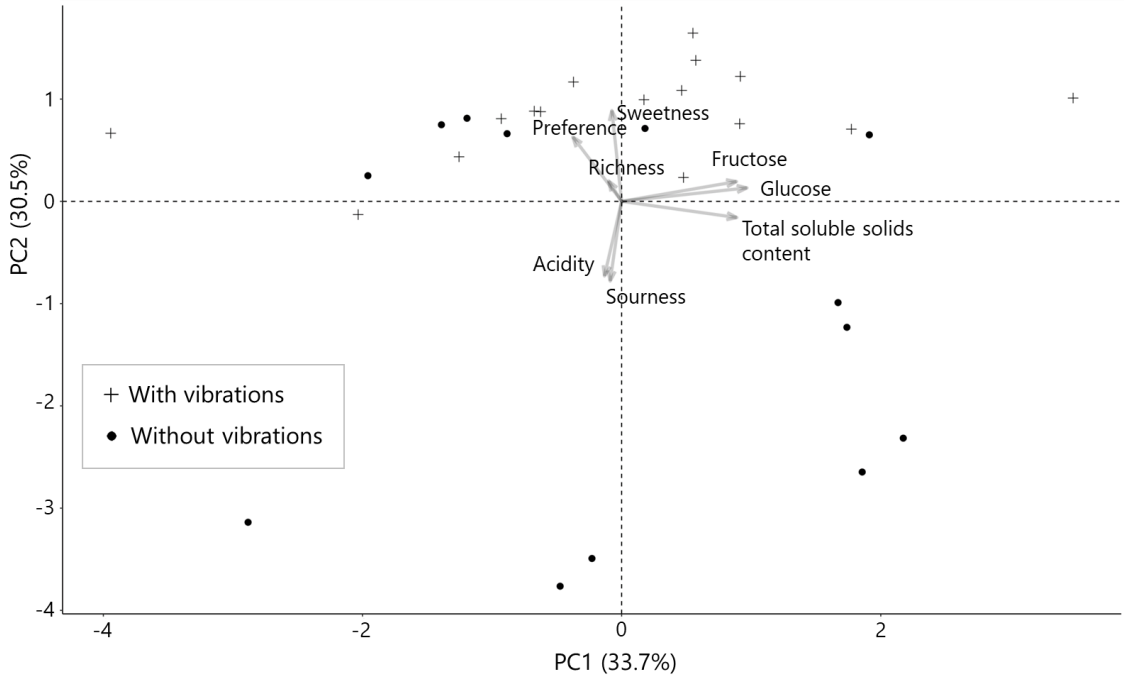

Fig. 20

Principal component analysis (PCA) plot of parameters for each fruit in the sensory tests. Crosses and circles represent each fruit in the treatment with and without vibrations. Each arrow represents each parameter of principal component loading.

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