

# Chapter 20

## Exploitation of Vibration Sensing for Pest Management in Longicorn Beetles



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**Abstract** Many insects exhibit sensitivities to substrate-borne vibrations. Some beetles detect vibrations via leg chordotonal organs and respond with predator avoidance or sexual communication. Because vibrations modify insect behaviors, vibrations could be exploited for physical pest control to reduce insect damage to plants. Here we review the abilities of beetles to sense vibrations and the use of vibrations as a pest management option for the longicorn beetle *Monochamus alternatus*, a vector of the lethal pine wilt disease, and other longicorn beetles. More specifically, we report new findings describing leg chordotonal organs and behavioral control methods using vibrations in *M. alternatus* and *Moechotypa diphysis*, a pest of mushroom bed logs. These beetles show freezing and startle responses when exposed to low-frequency vibration pulses. We characterize the morphologies of the femoral chordotonal organs and their central projections, and describe a new procedure for vibrational pest management, based on vibration sensitivities in longicorn beetles. For this method, a prototype vibration exciter that generates vibrations with large amplitudes is attached to a tree, and the vibrations from the exciter disrupt beetle feeding and walking by initiating startle and freezing responses by beetles. We believe that vibrations can be applied to plants to reduce future damage by various pests that are sensitive to vibrations.

### 20.1 Introduction

Many insects are sensitive to vibrations transmitted through substrates (Greenfield 2002; Hill 2008). These insects evolved chordotonal organs in their legs that are responsible for detecting vibrations (Field and Matheson 1998; Hill 2008), and many

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exhibit various behaviors and communications in response to vibrations (Hill 2008; Takanashi et al. 2019). For instance, larvae of the group-living beetle *Trypoxylus dichotomus* freeze in response to vibrations produced by approaching moles in the soil (Kojima et al. 2012a, b). Furthermore, they also freeze in response to vibrations that pupae of the beetle produce by drumming on their abdomen; as a result, pupal cells are protected from damage caused by approaching larvae (Kojima et al. 2012c). In the brown marmorated stink bug, *Halyomorpha halys*, egg-cracking vibrations promote synchronous hatching in a clutch of eggs in contact with each other on host plants (Endo et al. 2019).

In order to control pest insects, it may be possible to utilize their ability of sensing vibrations to disrupt communications and various behaviors, which would represent an environmentally friendly alternative to the synthetic pesticides that are currently used (Polajnar et al. 2015; Takanashi et al. 2019). A successful example of vibrational pest management is communication disruption in the American grapevine leafhopper, *Scaphoideus titanus* (Eriksson et al. 2012; Polajnar et al. 2016; Nieri and Mazzoni 2018). The mating frequency of this insect in field cages was decreased by disturbance vibrations at 300 Hz produced by an electromagnetic shaker placed in grapevines (Eriksson et al. 2012). Validation of the amplitude threshold for efficacy in mating disruption in a vineyard (Polajnar et al. 2016) was followed by pilot studies on mating disruption (Nieri and Mazzoni 2018). Similarly, communication disruption with vibrations has been reported in the Neotropical brown stink bug *Euschistus heros* (Laumann et al. 2018) and the Asian citrus psyllid *Diaphorina citri* (Lujo et al. 2016). Hosomi (1996) has reported a case of behavioral disruption in which vibrations at 5 to 40 Hz produced by a mechanical knocker suppressed feeding of the longicorn beetle *Apriona japonica* on fig trees. As noted by Takanashi et al. (2019), the vibration exciter hardware and software controlling the spectral, temporal, and amplitude characteristics of the vibrations are important for efficient disruption of communications and behaviors.

Longicorn beetles (Cerambycidae: Coleoptera) comprise more than 3600 species worldwide and include forest and agricultural pests that damage trees (Wang 2017). The beetles include invasive species spread by international trade and vectors of pathogens of serious tree diseases (Kobayashi et al. 1984; Wang 2017). Beetle adults feed on tree bark, and mate and oviposit on the host trees (Wang 2017). Because longicorn beetle larvae bore inside the trees for feeding, they can be difficult to control with pesticides. Long-range vibrational communication for mate localization, which is well known in hemipteran insects, has not yet been found in longicorn beetles (Takanashi et al. 2019). However, various behaviors, such as freezing and walking, are induced by vibrations (Takanashi et al. 2016, 2019).

In this chapter, we report recent findings on leg chordotonal organs and behavioral control methods using vibrations in two longicorn beetle pests, *Monochamus alternatus* and *Moechotypa diphysis*. We also report on previous findings on *Paraglenea fortunei*, a longicorn beetle that feeds on ramie and other plants (Tsubaki et al. 2014; Takanashi et al. 2016), and *M. alternatus*. *Monochamus alternatus* is the vector of the pine wilt nematode *Bursaphelenchus xylophilus*, which kills pine trees by causing water deficiency (Kobayashi et al. 1984; Kikuchi et al. 2011; Yazaki et al.

**Fig. 20.1** A female adult of *Monochamus alternatus* on a host pine twig



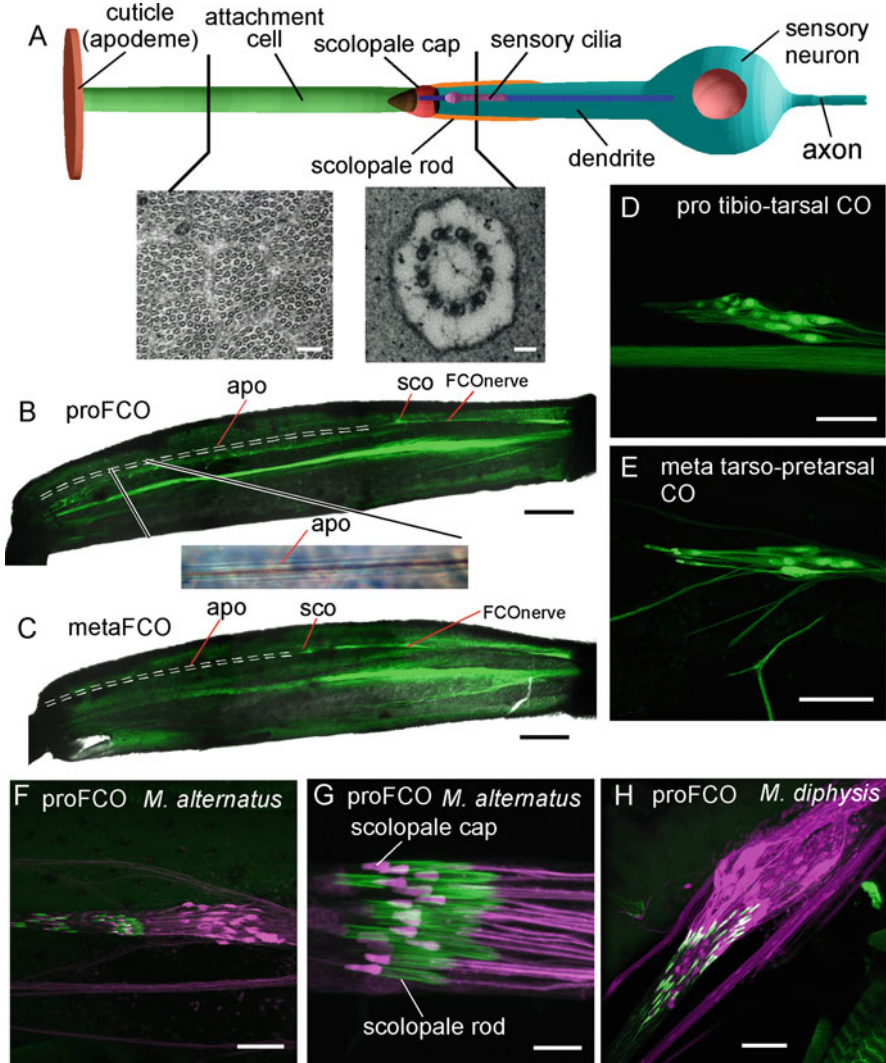
2018) (Fig. 20.1). *Monochamus alternatus* is distributed in Asia, including Japan, and the damage caused by this species is ca. 400,000 m<sup>3</sup> of pine trees per a year in Japan (Kobayashi et al. 1984; Forestry Agency 2018). *Moechotypa diphysis* is an invasive pest of Shiitake mushroom (*Lentinula edodes*) bed logs from oak trees in Japan (Furukawa and Nobuchi 1996). We discuss new technologies for vibration exciters and the potential use of vibrational pest management against longicorn beetles and other pests.

## 20.2 Vibration Sense Organs

### 20.2.1 Chordotonal Organs

Chordotonal organs in insects are internal proprioceptors that measure positions and movements of limb joints or the body wall, and they are distributed ubiquitously in body appendages/segments (Field and Matheson 1998). As schematized in Fig. 20.2a, the sensing unit of a chordotonal organ is a bipolar sensory neuron, in which stretch-sensitive mechanosensory channels are distributed in the sensory cilia of the dendrite. The dendritic tip is inserted in the scolopale cap supported by the scolopale rod, which is further connected to a chord-like attachment cell enriched with microtubules.

The number of sensory neurons varies among distinct chordotonal organs located in different body positions in the same species. Some proprioceptive chordotonal organs are able to detect fast and small displacements (i.e., vibrations) transmitted through attachment cells (e.g., Field and Pflüger 1989; Pflüger and Field 1999). The vibrational sensitivities of chordotonal organs are usually higher than those of other



**Fig. 20.2** Leg chordotonal organs in longicorn beetles. (a) The basic structure of a chordotonal organ (CO) showing the ciliated dendrite with  $9 \times 2 + 0$  axoneme configuration (right inset, derived from the tree weta, *Hemideina femorata*) and the microtubule-rich attachment cell (left inset, derived from *H. femorata*). (b, c) Low-magnification images of pro- and metathoracic femoral chordotonal organs (FCOs) in *Monochamus alternatus*, showing that the scoloparium (sco) containing sensory neurons is connected to the long cord-like cuticular apodeme (apo). The scoloparium is located more distally in the metathoracic FCO than in the pro- and mesothoracic FCOs. Adapted from photos of Takanashi et al. (2016). (d, e) Tibio-tarsal CO and tarso-pretarsal COs contain approximately 16 and 10 sensory neurons, respectively, and have cell bodies of varied sizes. (f–h) Differential labeling with rhodamine and phalloidin showing sensory neurons (magenta) and scolopale rods (green) in the prothoracic FCOs in *M. alternatus* (f, g) and *Moechotypa diphysis* (h). Scale bars = 100 nm in left inset; 50 nm in right inset; 500  $\mu$ m in b and c; 50  $\mu$ m in d, e, f, h; 10  $\mu$ m in g

types of sense organs such as campaniform sensilla and hair sensilla, which detect low-frequency vibrations (Kühne 1982; Ai et al. 2010).

The chordotonal organs in coleopterans have been one of the least studied sense organs because the thick exoskeleton hinders dissection and direct observations of these organs. Our extensive survey using retrograde labeling of nerve trunks originating from thoracic ganglia in *M. alternatus* revealed that in the periphery of the ganglion, there are no subgenual organs or tympanal organs specialized for detecting substrate vibrations or airborne sounds, respectively (Takanashi et al. 2016). This view is in agreement with a previous report on coleopteran species (Schneider 1950).

By backfill staining of the leg nerve of longicorn beetles, we identified on each of the six legs a femoral chordotonal organ (FCO), a tibio-tarsal chordotonal organ, and a tarso-pretarsal chordotonal organ (Fig. 20.2b–e; Takanashi et al. 2016), all three of which potentially detect substrate vibrations (Field and Pflüger 1989; Goodwyn et al. 2009). Among these chordotonal organs, the FCO contained far higher numbers of sensory neurons (Fig. 20.2d–h; Takanashi et al. 2016). We further investigated whether the FCO is the primary vibration detector in the legs.

## 20.2.2 Morphologies of Femoral Chordotonal Organs

The FCOs of *M. alternatus* share fundamental structures with those in other insect species (Takanashi et al. 2016). The sensory neurons are distally connected via a bundle of attachment cells to the cuticular apodeme, which extends from the dorsal region of the joint pivot of the tibia (apo, Fig. 20.2b, c; Shelton et al. 1992). Therefore, the tibial flexion and extension are converted to distal and proximal apodemal displacements and, in turn, evoke mechanical distortion on the dendrites of sensory neurons. Sensory neurons are functionally specialized for detecting position, velocity, and/or acceleration in particular ranges of femoro-tibial angles; this functional specialization is called range fractionation (Hofmann et al. 1985; Matheson 1990; Büschges 1994; Sauer and Stein 1999).

The main body of the FCO that embeds sensory neurons is called the scoloparium (sco, Fig. 20.2b, c; Takanashi et al. 2016). The FCO in each leg of *M. alternatus* has a single scoloparium, and no clear anatomical subdivision is detectable within it. The scoloparial location somewhat differs between legs: it is located in the proximal third of the femur for pro- and mesothoracic FCOs (Fig. 20.2b) but in the proximal half for the metathoracic FCO (Fig. 20.2c; Takanashi et al. 2016). The different lengths of the apodeme resulting from distinct scoloparial locations may affect the resonant frequency at which each apodeme transmits substrate vibrations, but their functional differences remain to be studied.

The number of sensory neurons embedded in the scoloparium is nearly identical between pro-, meso-, and metathoracic FCOs (Takanashi et al. 2016). As in FCOs of other insects, two neighboring sensory neurons are paired to extend dendrites into a common scolopale cap; these paired neurons are referred to as heterodynal (Fig. 20.2g; Field and Matheson 1998). The number of sensory neurons in the

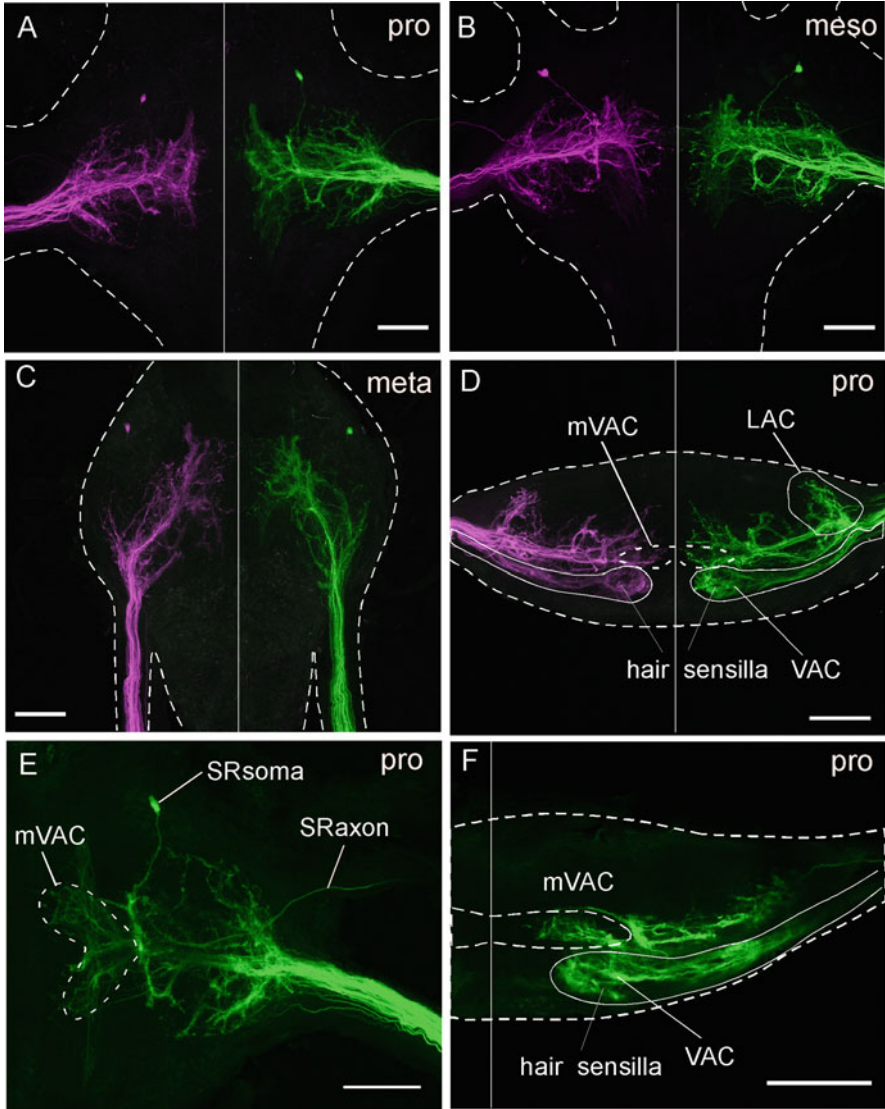
scoloparium is, therefore, countable by checking the number of scolopale caps or rods. The scolopale rods are rich with actin filaments, so they can be visualized by phalloidin staining (Fig. 20.2f–h; Nishino et al. 2016). For example, the number of scolopale rods in the prothoracic FCO of *M. alternatus* is 37, and the estimated number of sensory neurons is therefore 74 (Fig. 20.2f). In *M. diphysis*, the number of scolopale rods in the prothoracic FCO is 41, so there are an estimated 82 sensory neurons (Fig. 20.2h). We found FCOs of the longicorn beetle *Mesosa longipennis*, a pest of oak and conifer trees, in similar locations on all legs; although the exact number of sensory neurons in each FCO has not yet been determined.

### 20.2.3 *Central Projections of Sensory Neurons in the Femoral Chordotonal Organs*

Since the FCO of *M. alternatus* is innervated by a long sensory nerve diverged from the main leg nerve (FCO nerve, Fig. 20.2b, c; Takanashi et al. 2016), by immersing the cut end of the FCO nerve into a dye-filled capillary, we could trace sensory axonal fibers projecting to the central nervous system. The FCO nerve contains sensory axons of the FCO, in addition to those of hair sensilla located in the antero-dorsal surface of the femur. Bilateral labeling of FCO nerves in the pro-, meso-, and metathoracic legs revealed extensive projections of FCO axons from lateral to medial in the ipsilateral ganglion (Fig. 20.3a–c). These projections, resembling those of FCOs in other insects (Field and Pflüger 1989; Nishino 2003), were entirely segregated from those of hair sensilla, which nearly exclusively occupy the ventral association center (Fig. 20.3d, f; Pflüger et al. 1981; Newland 1991).

Most characteristically, the FCO axon terminals enter the medio-ventral association center specialized for vibratory/auditory processing (Fig. 20.3d, e), in addition to the motor association neuropil, the lateral association center located more laterally to the medio-ventral association center (Fig. 20.3d; Pflüger et al. 1981; Pflüger et al. 1988; Mücke and Lakes-Harlan 1995). The medio-ventral association center is conserved among different insect orders (Boyan 1993). Retrograde labeling of nerve trunks originating from thoracic ganglia indicated that in longicorn beetles, the FCO is the primary source that feeds axons to the medio-ventral association center region (Nishino, unpublished observation). There is no indication that single neurons project exclusively to the medio-ventral association center, but they appear to possess side branches to other areas, including the lateral association center. Thus, the overall morphologies of single sensory axons resemble those of subgenual organs in orthopteran insects (Mücke and Lakes-Harlan 1995; Stein and Sauer 1999; Nishino and Field 2003; Stritih Peljhan et al. 2019).

Together with the lack of anatomical subdivision in the FCO of *M. alternatus* (Takanashi et al. 2016), we presume that individual sensory neurons might be bifunctional, detecting low-frequency vibrations as well as mediating some



**Fig. 20.3** (a-c) Central projections of femoral chordotonal organs in *Monochamus alternatus*. Bilateral labeling of femoral chordotonal organ (FCO) nerves with different fluorescent dyes (rhodamine, FITC) in pro-, meso-, and metathoracic legs show mirror-image projections on either side of a midline (solid line). (d, f) Transverse sections show extensive projections of FCO afferents included in the lateral association center (LAC) and the medio-ventral association center (mVAC) of the ipsilateral ganglion, which are entirely segregated from those of hair sensilla projecting exclusively to the ventral association center (VAC). (e) A presumed strand receptor (SR) with a cell body in the central nervous system (Bräunig 1982) is present in each hemisphere of the ganglion. The ganglion was outlined by broken lines in (a-d) and (f). Scale bars = 100 μm

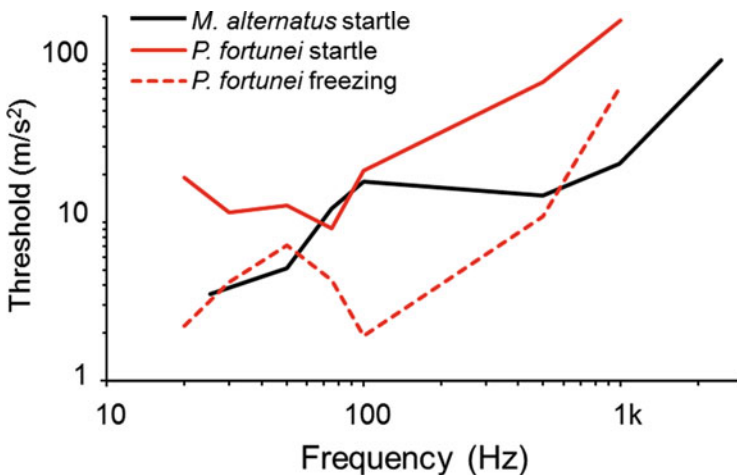
proprioceptive feedbacks, such as resistance reflex in tibial extensor/flexor muscles (Field and Burrows 1982; Sauer and Stein 1999).

## 20.3 Behavioral Control with Vibrations

### 20.3.1 Freezing and Startle Responses and Related Sense Organs

Freezing and startle responses to vibrations are found in *M. alternatus*, *P. fortunei*, and the house longhorn beetle *Hylotrupes bajulus* (Breidbach 1986; Tsubaki et al. 2014; Takanashi et al. 2016). Beetles respond to vibrations while walking by freezing. A startle response—that is, small movement of the legs and antennae—is induced when vibrations are applied to beetles under quiescence. This response is similar to the vibration-induced startle response in the locust *Schistocerca gregaria* under quiescence (Friedel 1999). *Monochamus alternatus* and *P. fortunei* show high sensitivity to frequencies of 20–500 Hz, with response thresholds of 2–20 m/s<sup>2</sup> (Fig. 20.4). In *P. fortunei*, vibrations from approaching conspecifics are far enough above the behavioral thresholds to induce the responses, which allow them to recognize approaching conspecifics or predators (Tsubaki et al. 2014; Takanashi et al. 2019). In addition to these two responses, *M. alternatus* begins walking in response to vibrations at 100 Hz (Takanashi et al. 2016).

To confirm that FCOs detect vibrations, the scoloparia attached to the apodemes of all six femora of beetles were removed with microscissors to produce



**Fig. 20.4** Thresholds of behavioral responses to vibrations in *Monochamus alternatus* and *Paraglenea fortunei*: startle responses during quiescence (solid lines) in both species and freezing response during walking in *P. fortunei* (dashed line). Reproduced from Takanashi et al. (2019)





**Fig. 20.5** *Moechotypa diphysis* exhibiting tonic immobility after dropping from an oak log in response to vibrations. Courtesy of Shuji Fukui

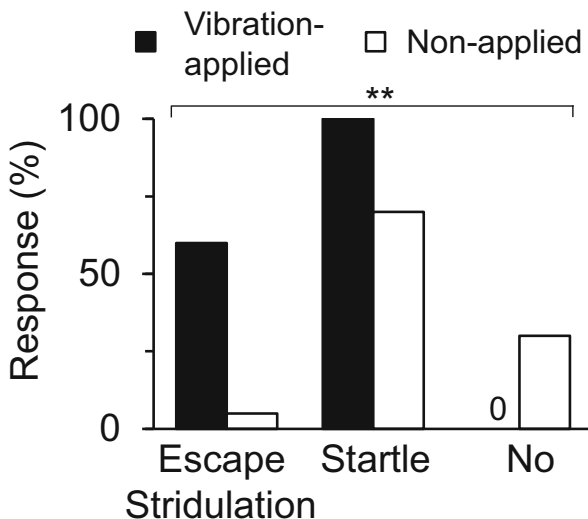
dysfunctional FCOs (Takanashi et al. 2016). *Monochamus alternatus* individuals with altered FCOs on all legs did not freeze in response to 100 Hz and 1 kHz while walking, whereas intact and sham-operated beetles froze. These findings indicate that the FCO is responsible for detecting low-frequency vibrations. Thus, the sense organ involved in the freezing behavior has been identified in a coleopteran species.

In *M. diphysis*, we investigated startle response thresholds to vibrations of 100 and 120 Hz. We observed a startle response in individual adults during a period of quiescence on a steel plate attached to a vibration exciter with variable accelerations, as previously reported in Tsubaki et al. (2014). Mean response thresholds were  $0.86 \text{ m/s}^2$  at 100 Hz and  $0.80 \text{ m/s}^2$  at 120 Hz ( $n = 10$ ); thus, *M. diphysis* is more sensitive to vibrations than *M. alternatus* and *P. fortunei*. This finding is in agreement with casual observations in the field that even small-amplitude vibrations that do not evoke any detectable reactions in the two species, *M. alternatus* and *P. fortunei*, can induce drop-off behaviors in *M. diphysis* (Furukawa and Nobuchi 1996; Tsubaki et al. 2014; Takanashi et al. 2019). Immediately after dropping to the ground, *M. diphysis* often displays tonic immobility (thanotaxis) with femoro-tibial joints in extended positions at angles of various degrees (Fig. 20.5).

### 20.3.2 Behavioral Manipulation with Vibrations

Low-frequency vibrations can be used to manipulate *M. alternatus*, *P. fortunei*, and *M. diphysis* to produce startle, freeze, and walk responses. Furthermore, low-frequency vibrations are predicted to disrupt feeding, oviposition, and other behaviors because freezing entails the sudden cessation of any ongoing behavior. A new procedure for pest management that uses vibrations to control behaviors of longicorn beetles has been developed (Takanashi et al. 2019). A prototype of a weather-resistant vibration exciter was made from giant magnetostrictive material (GMM) as a new technology for generating large-amplitude vibrations. In GMM, an

**Fig. 20.6** Rates of various behavioral responses in vibration-applied and non-applied individuals of *Monochamus alternatus*. The number of responses was significantly different between the two groups according to Fisher's exact test (\*\* $p < 0.001$ ,  $n = 20$ )

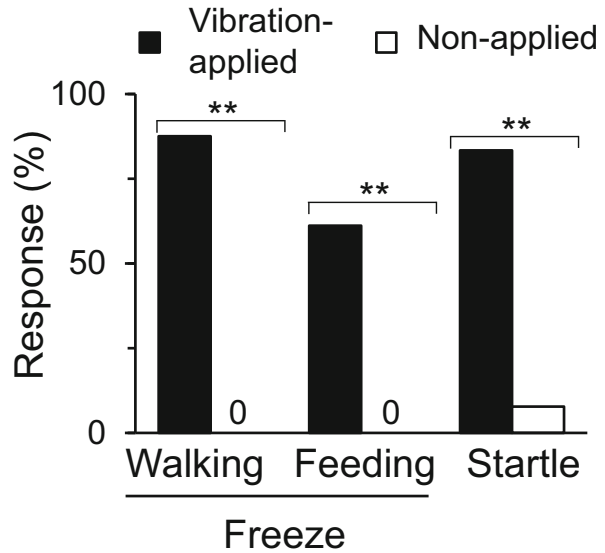


alloy of iron and rare metals, a large strain, called magnetostrain, is induced by a magnetic field (Söderberg et al. 2004).

Behavioral responses to vibrations were evaluated in *M. alternatus* individuals when they are quiescent by using a custom-made GMM vibration exciter (90 mm in length, 15 mm in diameter; Fig. 20.8). Vibrations at 100 Hz were generated for 1 to 2 s by the amplifier and function generator of the GMM vibration exciter. The vibration stimuli were applied to the femur of a prothoracic leg via a steel wire (0.6 mm in diameter, 85 mm in length; Fig. 20.2). An accelerometer with an attached data logger recorded the amplitude and other characteristics of the vibrations, as described in Takanashi et al. (2016). Amplitude at the tip of the wire was set to  $5 \text{ m/s}^2$ , which is larger than the threshold amplitude of the startle response in *M. alternatus*. In vibration-applied individuals, 60% showed escape behavior or stridulation (i.e., sound production) and 100% showed a startle response to vibrations transmitted through the wire (Fig. 20.6). In contrast, in non-applied individuals, only 5% showed escape behavior or stridulation and 70% showed a startle response to the mechanical stimulation of contacting the wire without vibrations. No response was observed in 30% of non-applied individuals. These results suggest that vibrations from the GMM vibration exciter enable behavioral manipulation of this species.

Behavioral responses of *M. diphyis* were observed to respond to vibrations produced by a different GMM vibration exciter (200 mm in length, 50 mm in diameter) (Fig. 20.7; Takanashi et al. 2019). Pulsed vibrations at 100 Hz for 1 s generated at intervals of 9 s by the amplifier and function generator of the GMM vibration exciter were applied to the bottom of a vertically oriented mushroom bed log (*Quercus crispula*; 80 mm in diameter, 740 mm in length). The accelerometer described above was used to adjust the acceleration of the vibration at the middle of the log to  $>1.5 \text{ m/s}^2$ , which is larger than the thresholds of the startle response in

**Fig. 20.7** Rates of various behavioral responses in vibration-applied and non-applied individuals of *Moechotypa diphysis*. The vibration-applied individuals showed significantly higher responses than the non-applied individuals by Fisher's exact test (\*\* $p < 0.001$ ,  $n = 8-18$ )



*M. diphysis*. Overall, the vibration-applied individuals showed much higher rates of behavioral responses than the non-applied individuals (Fig. 20.7). Freeze during walk, freeze during feeding, and startle response during quiescence were induced in 61–88% of vibration-applied individuals and in 0–8% of non-applied individuals. These experiments reveal that a GMM vibration exciter can be used to manipulate behaviors of *M. alternatus* and *M. diphysis* and suggest that this procedure may be useful for behavioral disruption in pest management.

## 20.4 Pest Management with Vibrations

Many species belonging to both hemimetabolous and holometabolous insect orders, including Hemiptera, Coleoptera, Diptera, and Lepidoptera, exhibit sensitivity to vibrations from the larval to adult stages (Greenfield 2002; Cocroft and Rodríguez 2005; Hill 2008; Scott et al. 2010; Mazzoni et al. 2013; Takanashi et al. 2019; Kishi and Takanashi 2019b; Hofstetter et al. 2019). Since these insects are sensitive to vibrations, artificial vibrations can be applied to disrupt their various behaviors. Low-frequency vibrations at high acceleration that are above behavioral thresholds are necessary for the procedure to succeed (Takanashi et al. 2016). A GMM vibration exciter is suitable for generating vibrations to disrupt the behaviors of target pests. In addition, intermittent application of vibrations at sufficient intervals can help avoid the problem of sensory adaptation and behavioral habituation to vibrations (Fig. 20.8) (Friedel 1999; Kishi and Takanashi 2019a).



**Fig. 20.8** A schematic of the vibrational pest control procedure. A vibration exciter made with giant magnetostrictive materials is attached to a tree, and it generates vibrations to disrupt behaviors of insects. Scale bar = 50 mm. Adapted from a photo of Takanashi et al. (2019)

In this study, vibrations from a GMM vibration exciter disrupted feeding and walking in *M. diphyis*, because freezing results in the cessation of ongoing behavior. Vibrations also promoted startle and escape responses in *M. alternatus* and *M. diphyis*. Disruption of residence in *M. alternatus* was demonstrated by using an exciter attached to a tree in the field (H. Sakamoto, T. Koike, N. Fukaya, T. Takanashi, in preparation). In addition, preliminary experiments have shown that the feeding of *M. alternatus* is disrupted by vibrations (T. Takanashi et al., unpublished data). The GMM vibration exciter appears to be a useful tool for vibrational management of longicorn beetle pests (Fig. 20.8). Low-frequency vibrations from the exciter are expected to be able to disrupt feeding, oviposition, and residence of longicorn beetles on host trees. It is also possible to use vibrations to enhance repellency by inducing the pests to escape from the host tree, thus causing disruption of residence.

Furthermore, a vibration exciter that uses GMM technology is able to generate vibration on various substrates (e.g., crops in greenhouses). We predict that vibrations can be applied in trees and crops damaged by various pests that exhibit vibration sensitivities. In the future, studies should explore and resolve installation of exciters and jigs for vibrational transmission, as well as ways to reduce the cost of the exciter to make it commercially practical. Vibrational pest management technologies that help to reduce the use of pesticides may become part of integrated pest

management in the future (Polajnar et al. 2015; Takanashi et al. 2019). Potential negative side effects of the vibrations on plants and non-target beneficial insects must be minimized (Mitchell 1996; Polajnar et al. 2015; Nieri and Mazzoni 2018; Takanashi et al. 2019), and the most appropriate procedures need to be selected for various pests and plants. Vibrational pest management can promote integrated pest management by combining it with several existing and newly developed procedures, including physical technologies (Vincent et al. 2009; Shimoda and Honda 2013).

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