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Acacia Ants Respond to Plant-Borne Vibrations Caused by Mammalian Browsers

Highlights

- Acacia ants discriminate browser-induced vibrations from wind-induced vibrations
- Browser-induced vibrations serve as a long-distance alarm cue for ants
- Acacia ants make use of tropotactic directional vibration sensing

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In Brief

Hager and Krausa show that the acacia ant *Crematogaster mimosae* defends its host tree by exploiting plant-borne vibrations caused by browsers feeding on the tree. Browser-induced vibrations serve as a long-distance alarm cue, and the ants make use of tropotactic directional vibration sensing to orient to the attacked part of the tree.



Acacia Ants Respond to Plant-Borne Vibrations Caused by Mammalian Browsers

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SUMMARY

Living in the African savanna is dangerous, especially for plants. Many plants therefore engage in mutualism with ants, in which plants provide food and shelter in exchange for protection against herbivores. Ants become alarmed when the plant takes on some sort of damage. They immediately emerge from their plant shelter and aggressively defend the plant. Mammalian herbivores can have devastating effects on trees by browsing, breaking tree branches, stripping bark, and pushing over entire trees. However, mutualistic ants substantially reduce the amount of damage. To efficiently protect the tree, ants need to rapidly react together when the tree is under attack. Here, we show that the acacia ant *Crematogaster mimosae* defends its host tree by exploiting plant-borne vibrations caused by browsers feeding on the tree. Experiments with controlled vibrations show that ants discriminate browser-induced vibrations from those induced by wind, become alarmed, and patrol on the branches. Browser-induced vibrations serve as a long-distance alarm cue. The vibrations propagate through the whole acacia tree and trigger ants' defensive behavior, even on the other side of the tree. Furthermore, the ants make use of tropotactic directional vibration sensing to orient to the attacked part of the tree and fight back the attacker.

INTRODUCTION

Acacia trees are prominent and integral features of the East African savanna. In this region, the genus *Acacia* comprises 62 species and is one of the most common plant genera [1]. Many browsers rely on acacia trees. Both domestic livestock and wild herbivorous mammals feed on acacia leaves and branches [1]. Acacias have evolved an array of defense mechanisms to protect against herbivore damage. These include straight and hooked thorns, secondary metabolites that act as repellents or toxins, and the engagement in a mutualistic relationship with ants [2, 3]. Many studies have demonstrated the ability of ants to protect plants against damage [3–6]. By deterring browsing herbivores, ants influence several parameters of plant fitness, as plants without ants suffer a reduction in biomass, leaf production, and reproduc-

tion [5]. In return, the acacia provides food and nesting space. Ants find year-round carbohydrate-rich food offered in extrafloral nectaries. In the growth season, ants drill holes in newly produced swollen thorns when their tissue is still soft. The acacia's swollen thorns are then used as domatia in which ants live and rear their brood [7]. About eleven African acacia species are so-called swollen-thorn acacias that are associated with around 20 ant species mainly belonging to the genus *Crematogaster* [7].

While the acacia's protection by thorns is continuous, the indirect ant-mediated defense is an induced behavioral response. Once ants notice an herbivorous attack, they immediately emerge from their plant shelter and aggressively defend the plant [8]. Ants deter both vertebrate and invertebrate herbivores [3]. Ants defend the tree by swarming on the body of the herbivore and biting or stinging into tissue (Figure 1). For an efficient defense against mammalian herbivores, ants must detect cues associated with browsing, orient to the browser, and fight the attacker as quickly as possible. In studies of animal communication, cues are defined as incidental sources of information that are detected by unintended receivers [9, 10]. Ants could detect the presence of herbivores by using visual, chemical, or mechanical cues. Many browsers are active during the night, when it is too dark to use visual cues. Moreover, for ants inside the domatia, visual cues are generally not available. Therefore, the fast defense reaction of the ants cannot be explained by the usage of visual cues.

Several studies found that ants are attracted by plant sap or solvent extracts of plant parts. After a leaf is damaged or ants are exposed to extracts of volatile compounds from leaf tissue, the number of ants patrolling on the site of damage increased after some minutes [11–16]. The propagation of chemical cues is relatively slow compared to the propagation of visual or mechanical cues. Furthermore, the detectability and localizability of chemical cues highly depends on air currents [17]. Thus, volatiles might spread too slowly to inform ants in time about the herbivore's presence. Especially under windy conditions, chemical cues may not reach ants located on the windward side of the tree. Cues that would overcome those limitations are mechanical vibrations associated with browsing. Some studies indicate that the shaking of acacia branches results in ants quickly swarming out to defend [13, 18–20]. However, the effects of these non-standard shakes are described rather anecdotally, and the studies do not exclude other cues accompanying the plant-borne vibrations. Whether plant-borne vibrations induced by browsing serve as a cue to detect the browser and defend the tree has not been investigated so far [21].

Mechanoreception is suggested to be evolutionarily ancient among animals [22, 23]. The usage of vibrational cues and signals





Figure 1. Acacia Ants on their Host Tree and a Browsing Goat

(A) *Crematogaster mimosae* feeding at an extrafloral nectary of *Acacia zanzibarica*.
 (B) *C. mimosae* patrolling on an acacia branch.
 (C) A goat browsing on leaves of *A. zanzibarica*. Note that ants swarm on the goat's head to deter it.

RESULTS

Ant Activity

In our study area, most *Acacia zanzibarica* trees have a single resident ant colony, although the colony may occupy multiple trees. Two ant species are common residents on the acacia trees. Most trees (70.2%) are occupied by *Crematogaster mimosae* (*C. mimosae*), fewer

is widespread in insect social and ecological interactions. Only recently was the term biotremology introduced to formalize this field of study [24]. It is estimated that 92% of insect species use substrate vibrations alone or with other forms of mechanical signaling [25]. Insects not only use vibrational cues in the context of prey localization and predator avoidance, but also employ vibrational signals to communicate with potential mates and nest-mates [25–27]. Ants use vibrational cues and signals in the context of recruitment, nest excavation, and alarm sounding [28–32].

In insects, vibration receptors are mainly positioned in all six legs [33]. Thus, insects are particularly sensitive to vibrations traveling through the substrate they stand on. The vibrations could bear information that enables the receiver to localize and orient toward their source.

To date, more than 50 behavioral studies demonstrate the ability of insects to localize the source of vibration. Insects could use different behavioral strategies to encode directional information. They could either compare measurements over time (klinotaxis) or compare the inputs of at least two receptors (tropotaxis) [34]. For a leaf-cutting ant, for example, it was shown that they are capable of vibrotropotactic orientation by comparing time-of-arrival differences of the vibrations between their legs [35].

In this study, we asked whether the African acacia ant *Crematogaster mimosae* uses plant-borne vibrations to protect its host tree *Acacia zanzibarica*. In the natural environment, plant-borne vibrations are elicited by various sources that create noise. Noise creates problems in reliable detection and deciphering of information [36]. For an efficient defense of the host tree, ants should be able to discriminate wind-induced vibrations from vibrations induced by browsing herbivores. Browser-induced vibrations should elicit defensive responses, whereas wind-induced vibrations should not elicit any reaction. The detection of vibrations by ants could simply lead to an increased patrolling activity on the tree and thus to an increased probability of finding the source of vibration, i.e., the browser, or the vibrations could provide directional information. The efficiency of the plant defense would be increased if ants could extract directional information from the plant-borne vibrations. Therefore, we also asked if ants show vibrotropotactic orientation.

trees (13.8%) host *Crematogaster sjostedti*, and 16.0% of the trees do not host ants ($N = 181$). We first assessed the ants' diel activity pattern in relation to wind velocity and temperature. During our observation period, the average wind velocity close to the focal acacia was 2 ms^{-1} (mean, $SD = 0.59$, $n = 57$ h) and on average 16 wind bursts exceeding 4 ms^{-1} per hour occurred (mean, $SD = 15$, $n = 57$ h). Ants of the focal *C. mimosae* colony are active throughout the day and night with only slight variations (Figure 2). The number of *C. mimosae* feeding on extrafloral nectaries increased with increasing temperature ($r = 0.282$, $p < 0.001$, $n = 249$) but did not correlate with average windspeed measured during a 1-min observation period ($r = -0.012$, $p = 0.867$, $n = 215$). The number of ants walking over the branches decreased with both increasing temperature ($r = -0.128$, $p < 0.043$, $n = 249$) and increasing windspeed ($r = -0.197$, $p < 0.004$, $n = 215$).

Plant-Borne Vibrations

We mounted accelerometers on five branches of a tree and measured the plant-borne vibrations induced by wind and a browsing goat. Plant-borne vibrations induced by wind bursts exceeding 4 ms^{-1} were characterized by low frequencies, with most energy below 2 kHz (Figures 3B and 3E). The mean peak amplitude is $1.0 \pm 0.9 \text{ ms}^{-2}$ ($\pm SD$, $n = 25$, $N = 5$). The vibrational amplitude of branches shaking in the wind highly depends on the accelerometer's mounting place on the branch, with increasing amplitude toward the tip of the branch. Plant-borne vibrations induced by a browsing goat clearly show higher-frequency components up to 6 kHz (Figure 3C). A video analysis showed that the highest vibrational amplitudes are produced when the goat removes a leaf from the branch. The mean peak amplitude is $26.7 \pm 10.1 \text{ ms}^{-2}$ ($\pm SD$, $n = 25$, $N = 5$). To produce plant-borne vibrations in a reproducible manner, we used a custom-made vibration exciter (henceforth, artificial browser; Figure 3A) that was calibrated to imitate browser-induced vibrations. The frequency components of plant-borne vibrations induced by the artificial browser are in the same range as those induced by the browsing goat (Figures 3C, 3D, 3F and 3G). The mean peak amplitude is $1.84 \pm 1.4 \text{ ms}^{-2}$ ($\pm SD$, $n = 25$, $N = 5$).

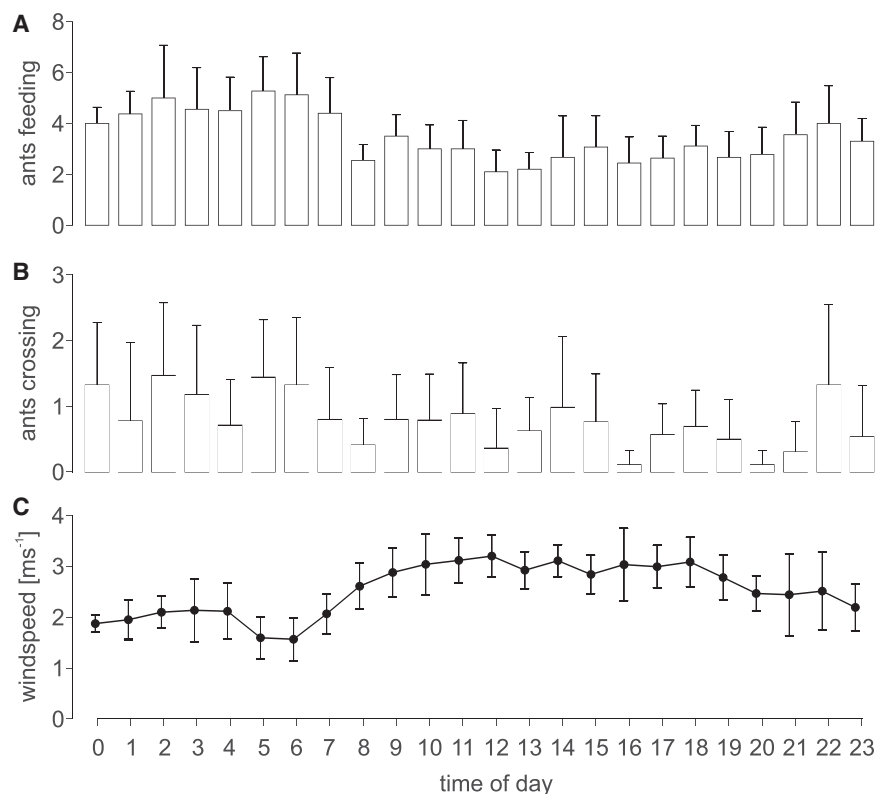


Figure 2. Diel Activity of *Crematogaster mimosae* and Windspeed

Observations were made in intervals of 20 min throughout day and night.

(A) Number of ants feeding on extrafloral nectaries immediately before 1-min observation periods.

(B) Number of ants crossing a ring marking within 1-min observation periods.

(C) Average windspeed during 1-min observation periods.

The bars and line indicate the mean; error bars indicate the standard deviation, $n = 8\text{--}14$ per h.

The peak correlation coefficient resulting from cross-correlation of frequency spectrograms was used as a measure for similarity between different sources of plant-borne vibrations (Figure 3H). If wind-induced vibrations are compared with vibrations caused by the browsing goat or by the artificial browser at the same branch, the correlation coefficient takes relatively low values of $r = 0.465 \pm 0.081$ (mean \pm SD, $n = 25$) and $r = 0.45 \pm 0.105$ (mean \pm SD, $n = 25$), respectively, indicating a relatively low similarity of frequency spectrograms. A comparison between goat- and artificial browser-induced vibrations instead reveals that the frequency spectra are relatively similar ($r = 0.75 \pm 0.099$, $n = 25$). If goat- and artificial browser-induced vibrations are compared among themselves, the correlation coefficient takes similarly high values of $r = 0.775 \pm 0.113$ (mean \pm SD, $n = 20$) and $r = 0.742 \pm 0.172$ (mean \pm SD, $n = 20$), respectively. This analysis shows that the vibration exciter (artificial browser) is suitable to imitate vibrations induced by a browser.

Attenuation of Browser-Induced Plant-Borne Vibrations

With increasing distance to the impact point, i.e., to the artificial browser, the peak amplitude of the vibration traveling through the branches decreases (Figure 4; Pearson's, $r = 0.987$, $p < 0.001$, $n = 70$). The attenuation per centimeter along the acacia branches is 0.15 dB (linear regression analysis ANOVA, $F = 2650.107$, $p < 0.001$, $n = 70$).

Plant-Borne Vibrations and Ant Activity

Single wind gusts above 4 ms^{-1} are strong enough to visibly shake the branches. Ants should be able to discriminate wind-induced vibrations from browser-induced vibrations. Vibrational stimuli

induced by the artificial browser led to an increasing number of ants moving on the branches close to the impact point. During 30 s of vibrational stimulation, significantly more ants crossed a ring marking compared to the previous 30 s without stimulation (Figure 5; Wilcoxon signed-rank test, $z = 5.894$, $p < 0.001$, $n = 100$, $N = 10$). In contrast, vibrations induced by wind did not have such effect on the ants' behavior (Figure 5; Wilcoxon signed-rank test, $z = 0.545$, $p > 0.586$, $n = 100$, $N = 10$). These two experiments do not only differ in the frequency components of the plant-borne vibrations but

also in the wind velocity. The wind velocity could be utilized by the ants directly to decide whether to patrol on the branches after the detection of plant-borne vibrations. If this would be the case, ant activity in the artificial browser experiments should correlate negatively with wind velocity, which is not the case (Pearson's correlation coefficient, $r = -0.042$, $p = 0.836$, $N = 37$).

Long-Distance Alarm Cues

We furthermore assessed whether the plant-borne vibrations may suit as a long-distance alarm cue. Artificial browser-induced plant-borne vibrations led to an increase in ant activity far away from the vibration source. On branches around 2 m away from the vibration source, significantly more ants patrol on the branches after the onset of vibrational stimulation compared to the previous 30 s without stimulation (Figure 6A; Wilcoxon signed-rank test, $z = 2.972$, $p < 0.003$, $n = 60$, $N = 6$). Moreover, the ants show a directional defense response: more ants walk toward than away from the source of vibration (one-sample t test, $t = 4.811$, $p < 0.005$, $n = 60$, $N = 6$). In a control experiment without vibrational stimulation, ants cross the ring marking in both directions (toward and away from the vibration source) without a preference for one side (one-sample t test, $t = 1.711$, $p < 0.148$, $n = 60$, $N = 6$). This reveals that ants are capable of extracting directional information from the plant-borne vibrations. However, this experiment does not enable us to tell whether the ants' orientation is a klino- or tropotaxis.

Directional Vibration Sensing

To find out whether the ants orient through klino- or tropotaxis, we conducted a focal ant experiment. We observed the

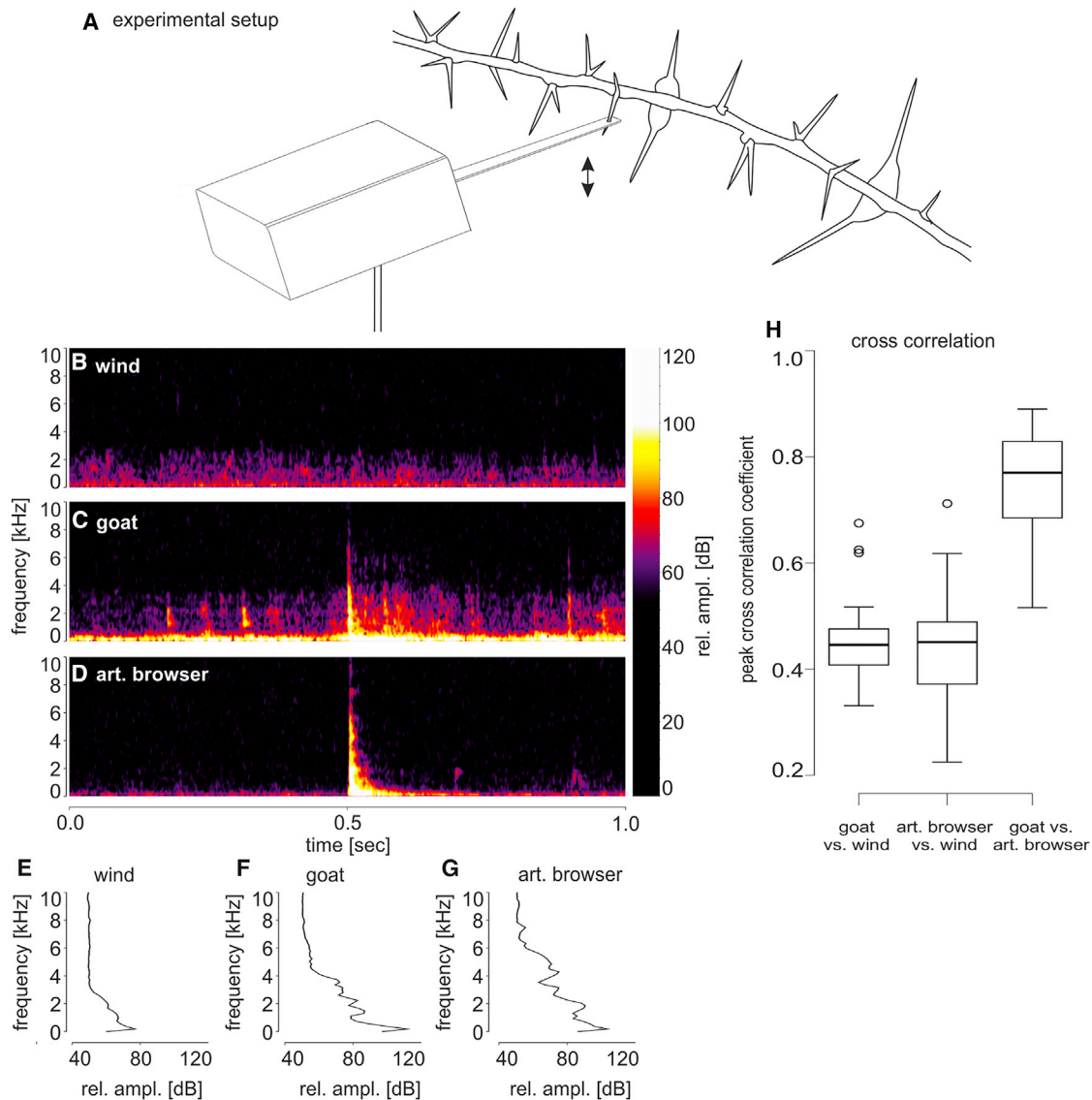


Figure 3. Plant-Borne Vibrations of *Acacia zanzibarica* Branches

(A) Artificial browser tightly coupled to a thorn.

(B) Frequency spectrograms of vibrations induced by a wind burst exceeding 4 ms^{-1} . Most energy is found below 2 kHz.

(C and D) Frequency spectrograms of vibrations induced by a browsing goat (C) and the playback device (D), i.e., the artificial browser, show higher-frequency components (band-pass filter, 10 Hz to 10 kHz; 256 samples).

(E–G) Frequency spectra of vibrations induced by wind (E), a browsing goat (F), and the playback device (G).

(H) Similarity of wind-, goat-, and artificial browser-induced plant-borne vibrations. Five recordings of plant-borne vibrations induced by different sources were made on five branches ($N = 5$, $n = 25$) and peak cross-correlation coefficients of frequency spectrograms (256 samples) were calculated. The plant-borne vibrations induced by a browsing goat are similar to those induced by the artificial browser. Goat-wind and artificial browser-wind comparisons show smaller similarities. Boxes show first and third quartiles; bold lines indicate medians; whiskers represent $1.5 \times$ the interquartile range; and circles indicate outliers.

directional response of individual ants to vibrational stimulation in 200 trials, equally distributed over ten branches. Focal ants fed at extrafloral nectaries. As a response to vibrational stimulation, ants could either walk toward the source of vibration or away from the vibration source. In only six cases, the focal ant walked neither toward nor away from the artificial browser within the 10 s of observation. Ants reacted to the vibration stimuli after 4.5 s (mean; $SD = 4$, $n = 194$). Significantly more ants turned toward the source of vibration than to the other side (Figure 7;

t test, $t = 5.594$, $p < 0.001$, $n = 200$, $N = 10$). In the control experiment, ants do not show a side preference (Figure 7; one-sample t test, $t = 1.013$, $p < 0.337$, $n = 200$, $N = 10$).

DISCUSSION

The mutualism between ants and the acacias has a stabilizing effect on the megaherbivore-driven landscape change in the African savanna, and its disruption has dramatic ecological

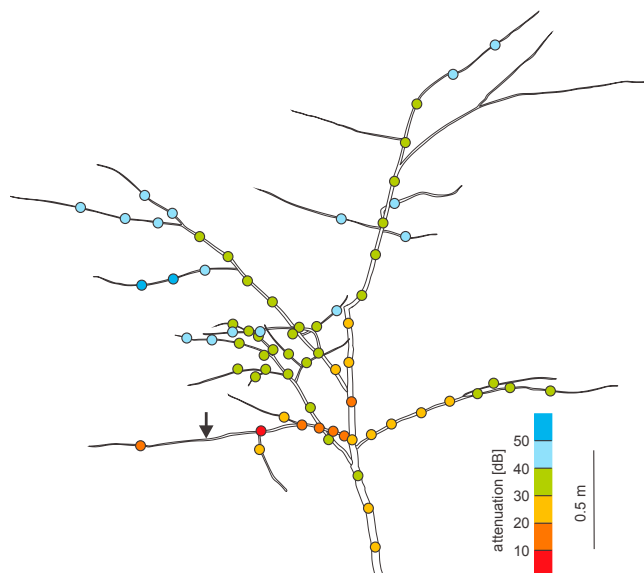


Figure 4. Attenuation of Vibrations Induced by the Artificial Browser along Branches of an *Acacia zanzibarica* Tree

Arrow indicates the impact point and the position of the reference accelerometer; circles indicate positions of a second accelerometer. Note that the tree is perspective-pictured, and the given scale applies only to the vertical axis. Measurement positions were either 20 cm apart from each other or at a 20-cm distance to a branching point.

effects [37]. After 10 years of mammalian herbivore exclusion, trees reduced their investment in nectar and domatia production. Consequently, the nectar-dependent and defending mutualistic ants disappeared. Trees in the following period suffered increased attacks by stem-boring beetles, grew more slowly, and experienced doubled mortality relative to that of trees occupied by the mutualistic ant [38]. The mutualistic ants strongly defend trees against elephants, which can have dramatic impacts on tree cover. The loss of mutualistic and defending ants leads to a 5- to 7-fold increase in the number of trees catastrophically damaged by elephants [39]. Ants act as an indirect defense by controlling the damage of herbivores, which ultimately increases the trees' fitness [40]. Vibrational cues play a crucial role for the defensibility of this mutualistic relationship.

Plant-Borne Vibrations

To consume acacias with thorns, giraffes and many other browsers nimbly work around the thorns to pull off the leaves. All our attempts to record plant-borne vibrations induced by browsing giraffes failed, and to our knowledge, there are no studies available concerning plant-borne vibrations due to browsing by large mammals. The video analysis of our browsing goat reveals that the highest amplitudes are emitted when a leaf is pulled off and the branch pushes back. The frequency range of vibrations induced by the browsing goat while removing a leaf from a branch is broadband, with high-frequency components up to 6 kHz and high amplitudes of up to 46 ms^{-2} . We expect that giraffes and other large mammals, which browse in a like manner, emit similar plant-borne vibrations.

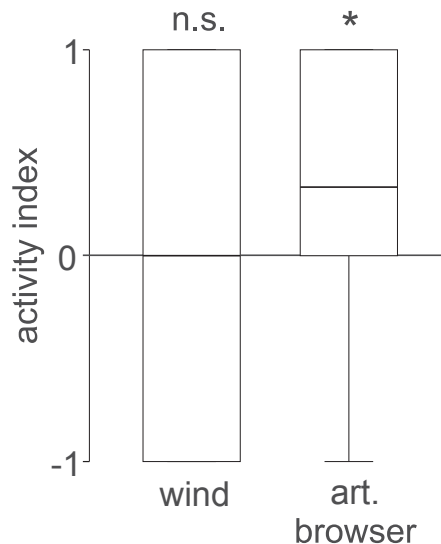


Figure 5. Activity Indices of *Crematogaster mimosae* Related to Two Different Kinds of Plant-Borne Vibrations

After the onset of vibrations induced by wind bursts, ants show neither more nor less activity compared to that during the previous 30 s (Wilcoxon signed-rank test, $z = 0.545$, $p > 0.586$, $n = 100$). After the onset of browser-induced vibrations, significantly more ants crossed the ring marking compared to the previous 30 s without stimulation (Wilcoxon signed-rank test, $z = 5.894$, $p < 0.001$, $n = 100$). Boxes show first and third quartiles; bold lines indicate medians; whiskers show the minimum and maximum; and the asterisk indicates statistical difference.

Besides mechanical vibrations, several other potential cues may indicate the feeding activity of the browser. When the browser damages leaves and breaks branches, plant volatiles are released, and the browser may be sensed by its odor or visually. We used a playback device (artificial browser) to elicit vibrations in a reproducible manner without producing other cues. The similarity analysis shows that artificial browser-induced vibrations closely resemble goat-induced vibrations. Thus, the artificial browser is well suited to imitate browser vibrations.

Without much doubt, chemical cues or signals, such as alarm pheromones, can indicate the presence of a browser. However, these cues might not always be sufficient to be detected by the ants in time. The propagation velocity of volatiles depends on diffusion and air current. Volatiles are relatively slow communication signals. In still air, most pheromone molecules have diffusion coefficients of a few square millimeters per second [17]. The propagation velocity can be increased by the presence of wind, which is an important environmental parameter in determining the structure of a volatile plume [41]. However, air currents may create problems for the detectability and localizability of the volatiles [17, 41].

Volatiles might spread too slowly to inform nestmates in time about an attack. Especially under windy conditions, chemical signals and cues may not reach ants located at the windward side of the tree. Vibrations instead propagate much more quickly. Plant-borne vibrations mostly propagate as bending waves [42–44]. The propagation velocity of bending waves is proportional to the square root of frequency and depends on plant properties, with a velocity of tens to hundreds of meters

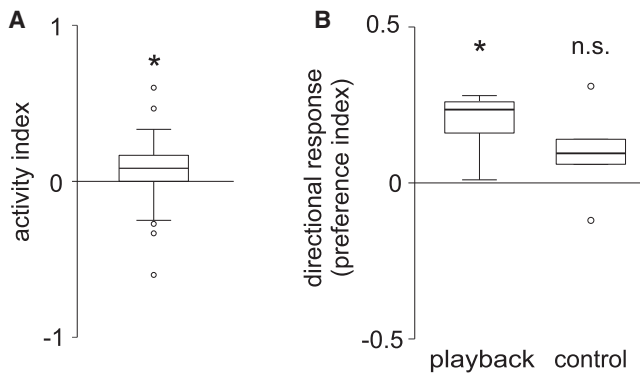


Figure 6. Long-Distance Alarm Cues

Behavioral observations were conducted about 2 m from the vibration source. (A) After the onset of vibrational stimulation (30 s), significantly more ants patrol on the branches compared to the amount during the previous 30 s without stimulation (Wilcoxon signed-rank test, $z = 2.972$, $p < 0.003$, $n = 60$, $N = 6$). (B) Directional defense response. In the control experiment without vibrational stimulation, ants cross the ring marking in both directions (toward and away from the potential vibration source) without a preference for one side (one-sample t test, $t = 1.711$, $p < 0.148$, $N = 6$). Playback of browser vibrations leads to significantly more ants walking in the direction of the source of vibration than in the other direction (one-sample t test, $t = 4.811$, $p < 0.005$, $N = 6$). Boxes show first and third quartiles; bold lines indicate the median; whiskers represent 1.5 \times the interquartile range; the asterisk indicates statistical difference; and circles represent the outliers.

per second [45]. Thus, within a fraction of a second, the vibrations arrive on the other side of the tree and may thereby facilitate a quick alert of ants.

For the many insects that communicate using plant-borne vibrations, wind is considered to be the major source of environmental noise [36]. Male treehoppers, for example, use gap detection to initiate signaling during relatively wind-free periods, and females respond less to signals given in the presence of wind-induced vibrations [36]. However, in our case, a browser induces incidental mechanical vibrations, which serve as cues for the defending ants. It cannot be assumed that browsers show any considerations toward a good cue-noise ratio. As the ants rely on the health of their host trees, there should be a strong selective pressure on adaptations enabling the ants to discriminate wind-induced from browser-induced vibrations.

Ant Patrolling Behavior

Wind peak velocities of 4 ms^{-1} were strong enough to visibly shake acacia branches, and they occurred frequently in our study area. The experiments show that the ants do not increase their patrolling behavior in response to wind-induced vibrations. In contrast, the number of patrolling ants increases as a response to browser-induced vibrations. This clearly shows that the acacia ant *C. mimosae* solves the discrimination task. Furthermore, records of diel activity patterns show that the number of ants walking over the branches decreased with increasing windspeed. If the ants could not discriminate wind-induced from browser-induced vibration, we would find the opposite effect. It is unlikely that the ants make use out of any amplitude differences between wind-induced and browser-induced vibrations, as the vibrational amplitude highly depends on the dis-

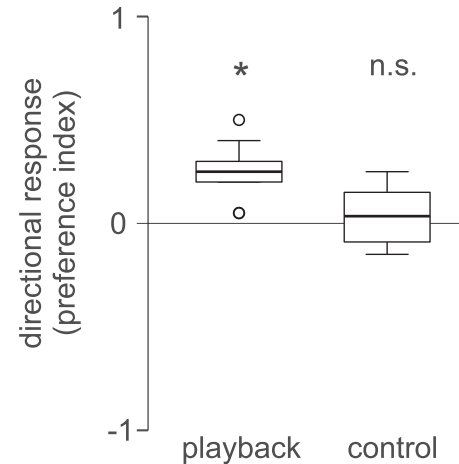


Figure 7. Directional Response of *Crematogaster mimosae*

In the playback experiment, focal ants on acacia branches turn significantly more frequently toward the source of vibration than to the other side (one-sample t test, $t = 5.590$, $p < 0.001$, $n = 200$; $N = 10$). In the control experiment without vibrational stimulation, ants do not show a side preference (one-sample t test, $t = 1.013$, $p < 0.337$, $n = 200$; $N = 10$). Boxes show first and third quartiles; bold lines indicate median; whiskers represent 1.5 \times the interquartile range; asterisks indicate statistical difference; and circles represent the outliers.

tance between the source of vibration and the receiving ant and is therefore not a reliable cue. In contrast, frequency differences have the potential to be discriminated. Wind-induced vibrations are characterized primarily by low-frequency components ($< 2 \text{ kHz}$), whereas browser-induced vibrations have higher-frequency components.

Many behavioral studies show that vibrational communication plays an important role in the context of mate recognition and localization. By exploiting very small differences in the temporal pattern and the frequency components, many insects can discriminate signals from conspecific and non-conspecific insects [26, 27]. In this light, the task of discriminating low-frequency wind-induced vibrations from vibrations caused by browsing animals, which have clearly higher-frequency components, does not seem too difficult.

The motion of branches can occur along any axis within a plane perpendicular to the long axis of the branch [44]. Our behavioral observations reveal that *C. mimosae* ants are preferentially active on the upper side of the branches. Therefore, we measured vibrations perpendicular to the long axis of the branches' upper side. In this way, the branches' maximum vibration amplitude may be underestimated [46], but we gain some insights into which vibrational cues are available to the ants.

Browser vibrations traveling through the acacia are attenuated by about 15 dBm^{-1} . While browsing, the goat produced plant-borne vibrations with an average amplitude of 26 ms^{-2} close to the feeding site. This would lead to vibrational amplitudes at a 3-m distance (on the other side of the tree) of about 0.15 ms^{-2} . There is not much information available about the vibration perception threshold of ants, except for one study about leafcutter ants [47], which are very sensitive to frequencies between 0.05 and 4 kHz and amplitudes down to 0.02 m^{-2} . Assuming a perceptual threshold of *C. mimosae* in the same

range, vibrations produced by a browser feeding on the acacia can be perceived by ants all over the tree. Indeed, our playback experiments reveal that more ants patrol not only on the branches near the vibration source, but also on the other side of the tree.

The playback of browser-induced vibrations leads to an increase of ant patrolling, whereas wind-induced vibrations do not influence the ants' activity. The ants' activity in response to browser-induced vibrations is not affected by wind velocity during the playback experiment. This suggests that the ants in the presence of plant-borne vibrations do not directly measure the wind velocity to decide whether to patrol, but rather make use of different frequency components to discriminate wind- from browser-induced vibrations.

The number of ants patrolling on the acacia branches might increase solely because of the presence of browser-induced vibrations, or they might be reinforced by recruitment of alerted ants. Many studies focusing on the mutualism of ants and acacias show that the number of ants increases at the site of attack [11–16, 18–20]. However, the studies do not enable us to state whether the increasing number of ants is simply the result of an increase in activity and search behavior, is a directed response to cues such as plant sap, or is the result of recruitment. The ability to communicate and thereby recruit nestmates is a prominent feature of social insects. Wilson [48] defined recruitment as communication that brings nestmates to some point in space where work is required. To communicate with each other, ants use a number of chemical and mechanical signals that have evolved specifically to alter a receiver's behavior [49, 50].

In acacia ants, recruitment of nestmates to the attacked site would lead to an even more efficient defense. Ants perceiving browser-induced vibrations may subsequently recruit nestmates by releasing alarm signals. Like most other Myrmicinae, ants of the genus *Crematogaster* have a stridulatory organ [50]. Ants stridulate by raising and lowering their gaster so that a cuticular file located on the first gastric tergite is rubbed against a scraper, thereby producing mechanical vibrations [29]. Many studies show that ants make use of vibrational signals to recruit nestmates in various social contexts [50].

Besides using mechanical signals, ants may use chemical signals such as pheromones to recruit nestmates. *C. mimosae* displays alarm behavior when it is confronted with the crushed heads of workers, which are believed to contain alarm pheromones inside of their mandibular glands [51, 52]. To our knowledge, the recruitment behavior of *C. mimosae* has not been studied in detail so far.

Directional Vibration Sensing

For an efficient defense of the host tree, it would be beneficial for the ants, and of course for the tree, if directional information could be extracted out of the plant-borne vibrations. Among ants, the leaf-cutters are particularly well studied in this context. Leaf-cutter ants communicate with the substrate-borne component of the vibratory emission produced by stridulation [29]. Workers stridulate when they cut an attractive leaf. The vibrations migrate along the body of the leaf-cutter and are transmitted from the ant's head to the substrate. Nearby workers respond to the vibrations transmitted through the plant material

by orientating toward the source of the vibration and subsequently join in leaf cutting [29, 31]. Workers also stridulate while engaged in nest digging and when they are buried by a cave-in of the nest, thereby attracting other workers [32, 53]. Experiments with two movable bridges vibrating the ants' legs independently revealed that the leaf-cutting ant *Atta sexdens* uses time-of-arrival delays as small as 0.1 ms of the vibrational signals for tropotactic orientation [35].

Our experiments show that browser-induced plant-borne vibrations lead to an increase in patrolling behavior all over the tree. Moreover, more ants orient toward the source of vibration than in the other direction. This suggests that ants gain directional information from the plant-borne vibrations. However, in our first experiment, ants could move freely on the branches. Gradients in vibrational amplitude or the whirling motion of the branch are influenced to varying degrees by source distance and by local stem properties [44, 46]. By sampling at different locations, ants could experience these gradients and thereby successively update directional information. Therefore, this experimental setup does not allow us to tell whether ants orient via klino- or tropotaxis.

For a tropotactic orientation, ants need to compare the inputs of at least two vibration receptors. Theoretically, a vibrational wave should arrive at each receptor at different times and with different intensities. To reveal whether ants make use of klino- or tropotaxis, we observed directional orientation based on plant-borne vibrations on an individual level.

The experiments clearly show that *C. mimosae* is capable of a vibrotropotactic orientation toward the source of vibrations, i.e., the browser. The ants' first movement is significantly more often directed toward the source of vibration than away from the source. The most obvious directional cues that could be used are differences in time of arrival and amplitude [35, 54–56]. Vibration transmission in living plants presents challenges for localization: waves travel at very different speeds on different parts of the plant and at different frequencies; adding to that complexity, at any given location, the stem moves in a whirling path, whose properties also vary among different frequencies in the signal. These difficulties are especially great for small insects, whose vibration sensors are separated by only a few millimeters [44].

Our measurements show that the amplitude decreases with distances traveled through the acacia branches. However, at small distances, amplitude does not attenuate reliably [45]. Moreover, plants are highly heterogeneous substrates for signal propagation; some gradients may occur as a consequence of plant structure rather than source distance, per se [44]. The utilization of amplitude differences for orientation by ants therefore seems less likely than the utilization of time delays. Measurements of plant species other than acacia trees show that the velocity of a bending wave is in the range of 36–220 ms⁻¹ depending on the frequency [45]. The distance between the vibration receptors on the ants' legs is crucial to assess whether these velocities lead to time delays that could be processed in their nervous system. In *C. mimosae*, the maximum distance between the legs is approximately 5 mm, leading to time delays of 0.02–0.14 ms between vibration receptors. Assuming a temporal resolution in the same range as in the leaf-cutter ant *Atta sexdens*, this time delay should be well above the perceptual

threshold. Time-of-arrival delays are most likely the directional cue that facilitates vibrotropotactic orientation by acacia ants.

Ants and acacias live in a mutualistic relationship in which both partners would benefit from a fast and efficient localization of browsers. A long coevolution has shaped this mutualistic relationship [57, 58]. It would not be surprising if the vibrational characteristics of the acacia branches and the ants' ability to detect, localize, and orient toward vibrations are tuned to meet the common interest of an efficient defense against browsers.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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AUTHOR CONTRIBUTIONS

Conceptualization, F.A.H. and K.K.; Methodology, F.A.H. and K.K.; Investigation, F.A.H. and K.K.; Writing – Original Draft, F.A.H. and K.K.; Writing – Review & Editing, F.A.H. and K.K.; Funding Acquisition, F.A.H.; Resources, F.A.H. and K.K.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Dharani, N. (2006). *Field Guide to Acacias of East Africa* (Penguin Random House).
2. Hanley, M.E., Lamont, B.B., Fairbanks, M.M., and Rafferty, C.M. (2007). Plant structural traits and their role in anti-herbivore defence. *Perspect. Plant. Ecol. 8*, 157–178.
3. Palmer, T.M., and Young, T.P. (2017). Integrating ecological complexity into our understanding of ant-plant-mutualism: ant-acacia interactions in African savannas. In *Ant-Plant Interactions. Impacts of Humans on Terrestrial Ecosystems*, P.S. Oliveira, and S. Koptur, eds. (Cambridge University Press), pp. 200–222.
4. Agrawal, A.A., and Rutter, M.T. (1998). Dynamic anti-herbivore defense in ant-plants: the role of induced responses. *Oikos* 83, 227–236.
5. Rosumek, F.B., Silveira, F.A.O., de S. Neves, F., de U. Barbosa, N.P., Diniz, L., Oki, Y., Pezzini, F., Fernandes, G.W., and Cornelissen, T. (2009). Ants on plants: a meta-analysis of the role of ants as plant biotic defenses. *Oecologia* 160, 537–549.
6. Trager, M.D., Bhotika, S., Hostetler, J.A., Andrade, G.V., Rodriguez-Cabal, M.A., McKeon, C.S., Osenberg, C.W., and Bolker, B.M. (2010). Benefits for plants in ant-plant protective mutualisms: a meta-analysis. *PLoS ONE* 5, e14308.
7. Hocking, B. (1970). Insect associations with the swollen thorn acacias. *Ecol. Entomol.* 122, 211–255.
8. Heil, M., Orón-Tamayo, D., Eilmus, S., Kautz, S., and González-Teuber, M. (2010). Chemical communication and coevolution in an ant-plant mutualism. *Chemoecology* 20, 63–74.
9. Maynard Smith, J., and Harper, D.G.C. (2003). *Animal Signals* (Oxford University Press).
10. Scott-Phillips, T.C. (2008). Defining biological communication. *J. Evol. Biol.* 21, 387–395.
11. Agrawal, A.A. (1998). Leaf damage and associated cues induce aggressive ant recruitment in a Neotropical ant-plant. *Ecology* 79, 2100–2112.
12. Agrawal, A.A., and Dubin-Thaler, B.J. (1999). Induced responses to herbivory in the Neotropical ant-plant association between *Azteca* ants and *Cecropia* trees: response of ants to potential inducing cues. *Behav. Ecol. Sociobiol.* 45, 47–54.
13. Lapola, D.M., Bruna, E.M., and Vasconcelos, H.L. (2003). Contrasting responses to induction cues by ants inhabiting *Maieta guianensis* (Melastomataceae). *Biotropica* 35, 295–300.
14. Bruna, E.M., Lapola, D.M., and Vasconcelos, H.L. (2004). Interspecific variation in the defensive responses of obligate plant-ants: experimental tests and consequences for herbivory. *Oecologia* 138, 558–565.
15. Christianini, A.V., and Machado, G. (2004). Induced biotic responses to herbivory and associated cues in the Amazonian ant-plant *Maieta poeppigii*. *Entomol. Exp. Appl.* 112, 81–88.
16. Romero, G.Q., and Izzo, T.J. (2004). Leaf damage induces ant recruitment in the Amazonian ant-plant *Hirtella myrmecophila*. *J. Trop. Ecol.* 20, 675–682.
17. Elkinton, J.S., and Cardé, R.T. (1984). Odor dispersion. In *Chemical Ecology of Insects*, W.J. Bell, and R.T. Cardé, eds. (Springer), pp. 73–91.
18. Madden, D., and Young, T.P. (1992). Symbiotic ants as an alternative defense against giraffe herbivory in spinescent *Acacia drepanolobium*. *Oecologia* 91, 235–238.
19. Federle, W., Maschwitz, U., and Fiala, B. (1998). The two-partner ant-plant system of *Camponotus (Colobopsis)* sp.1 and *Macaranga puncticulata* (Euphorbiaceae): natural history of the exceptional ant partner. *Insectes Soc.* 45, 1–16.
20. Palmer, T.M., and Brody, A.K. (2013). Enough is enough: the effects of symbiotic ant abundance on herbivory, growth, and reproduction in an African acacia. *Ecology* 94, 683–691.
21. Blatrix, R., and Mayer, V. (2010). Communication in ant-plant symbioses. In *Plant Communication from an Ecological Perspective*, F. Baluška, and V. Ninkovic, eds. (Springer Verlag), pp. 127–158.
22. Thurm, U. (2001). Evolutionary aspects of mechanoreception: from ciliates to man. In *Neuronal Coding of Perceptual Systems*, W. Backhaus, ed. (World Scientific Publishing), pp. 237–248.
23. Kung, C. (2005). A possible unifying principle for mechanosensation. *Nature* 436, 647–654.
24. Hill, P.S.M., and Wessel, A. (2016). Biotremology. *Curr. Biol.* 26, R187–R191.
25. Cocroft, R.B., and Rodriguez, R.L. (2005). The behavioral ecology of insect vibrational communication. *BioScience* 55, 323–334.

26. Virant-Doberlet, M., and Čokl, A. (2004). Vibrational communication in insects. *Neotrop. Entomol.* 33, 121–134.
27. Hill, P.S.M. (2008). *Vibrational Communication in Animals* (Harvard University Press).
28. Markl, H. (1965). Stridulation in leaf-cutting ants. *Science* 149, 1392–1393.
29. Roces, F., Tautz, J., and Hölldobler, B. (1993). Stridulation in leaf-cutting ants. Short-range recruitment through plant-borne vibrations. *Naturwissenschaften* 80, 521–524.
30. Roces, F., and Hölldobler, B. (1995). Vibrational communication between hitchhikers and foragers in leaf-cutting ants (*Atta cephalotes*). *Behav. Ecol. Sociobiol.* 37, 297–302.
31. Roces, F., and Hölldobler, B. (1996). Use of stridulation in foraging leaf-cutting ants: mechanical support during cutting or short-range recruitment signal? *Behav. Ecol. Sociobiol.* 39, 293–299.
32. Pielström, S., and Roces, F. (2012). Vibrational communication in the spatial organization of collective digging in the leaf-cutting ant *Atta vollenweideri*. *Anim. Behav.* 84, 743–752.
33. Lakes-Harlan, R., and Strauß, J. (2014). Functional morphology and evolutionary diversity of vibration receptors in insects. In *Studying Vibrational Communication*, R.B. Cocroft, M. Gogala, P.S.M. Hill, and A. Wessel, eds. (Springer), pp. 277–302.
34. Virant-Doberlet, M., Čokl, A., and Zorović, M. (2006). Use of substrate vibrations for orientation: from behavior to physiology. In *Insect Sounds and Communication*, S. Drosopoulos, and M.F. Claridge, eds. (Taylor and Francis), pp. 81–97.
35. Hager, F.A., Kirchner, L., and Kirchner, W.H. (2017). Directional vibration sensing in the leafcutter ant *Atta sexdens*. *Biol. Open* 6, 1949–1952.
36. McNett, G.D., Luan, L.H., and Cocroft, R.B. (2010). Wind-induced noise alters signaler and receiver behavior in vibrational communication. *Behav. Ecol. Sociobiol.* 64, 2043–2051.
37. Goheen, J.R., and Palmer, T.M. (2010). Defensive plant-ants stabilize megaherbivore-driven landscape change in an African savanna. *Curr. Biol.* 20, 1768–1772.
38. Palmer, T.M., Stanton, M.L., Young, T.P., Goheen, J.R., Pringle, R.M., and Karban, R. (2008). Breakdown of an ant-plant mutualism follows the loss of large herbivores from an African savanna. *Science* 319, 192–195.
39. Riginos, C., Karande, M.A., Rubenstein, D.I., and Palmer, T.M. (2015). Disruption of a protective ant-plant mutualism by an invasive ant increases elephant damage to savanna trees. *Ecology* 96, 654–661.
40. Karban, R., and Baldwin, I.T. (1997). *Induced Responses to Herbivory* (University Press of Chicago).
41. Beyaert, I., and Hilker, M. (2014). Plant odour plumes as mediators of plant-insect interactions. *Biol. Rev. Camb. Philos. Soc.* 89, 68–81.
42. Casas, J., Magal, C., and Sueur, J. (2007). Dispersive and non-dispersive waves through plants: implications for arthropod vibratory communication. *Proc. Biol. Sci.* 274, 1087–1092.
43. Miles, R.N. (2016). An analytical model for the propagation of bending waves on a plant stem due to vibration of an attached insect. *Heliyon* 2, e00086.
44. Gibson, J.S., and Cocroft, R.B. (2018). Vibration-guided mate searching in treehoppers: directional accuracy and sampling strategies in a complex sensory environment. *J. Exp. Biol.* 221, 175083.
45. Michelsen, A., Fink, F., Gogala, M., and Traue, D. (1982). Plants as transmission channels for insect vibrational songs. *Behav. Ecol. Sociobiol.* 11, 269–281.
46. McNett, G.D., Miles, R.N., Homentcovschi, D., and Cocroft, R.B. (2006). A method for two-dimensional characterization of animal vibrational signals transmitted along plant stems. *J. Comp. Physiol. A Neuroethol. Sens. Neural Behav. Physiol.* 192, 1245–1251.
47. Markl, H. (1970). Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. III. Die Empfindlichkeit für Substratvibrationen. *Z. Vgl. Physiol.* 69, 6–37.
48. Wilson, E.O. (1971). *The Insect Societies* (Belknap Press of Harvard University).
49. Hölldobler, B., and Wilson, E.O. (1990). *The Ants* (Harvard University Press).
50. Golden, T.M.J., and Hill, P.S.M. (2016). The evolution of stridulatory communication in ants, revisited. *Insectes Soc.* 63, 309–319.
51. Wood, W.F., and Chong, B. (1975). Alarm pheromones of the East African acacia symbionts; *Crematogaster mimosae* and *C. negriceps*. *J. Georgia Entomol. Soc.* 10, 332–334.
52. Wood, W.F., Palmer, T.M., and Stanton, M.L. (2002). A comparison of volatiles in mandibular glands from three *Crematogaster* ant symbionts of the whistling thorn acacia. *Biochem. Syst. Ecol.* 30, 217–222.
53. Markl, H. (1967). Die Verständigung durch Stridulationssignale bei Blattschneiderameisen. I. Die biologische Bedeutung der Stridulation. *Z. Vgl. Physiol.* 60, 103–150.
54. Hager, F.A., and Kirchner, W.H. (2014). Directional vibration sensing in the termite *Macrotermes natalensis*. *J. Exp. Biol.* 217, 2526–2530.
55. Brownell, P., and Farley, R.D. (1979). Orientation to vibrations in sand by the nocturnal scorpion *Paruroctonus mesaensis*: mechanism of target localization. *J. Comp. Physiol.* 131, 31–38.
56. Prešern, J., Polajnar, J., de Groot, M., Zorović, M., and Virant-Doberlet, M. (2018). On the spot: utilization of directional cues in vibrational communication of a stink bug. *Sci. Rep.* 8, 5418.
57. Bronstein, J.L., Alarcón, R., and Geber, M. (2006). The evolution of plant-insect mutualisms. *New Phytol.* 172, 412–428.
58. Janzen, D.H. (1966). Coevolution of mutualism between ants and acacias in Central America. *Evolution* 20, 249–275.
59. Vesala, R., Niskanen, T., Liimatainen, K., Boga, H., Pellikka, P., and Rikkinen, J. (2017). Diversity of fungus-growing termites (*Macrotermes*) and their fungal symbionts (*Termitomyces*) in the semiarid Tsavo Ecosystem, Kenya. *Biotropica* 49, 402–412.
60. van Wijngaarden, W. (1985). *Elephants, trees, grass, grazers: relationships between climate, soils, vegetation and large herbivores in a semi-arid savanna ecosystem (Tsavo, Kenya)*. PhD thesis (University of Wageningen).

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Experimental Models: Organisms/Strains		
<i>Crematogaster mimosae</i>	Sagala Wildlife Sanctuary, Kenya	S3°24'0, E38°34'0
<i>Acacia zanzibarica</i>	Sagala Wildlife Sanctuary, Kenya	S3°24'0, E38°34'0
Software and Algorithms		
Raven Pro 1.4	Cornell Lab of Ornithology	N/A
SPSS	IBM	N/A
Field Data	Neukadye	N/A
Mephisto Scope 1	Meilhaus	N/A
Other		
Custom-made vibration exciter	Ruhr University Bochum	N/A
Anemometer PCE-FST-200-201-I	PCE Instruments	N/A
EasyLog EL-USB-4	Lascar Electronics	N/A
Thermometer PL-125-T4-USB, type K sensors	Voltcraft	N/A
Accelerometer B&K 4369	Brüel and Kjær	N/A
Accelerometer KS94B.100	Metra	N/A
Preamplifier B&K 2635	Brüel and Kjær	N/A
Audiorecorder DR 40	Tascam	N/A
Camera D500	Nikon	N/A
Digital oscilloscope UM202	Meilhaus	N/A

CONTACT FOR REAGENTS AND RESOURCE SHARING

Further information and requests for resources and reagent should be directed to and will be fulfilled by the Lead Contact, Felix A. Hager (felix.hager@rub.de).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

The study was conducted at the Sagala Wildlife Sanctuary in the semi-arid Tsavo ecosystem in south-east Kenya. The sanctuary (S3°24'0, E38°34'0) is part of a wildlife corridor between the Tsavo national parks, located at an elevation of 540 m above sea level. The mean annual temperature is ca. 25°C and the mean annual precipitation is ca. 600 mm, with two rainy seasons from November to December and March to May [59]. The study site is characterized by Acacia-Commiphora bushland and well drained dark red fire sandy clay, intersected by deep dark brown sandy clay [60]. *Acacia zanzibarica* is the dominant swollen-thorn acacia and hosts *Crematogaster* ants. Experiments were carried out with *A. zanzibarica* hosting *C. mimosae* in the dry seasons between June 2017 and June 2018.

METHOD DETAILS

Ant Activity

First, we probed a transect, 100 m from west to east and 10 m wide, throughout the Acacia-Commiphora bushland, monitoring all *A. zanzibarica* trees higher than 0.5 m for inhabiting ant species. The diel activity of *C. mimosae* inhabiting a medium-sized tree of 2.5 m height was assessed by behavioral observations in intervals of 20 min throughout day and night. The number of ants feeding at nectaries (Figure 1A) and walking along branches (Figure 1B) were chosen as measures of ant activity. One branch (ca. 120 cm) was scanned from the tip to the stem for ants feeding on nectaries. On the same branch we subsequently counted the number of ants crossing a ring marking at 15 cm distance to the tip of the branch within one minute (Neukadye Timestamped Field Data Application). Simultaneously windspeed and temperature were recorded at 2.0 m distance to the tree and 1.5 m above ground (sample rate 1 Hz, PCE-FST-200-201-I; Lascar Electronics, EasyLog, EL-USB-4; Voltcraft PL-125-T4-USB, type K sensors). The average wind velocity and number of wind bursts exceeding 4 ms⁻¹ per hour were used for further calculations.

Artificial Browser

A custom-made vibration exciter (artificial browser, Ruhr University Bochum) was designed to elicit browser-like vibrations in a reproducible manner and to avoid all the problems large herbivores would have caused. The artificial browser operates as follows: a cooper spring sheet was tensioned by means of an electromagnet, which hit the tip of an acacia thorn with a 15 cm long metal rod. An acacia branch was tightly coupled by inserting a thorn in a hole at the tip of the metal rod. To calibrate the artificial browser we successively adjusted the amplitude, the impact time and the mounting method till the plant-borne vibrations resembled the vibrations caused by a natural browser. Therefore, an accelerometer was mounted on acacia branches and plant-borne vibrations were recorded (sampling rate 48 kHz, B&K 4369, B&K 2635, Tascam DR40) and compared in the time and frequency domain with recordings of browser-induced plant-borne vibrations using cross-correlation (Raven Pro 1.4).

Plant-Borne Vibrations

Plant-borne vibrations induced by wind, the artificial browser and a browsing goat were recorded in the following way: a spring steel clamp was used to tightly mount an accelerometer on five branches (diameter 9–14 mm, $N = 5$) of a medium sized *Acacia zanzibarica* (sampling rate 48 kHz, B&K 4369, B&K 2635, Tascam DR40). The accelerometer was mounted vertically on branches at a distance of 0.8–1.1 m from the main stem. Wind-induced vibrations were recorded continuously for 1 hour. Simultaneously wind velocity was recorded at 2 m distance to the tree and 1.5 m above ground (sampling rate 1 Hz, PCE-FST-200-201-I; Lascar Electronics, EasyLog, EL-USB-4). Afterward five wind bursts exceeding 4 ms^{-1} per branch were identified and the corresponding vibrations were analyzed. The artificial browser was attached in five different positions to the same acacia branches used to record the wind-induced vibrations. The accelerometer was mounted in the same way as stated before. Afterward, with the accelerometer still mounted at the same place, a goat was allowed to feed on the same five branches used for the previous recordings. The browsing goat (Figure 1C) was videotaped (Nikon D500) to link the recorded plant-borne vibrations to the goat's browsing behavior. The video camera was synchronized with the vibration recordings via the external microphone input of the camera. Five events in which the goat removed a leaf were analyzed per branch. The vibrations induced by wind, the artificial browser and the goat were compared with each other by correlating spectrograms (256f, Raven Pro 1.4). The peak-correlation coefficient is a measure for the similarity between the spectrogram images. The correlation coefficient can take values between 0 and 1, whereby 0 means the two spectrograms do not coincide, a correlation value of 1 indicates that both spectrograms are identical. To provide a better understanding of the peak-correlation values, vibrations were also compared within groups.

We measured the propagation and attenuation of browser-induced vibrations on an *A. zanzibarica* tree. The artificial browser was coupled to a thorn on a branch in reach of a natural browser i.e., 1 m above ground. The peak amplitude of the vibrations was measured at a reference accelerometer (PtP_{Ref}) mounted next to the impact point and a second accelerometer (PtP_x) coupled on 70 different locations on the tree (Metra KS94B.100, Tascam DR40, Meilhaus Mephisto Scope 1 UM202).

The measurement points were either 20 cm apart from each other or at 20 cm distance to a branching point. The distance of the second accelerometer to the reference accelerometer, i.e., impact point, was measured along the respective branches and stem axis. The attenuation was calculated by $20 \cdot \log_{10}(\text{PtP}_x / \text{PtP}_{\text{Ref}})$ expressed in decibel (dB).

Wind-Induced Vibrations and Ant Activity

Wind bursts of 4 ms^{-1} were strong enough to visibly shake branches and occurred frequently in our study area. To test whether wind-induced vibrations cause a change in ant activity, we measured ant activity continuously for periods of 10-min by counting the number of ants crossing a ring-marking. Each count was captured with accurate time (Neukadye Timestamped Field Data Application). Windspeed was recorded simultaneously as described before. Afterward wind bursts exceeding 4 ms^{-1} were identified and time intervals of 1-min length around the wind bursts were analyzed in terms of ant activity. Ant activity within the 30 s before and the 30 s after wind velocity exceeded 4 ms^{-1} were compared. Wind bursts were analyzed in chronological order. Wind bursts that followed a wind burst within 1 min were not counted to avoid pseudoreplication. We chose 10 branches and analyzed 10 wind bursts per branch.

Browser-Induced Vibrations and Ant Activity

The artificial browser was tightly coupled to a thorn at 12 cm distance to a ring-marking. Vibrational stimuli were given with 1 Hz repetition rate and peak to peak amplitudes were calibrated to 2 ms^{-2} measured at 1 m distance to the impact point. The number of ants crossing the ring-marking was counted as a measure of ant activity. Ants crossing within 30 s before the onset of vibrational stimulation and during the subsequent 30 s with vibrational stimulation were counted (Neukadye Timestamped Field Data Application). After the initial setup of the artificial browser and after each trial a pause of at least 5 min was made. We chose 10 branches and conducted 10 trials per branch. Simultaneous wind velocity was recorded at 2 m distance to the tree and 1.5 m above ground (sampling rate 1 Hz, PCE-FST-200-201-I; Lascar Electronics, EasyLog, EL-USB-4).

Long Distance Alarm Cues

To test whether browser-induced vibrations alert ants over a long distance on other parts of the same tree a playback-experiment with the same set-up as described before was conducted. Ring markings were made at distances of 1.8–2.3 m to the impact point, measured along the axis of branches and the stem. To find out whether ants orient to the vibration source the number of ants crossing the ring-marking toward the source of vibration and in the other direction were counted before (control) and after the onset of the

vibrational stimuli (playback). After the initial setup of the artificial browser and after each trial a pause of at least 5 min was made. We chose 6 branches and conducted 10 trials per branch.

Vibrotropotactic Orientation

To test whether ants orient to the vibration source, the artificial browser was coupled to a thorn as described before. As focal ants we chose individuals feeding on extrafloral nectaries at a maximum distance of 25 cm to the vibrational impact point. The ant fed either at the tip-ward side ($n = 100$) or at the stem-ward side ($n = 100$) of the branch in relation to the impact point. After the onset of the vibrational stimuli the focal ant could walk toward the vibration source, it could walk away, or it could stay at the extrafloral nectary. The first direction in which the focal ant moved for at least 0.5 cm (one body length) was scored. The reaction time was measured as the elapsed time after the onset of the vibrational stimuli until the ant started to move. The experiment was stopped once the focal ant started to move or after 10 s, whichever came first. After the initial attachment of the artificial browser and between every trial a pause of at least 5 min was made. We chose 10 branches and conducted 20 repetitions (n) per branch. A control experiment was conducted in the same way except that the battery was not connected to the artificial browser and hence the branches were not vibrated.

QUANTIFICATION AND STATISTICAL ANALYSIS

In ant activity experiments the average windspeed and temperature of the 1-min-observation period was used for statistical analyses using Pearson's correlation coefficient.

Pearson's r was calculated to test whether the plant-borne vibration's amplitude attenuates as a function of the distance traveled through the acacia. Linear regression analysis was used to calculate the attenuation coefficient.

Experiments testing whether plant-borne vibrations affect ant activity were analyzed using Wilcoxon signed-rank test. In case of wind induced vibrations the two related groups were ant activity "before" (t_1) and "after" (t_2) the onset of a wind burst. In experiments with artificial browser induced vibrations the two related groups were the number of ants crossing a ring marking before (t_1) and during (t_2) vibrational stimulation. For visualization, activity indices (a_i) were calculated by: $a_i = (t_2 - t_1) / (t_1 + t_2)$. In this way, the index yields values between -1 and 1 with values < 0 , if the ants' activity decreases after wind bursts, and values > 0 , if the ants' activity increases (Figure 6). Pearson's correlation coefficient was used to analyze whether wind velocity has an impact on the ant's reaction to artificial browser-induced vibrations.

To analyze whether plant-borne vibrations serve as a directional long-distance information cue, preference indices (p_i) were calculated by $p_i = (t_{\text{to-away}}) / (t_{\text{to+away}})$. In this way, the index yields values between -1 to 1 , with values < 0 , if the ants moved away from the source of vibration, and values > 0 , if they moved toward the source of vibration. Mean preference indices were calculated for each branch for further statistical analysis. One sample t test was used to test whether ants show a side preference (Figure 7).

In experiments examining the vibrotropotactic orientation, preference indices (p_i) were calculated for each branch by $p_i = (t_{\text{to-away}}) / n$. In this way, the index yields values between -1 to 1 , with values < 0 , if the ants' moved away from the source of vibration, and values > 0 , if they moved toward the source of vibration. One sample t test was used to test whether ants show a side preference (Figure 7).

Significance was determined when the p value is lower than 0.05. All statistical analyses were performed using IBM SPSS 25 software. All statistical details are indicated in the figure legends and results section.