

Projet de Fin d'Etudes (PFE) 2019

Advances in relationship between plants and sound waves

Possible innovative applications

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2019

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Le travail de recherche, dont l'objectif de base est d'acquérir une compétence méthodologique en matière de recherche, doit répondre à l'un des deux grands objectifs :

- Développer toute ou partie d'une méthode ou d'un outil nouveau permettant le traitement innovant d'un problème d'aménagement
- Approfondir les connaissances de base pour mieux affronter une question complexe en matière d'aménagement.

Afin de valoriser ce travail de recherche nous avons décidé de mettre en ligne sur la base du Système Universitaire de Documentation (SUDOC), les mémoires à partir de la mention bien.

REMERCIEMENTS

Nous tenons à remercier Mme Séraphine Grellier pour le temps qu'elle a passé à nous accompagner dans notre travail de recherche. Nous tenons également à lui témoigner notre gratitude pour sa patience et ses conseils qui nous ont été précieux afin de mener à bien notre travail.

Merci

Manon et Camille

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ABSTRACT

All living organisms have a vital need to know their environment. The sense of smell, the touch, the sight or the hearing... all species have developed organs or cellular sensitivity specialised in physical and chemical surrounding forces perception. Even if plants are living organisms, they have been considered as insensitive and quiet because they can move like animals we are. However, in 60s, Milburn and Johnson discovered that plants emitted sounds. Then, numerous scientists started to get interest to the relation of plants with, not only audible sounds but also ultrasounds and infrasounds. It has indeed been proven that plants were able to emit sound vibrations at different frequencies. Many theories (Ritman and Milburn, 1988; Laschimke et al., 2006; Gagliano et al., 2012a) tried to explain why plants can do this and if those sounds were intentional or not. Other studies have also shown that a extended exposure with specific frequencies could have effects (positives and negatives) on plant growth and its development at different levels/scales. These revelations can put in perspective not only the use of chemical pesticides applied on plants, but also open up potential applications in other areas. Many studies are interested in the relationship between plants and sounds but questions still remain : what is the organ or molecule which allows plants to apprehend sound waves? Are their emissions and responses only due to a physical mechanism or are they intentional ? Is the use of waves on a large scale without consequences ? Despite recent interest in plants bioacoustics, research needs further investigations.

Key words : Plant, sound wave, mechanosimuli, perception, audio, bioacoustic

INTRODUCTION

Communication in living organisms is one of the most studied topics in the behavioural sciences. Although the definition of communication is still source of debate, scientists generally agreed to say that communication is based on an intentional transfer of some kind of information or signals with mutual benefits for emitter and receiver (Gagliano, 2013). The ability to sense and respond to physical stimuli is common to all living organisms. Environmental stimuli detected by organisms are light, temperature, and many chemical signals. All living organisms appear to perceive these mechanical signals, no matter their taxonomic group (plant/animal) (Telewski, 2006). The difference between these signals is found in the sensory network of organisms and in their response to each specific mechanical stimulus. Several signals can be perceived by organisms: pressure created by the atmosphere and linked to the wind or to the gravity, or even pressure gradients in aquatic systems (Telewski, 2006). These stimuli can be transmitted in the air, soil or aquatic systems (Chowdhury et al., 2014; Mohanta, 2018). Regarding plants, we can talk about “touch”, also named thingo stimuli, which are reactions induced by some mechanical stimuli. They produce various of thigmo responses in plants, such as thigmomorphogenesis, thigmotropism, thigmonasty,

and thigmotactic response, which will influence the shape of the plant (Jaffe et al., 2002). Consequently, plants have a mechanosensing network, and so they can react to those external mechanical signals to adapt their system to the external environment, including sound waves.

Historically, acoustic communication had first focused on animals, because we hear them, we see them, so it was easier to perceive sound from animals than acoustic communication from plants. Studies on plant acoustics started back in 1950s with numerous ways of thinking concerning the effect of musical sound on plants. From the last decades, studies have focused on our understanding of plant ecology and specifically on chemical signalling in plant. Those research and their results have confirmed that plants indeed communicate (Gagliano, 2013). From an evolutionary point of view, no matter if an organism has developed an organ for sound perception or not, processing and response appeared to be almost similar. And the explanation of this comes from the physical properties of sound waves. As these can interact with any object interface with, vibration is the key of “hearing” process (Mishra et al., 2016).

From all living organisms’ point of view, communication by sounds is relatively advantageous. Indeed, sound travels faster and further in soil

because it's a dense substrate. For instance, a snake is in direct contact with the soil, so it is easier for it to capture information from distant sound sources (Gagliano et al., 2012a). Moreover, as vibrations are present in all environments, and connected with the ground, rooted plants receive all vibrations linked to sound (Gagliano et al., 2012a).

Apart from that, the signal of acoustic energy could be less costly than other pathways. For example, *Nepenthes hemsleyana*, a pitcher plant, changed her morphological structure to catch attention of bats with ultrasound waves to be pollinated with minimal fitness cost (Gagliano et al., 2012a). Evolutionarily, the reception of sound waves is advantageous because it permits to gain many information about the surrounding environment. Indeed, detecting sound waves could not only allow plants to increase their chances of survival, but also could develop a defence strategy. For instance, studies have emitted insights in which unstressed plants could be warning of a stress by their neighbour with acoustic signals. As a result, unstressed plants could have time to develop a defence strategy (Fernandez-Jaramillo et al., 2018; Mohanta, 2018). As sound propagates faster and allows for both a higher instantaneous transmission and quantity of information, it can be said it's more efficient than chemical diffusion. Additionally, the source localization could be more accurate than chemical signals (Gagliano, 2013).

First experiments of sound waves on plants has started on 1950's beginning with the effects of music on plants. For example, Singh et Ponniah (1954, 1955a, 1963) tested effects of violin during 25 min per day and every day on eudicotyledon plants like *Mimosa pudica* and *Tagetes erecta*. They found that there was an increase of the number of spines, leaves and branches after the experiment. However, at that time, results of those studies weren't enough significant to say there was a real effect of sound on plants. Nevertheless, scientists already mentioned the notion of specific frequency and intensity to obtain various responses of plants (Klein and Edsall, 1965; Measures and Weinberger, 1970; Ozkurt and Altuntas, 2018). Over time, studies and research have become more specific. Indeed, scientists started to put mechanic action and reaction aside from the studies of plants exposed to sound waves. They were more and more interested in the potential intentionality of plants and their responses to sound waves. Some scientists mention now a kind of "intelligence" of plants. Therefore, studies on the effects of sound waves on plants and their perception of sound are still

relatively new. For the moment, there are still a lot of questions and precisions needed responses.

This paper reviews the current knowledge of the relationship between plant and sound. However, some questions remain. To what extent plants could need sounds? What is the plant sensory mechanism dedicate to the sound perception? It seems that this new field of research could be interesting for human applications.

I- EMISSION

From the river's flow to the wind through leaves and the falling rain, our world is full of sounds. Animals are well known for their noisy communications too. We can recognise the genus, the species and even sometime the specimen by their sound. However, the idea of controlled communication is relatively new. For some "quiet" species we don't perceive the cry with our limited range frequency hearing organ. Human is able to detect sounds between 20 and 20 000 Hz. Indeed, before the creation of underwater sonar in the 60', we had no idea that cetaceans could "sing". Why then, plant couldn't be able to "speak" too and we can't hear it?

Perception device

Milburn and Johnson in the 60' were the first scientists to hear sound from plant. They emit the hypothesis of a communication through them (Milburn and Johnson, 1966). Since, some scientists have developed some systems to receive plant's sound. Sensors are positioned on different parts of the plant - it can be one on the root and one on the leaf or directly clamp to the bunk or the stem. They are linked to a device who calculates the vibration (*figure 1*) or the acoustic emission (*figure 2*) of the plant (Milburn and Johnson, 1966; Tyree and Sperry, 1989; Laschimke et al., 2006). Another machine transcodes afterward the signal into human range perceptible sound or recordable oscillations. Acoustic phenomena can also be counted and their occurrence analysed by statistics. Acoustic receptors can be linked with other devices such as transpiration loss

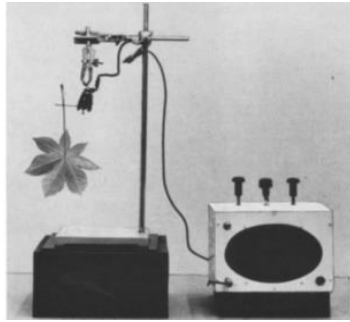


Figure 1 : Vibration detector device (left) linked with an amplifier and speaker device (right). (Milburn and Johnson 1966)

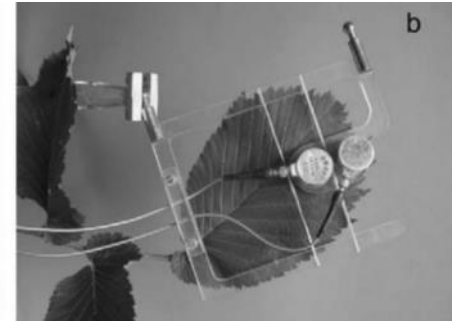


Figure 2 : Acoustic detection device pair with a loss transpiration detector : (a) all experimental installation (b) measuring sensors on the leaf of an *Ulmus glabra* (Laschimke, Burger, and Vallen 2006).

captors to explore links between several phenomenon and a possible explanation for plant's sound (figure 2b).

Plant emissions are recorded in the lower audio range (between 10 and 240 Hz) and in ultrasonic ranges (between 20 to 300 kHz) as well. Most of experiments are focused on Ultrasonic Acoustic Emissions (UAE) and use them in perceptions ability of plants as well. However, even though sound have been recording, the origin - emitter organ or kind of those - is still unknown and several scientists works on their possible provenance.

Discussion on sound emission by plants theories

One of the most popular theory about the sound emission in the plant word is linked to the very metamorphic structure of organism. Xylem channels link roots and leaves in order to feed the last ones with nutriments picked up by the first's ones. Water is the carrier of all these essential particles. However, in some cases - dry stressing periods for example - the negative pressure in the xylem isn't constant and gases concentrated within the solution begin to form bubbles by cavitation. Most of the plant sound emission studies link the abrupt release of these bubbles and the sound perceived by receptors.

This theory is supported by connections revealed between cavitation rate, temperature and sound emission.

The number of sound emissions related to cavitation have also been related to the species (Milburn and Johnson, 1966). Ultrasound emissions had also been noticed in case of freezing and dehydration stress among woody plants. After cavitation, the rupture of the water column structure produce ultrasound recorded by specific devices (Raschi et al., 1989).

In the 80', several team of research have been interested by the comparison of Audible Acoustic

Emissions (AAE) and UAE to understand their specific origins (Tyree and Dixon, 1983; Ritman and Milburn, 1988, 1991). It seems that AAE are most linked with the hydraulic channel and cavitation in large vessels. On the contrary, UAE seems to come from smaller channels with few hydraulic roles. They are less corelated with the cavitation than AAE. However, the similarity in all acoustics emissions and interference in the plant emission can allow us to use them to predict hydric stress (Kikuta et al., 1997).

Nonetheless, in the light of newer studies, scientists doubt that all the sound they perceived are only produced by a pressure re-establishing (Gagliano et al., 2012a; Jung et al., 2018). Studies counted a lot of sound phenomena. If each one of them leads to a "cavitation defect", the number of functional water channels will significantly decrease. Indeed, one sound would mean that the embolised channel should be malfunctional. However, for what we know, channels aren't dead and a fix mechanism has not been found yet.

Laschimke emitted another hypothesis: "the acoustic emission from plants are not caused by cavitation under negative pressure, but by sudden surface rearrangements of groups of wall adherent micro-bubbles under positive pressure" (Laschimke et al., 2006). He recorded several waveforms of acoustic emissions in audio (AAE) and ultrasonic (UAE) ranges. One of them is typical of the cavitation hypothesis. However, this kind is much less common than more regular emissions, which are impossible in the case of abrupt release of pression. Moreover, both AAE and UAE are emitted during transpiration of the plant but at different period of the process. Laschimke concluded with the lack of technology to solve the mystery of ultrasonic emission by plants at the moment.

Finally, in opposition on other theories, Monica Gagliano exposed molecular origin of the sound emission by plants (Gagliano et al., 2012b; Gagliano,

2013). She thought that the cytoplasmic streaming produced by cellular metabolism and ATP hydrolysis enzyme can be spread through the cell membrane and cell walls by vibrations. That gives information about the state of the organism. Vibrations are passed to neighbour cells in acoustic wave forms. Cells can vibrate in a similar frequency and amplified the signal collectively. This “coherent vibrational emission” can be spread beyond the organism, through leaf or root, and share information with other organisms (figure 3). This sound wave should theoretically be emitted around 150 and 200 kHz. Strangely or not, we will see later that acoustic vibration plant responses are better around this range of frequency.

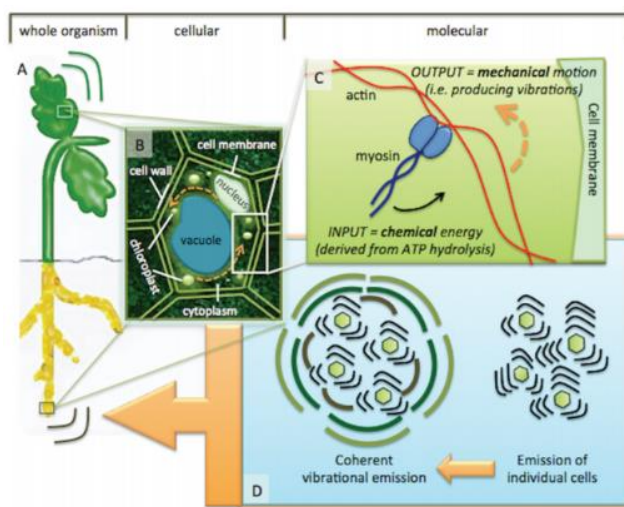


Figure 3 : Illustration of the theory of M. Gagliano about the cellular origin of sound emission by plants: (A) sound emission to neighbour, (B) cytoplasmic movements, (C) motor protein myosin movements create cytoplasmic streaming, (D) individual cells receive neighbour cell's vibration and coordinate themselves into an synchronise and strongest vibration (Gagliano 2013)

Do plants intend to emit sound?

As we said before, plants emit sound, directly by their metabolism or indirectly from wind for example. Most of the time, scientific explications of emissions of sound by plants are related to structural phenomenon (Milburn and Johnson, 1966). We still didn't know how the sound is transmitted inside the plant and the sensing network (Telewski, 2006). But in light of the visible effects of sound on plants, we can rationally ask if the emission of sound is on purpose or if it is a mechanical response of the organism. With our technical supplies and our current knowledge, the question remains (Jung et al., 2018). However, the reaction of plants, isolated each from others from all known forms of communication excepted vibration, seems to be correlated with the

species surrounding the organism. Therefore, information shared seems intentional at the first sight (Gagliano et al., 2012c).

To conclude, we still haven't enough research about the ability of plant to produce sound and the intentionality of this phenomenon is still to prove. Nevertheless, the actual way of research in this field and the technological advances can make possible a future understanding of the acoustic plant communication.

II- RECEPTION

Importance of exposition parameters

Sound waves technology have been applied on different plants. Responses of those depend, on one hand, on the exposure parameters and on the other hand, on the specific-sound frequencies and intensities. The last one is one of the most mentioned in scientific publications.

It has been proved that plants exposed to sound waves at a specific frequency, specific SPLs (Sound Pressure Level), or specific range of intensity and frequency, had effects on plant growth (Bochu et al., 2003; Chowdhury et al., 2014). Furthermore, some studies have taken into account more parameters like different exposure periods or different distances from the source of sound (Hassanien et al., 2014). They had also direct effects on the growth and development of plants (Yiyao et al., 2002; Chowdhury et al., 2014; Fernandez-Jaramillo et al., 2018; Mohanta, 2018). It's true that a lot of exposure methods have been tried. Some experiments were based on effects of classical, jazz and rhythmical music on plants. Others experiments dealt with natural environmental noises as birdsong (Creath and Schwartz, 2004). Depending on method, different results were extracted. Indeed, it has been proved that different styles of music and different sound frequencies and SPLs had different results on plant growth. However, there is a lot of confusion and contradictions in this topic (Hassanien et al., 2014). It has been found that the plant environment also played a role. An exposure in an open field and under greenhouse will not have the same effects on plant growth. For instance, the rice yield increased by 25.0% in pots experiment but only by 5.7% in open field (Hassanien et al., 2014). In addition, the medium in which the sound is propagated is also very important. Indeed, the sound does not go through solid, liquid or gas medium with

the same speed (Mohanta, 2018). It's also true that the speed of sound depends upon different environmental factors like temperature, humidity (Mohanta, 2018). The method the most used is still the Plant Acoustic Frequency Technology (PAFT). This generator has been manufactured by the Qingdao Physical Agricultural Engineering Research Centre in China and be used for the treatment of plant with a specific sound frequency. It has eight variable frequency levels from 0.06 to 2kHz and an intensity variable from 50 to 120dB for a distance about 50-100m. It has been particularly used in agri-wave technology to improve the yield and quality of plants. For instance, Ozkurt and Altuntas (2018), have showed an increase of 9.0% for total sugar in strawberries fruit treated by 105dB compared to control fruits. Respectively, strawberries treated at 100dB, 95dB have an increase of 7% and 2% (Ozkurt and Altuntas, 2018). Another study reported that tomatoes treated with agri-wave technology have 26.19% more sugar, 55.39% more vitamin A and 92.31% more niacin (Hou and Mooneyham, 1999).

Agri-wave technology is based on the fact of « broadcast intermittent pulses of sound waves by using the PAFT and spraying a compound of microelements fertilizer on the leaves once every week » (Hassanien et al., 2014).

The specificity of sound frequencies and intensities is a determining element on plant growth. Indeed, it's necessary to know the optimum threshold level at what intensity (SPL) and frequency (Hz) level a specific organism responds. Each species perceives sound levels differently. That is why sound waves with beneficial impact for one organism may be dangerous for the growth and the development of other organisms (Mohanta, 2018). For those reasons, sound reception experiments on plants should be done in closed environments.

Sound waves with specific frequencies and intensities have been shown to have significant effects on variable biological, physiological and biochemical activities in plants, including gene responses. However, it's necessary to discuss about the relationship between sound waves and plant responses: how these reactions happened and how plants can get these signals, and with which organ?

Reception organs

The perception of sound waves is well-known in animal studies and contributed significantly to the shaping behaviour, the ecology and the evolution of the organism. This evolution has permitted to

conserve communication mechanisms and to develop some specific organs like ear, antenna and eardrum. This evolution is also noticeable in plants in some way: it was found that plants perceive and respond to sound in their own way (Mohanta, 2018). In this way, they can identify signals that help them to detect and adapt them to their surrounding environment and thereby allow them to learn about it. The specific sensory mechanism available in plants for getting sound signals is still unclear at the moment. However, plant respond to environmental sounds. They can first feel mechanical perturbations and then respond to them. Indeed, Gagliano et al. (2017) proved that plants can locating water just by the sound it make through pipe. More than that, *Pisum sativum* seems to be able to distinguish water flowing through pipe to recording water noise or other noise. Having the choice between a compartment wrapped with a flexible tube full of running water and another compartment with other sound, the plant roots will be growing preferentially in the water surrounded compartment (figure 4).

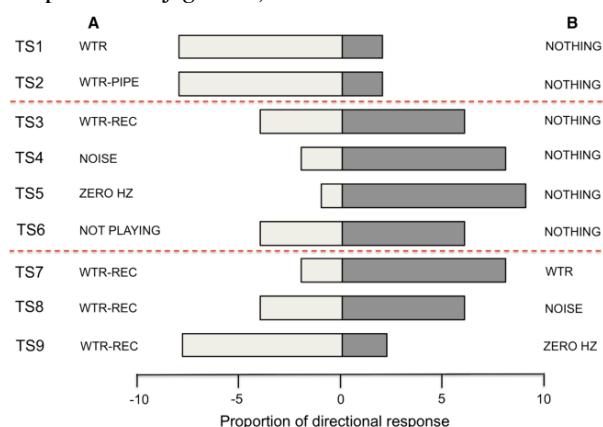


Figure 4 : *Pisum Sativum* Roots growth choosing between compartments A and B thanks to sound identification.. WTR : water, WTR-PIPE : *sound of water through pipe, WTR-REC : Recorded sound of water, NOISE : White noise, ZERO HZ : zero hertz, NOT PLAYING : sound equipment on but not playing. (Gagliano et al., 2017)

Even though plants are sessile organisms, they present a real sensitivity which has evolved to respond to different stimuli as light, temperature, gravity... Actually, if trees didn't felt in their cells gravity, they will grow in a spiral form (Bastien et al., 2013). Yet, they don't have like us an organ to perceive the gravity like our inner ear – which is linked with our sound perception organ as well... They can also perceive mechanical stimuli such as touch, wind, or rain without nervous network.

Organisms are inevitably leaving in noisy environment and, no matter the species, they have developed different mechanisms to sound perception

and processing and response appear to be more-and-less similar. The possible explanation for this comes from the properties of sound waves. Sound waves originate from vibrating objects and are transmitted by the oscillation of the particles in the medium they travel through (Mishra et al., 2016). These waves can potentially make vibrate any object they collide. It could be the origin of the process of hearing (Gagliano et al., 2012a). Even though we don't know the specific sensory organ, plants present an enormous number of mechano-sensitive channels that are believed to be responsive to mechanical vibrations (Mohanta, 2018; Haswell et al., 2011). In this idea, sound vibrations could directly have an impact in the tension in biological membranes and possibly evoke a signalling cascade through activation of sensory channels (Haswell and Meyerowitz, 2006). It was also proposed that plants can perceive and propagate sound waves with their root system because the sound moves faster in ground than in air due to the perfect molecular arrangement of soil texture (Mohanta, 2018). On the other hand, it was mentioned that sound waves make vibrate the plant leaves and that the activities of some enzymes were enhanced in different organs as stems and roots. Consequently, every organs of plants could be directly impacted by sound waves (Hassanien et al., 2014). The mechanism of how sound affects plants and which organ, or part of the plant is concerned need further research (Hassanien et al., 2014). Whatever, it was proved that sound waves have effects on plant growth and its development. Various elements of plants are concerned by the responses.

Plants reaction further to sound waves

Plants are complex multicellular organisms and their responses to sound waves are variable and depend on a lot of parameters as the species of plant, exposure parameters... It was found there were various responses at different stage of growth and more precisely at different levels: seed germination, cells, molecules, genes... including biochemical and physiological responses.

Seed germination responses

There are a lot of metabolic activities that occur during seed germination and sound waves are known to directly influence the germination. Creath and Schwartz (2004) have reported a study in which

musical sound and natural sounds have significantly enhanced the sprouting of okra and zucchini seeds than pink noises (closed to electronic noise). These effects did not depend on temperature, location of the experiments, seed type and the person doing the scoring. However, it was observed that natural sounds as birdsong had statistically the better significant effect on number of sprouted seeds. In another research, it was reported that *Echinacea angustifolia* seeds showed the highest germination rate with the least germination time when seeds were exposed to sound waves at 100 dB and 1000Hz (Chuanren et al., 2004). There were other research on the biological effect of sound waves on seed germination (Timonin, 1966). Bochu et al. (2003) have worked on paddy rice seeds. In this study, it was proved that the germination index, the height of stem relative increase rate of fresh weight, activity of root system, and the penetrability of cell membrane were significantly increased when the seeds were exposed to sound waves at 106dB and 0.4kHz. Moreover, it was mentioned that if the sound waves exceeded 4kHz and 111dB, the growth of paddy seeds was inhibited.

Physiological and biological responses

Responses of biological and physiological activities to sound waves are very complex and variable.

Collins and Foreman (2001) searched a correlation between the side of beans and impatiens leaves and the frequency of sound wave emission. They thought that sound waves are sweeping the stagnant air and increase the water transpiration of leaves. Their theory was more about the physical particularity of sound waves and the surrounding environment of the plant. But other scientists have been interested by the very intern effect of sound on plant. It was reported that specific-sound frequencies and intensities enhanced content of soluble proteins and sugar in the cytoplasm of *Dendranthema morifolium* callus (Zhao et al., 2003). Also, soluble proteins are particularly responsible for division and growth of plant tissues. Indeed, the increase of soluble proteins is linked with the increase of metabolization at cellular level thanks to enzymes. More metabolization enhances more cell division, and better growth of plant tissues (Yiyao et al., 2002). A study on effects of sound field on *Chrysanthemum* callus have reported that the optimum range of intensity and frequency of sound waves have enhanced the content of soluble proteins, the absorption rate of calcium, and the activity of SOD (Superoxide dismutase) in callus (Bochu et al., 2001). More generally, they think these

enhancements, prompt by moderate stress stimulation, can improve the physiological activity, accelerate the growth of plants, and as a result, is helpful to the growth of plant tissues. Sound stimulation also increases the cell wall and membrane fluidity, which facilitates cell division and growth (Chowdhury et al., 2014) and improved stronger resistance to the environment (Hassanien et al., 2014). The fluidity of the physical state of lipids in plasmalemma is also increased, which is the most external part of cells and the most sensible part to environment factors. All these structural changes are helpful on the modulation of membrane and metabolic activity acceleration (Yi et al., 2003). By electron microscopy (EM), it was observed that there was a difference in Ca^{2+} distribution between control and treated *Chrysanthemum* callus by sound waves. Indeed, treated plants shown both better concentration of Ca^{2+} ion in cytoplasm and better opening/closing Ca^{2+} channels. Thus, these changes modified ions concentration on both sides of the membrane and the membrane potential leading the growth of plant tissues. Ca^{2+} does not only play an important role on plant and development of plants, but also on the resistance to the various environmental stresses (Hassanien et al., 2014).

Cellular and molecular responses

Once again, it's difficult to search how plant cells can both receive and transfer the mechanic signals into the cells. Yiyao et al. (2002) studied the responses of four physiological and biochemical parameters indexes responsible for the growth of *Chrysanthemum* callus. They found that sound field had strong effects on the growth, development and cell division in this plant. For instance, the activities of the SOD, which is not only related to antivirus, anti-stress and anti-caducity but also has relation with differentiation and division of cells, was measured. Studies shown that SOD was responsible for the destruction of the cell membrane, which will cause cell death. Unfortunately, they found that the activity of SOD increased with the increasing of intensity and frequency. Once again, the optimum specific-sound frequencies and intensities is essential to have beneficial effects on plant growth and development. The study revealed that under 100dB and 800Hz, the content of soluble proteins was the highest, which is good conditions for tissue growth. Indeed, the accumulation of soluble proteins provides cell division, the content of enzymes and the metabolization level.

Another parameter is Indole Acetic Acid (IAA), which is an important element for cell division and growth. If its regulator, IAA oxidase, increases, the content of IAA decreases, so the growth of callus is inhibited. In their experiments, they revealed that the activity of IAA has decreased in *Chrysanthemum* callus after sound field stimulation in 100dB and 800Hz. Thus, it was beneficial for the growth of *Chrysanthemum* callus. In this study, they also talked about the transition temperature phase which decreases with a certain range of intensity and frequency. It was shown that the decrease of this parameter enhanced the fluidity of cell wall and membrane, which make the cell growing and splitting faster. Thus, a better cell fluidity might enhance plant growth by sound field (Yiyao et al., 2002). In another study, it was reported that sound waves could not only change the cell cycle of paddy rice cells, but also speed up its reproduction rate (Hassanien et al., 2014).

Gene responses

At gene responses level, it is true that there is not so much information about how sound waves could activate gene responsive on plants. However, there were studies about the gene regulation in response of application of sound stimulation on plants. The research of Jeong et al. (2008) has suggested that sound could represent an alternative to light as gene regulator. They made an experiment by 4 hours treatment applied to rice plants with different frequencies (50, 125, 250 Hz and 1kHz) and SPLs from 65 to 70dB. The principle of the experiment was based on the sound regulation through mRNA expression analysis which established the expression of the gene encoding the cytoplasmic protein aldolase (*ald*) in relation to specific frequencies. The experiment was done under both light and dark conditions. This experiment has demonstrated that sound up-regulated the expression of Rubisco small subunit (*rbcS*) and *ald*, which are also light-responsive gene.

Protective enzyme responses

In the mini-review published by Chowdhury et al. (2014), it was mentioned that responses of protective enzymes on plants treated by sound waves have also been studied. Specifically, sound waves stimulation increases the activity of various protective enzymes. They are responsible of the accumulation of active

oxygen species's (AOS) decreases. Reducing AOS may protect cells from oxidative damage.

Plants adaptation responding to the surrounding environment

From an evolution point of view, it is true that plants have adapted their system and their “intern” activities according to their surrounding environment. Sound waves have permitted plants to learn about this noisy environment. Sound relative to plants allow those organisms to develop adaptive mechanisms to survive to their environment.

Interspecific and intraspecific acoustic communication is well-known in animals, but has been neglected in plants (Schöner et al., 2016). Recently, studies have shown that relationships between some animals and plants existed such as bats and bat-pollinated plants. Plants can change their morphological structure in order to create echoes to attract bats. This adaptation appeared because of the cluttered habitats where both species are living. Indeed, it is a bat-dependent plant so it is necessary to the plant to adapt its structure to present the highest mutualistic relation to bats (Schöner et al., 2016).

This kind of adaptation can also be found in response to sound waves emitted by predatory insects. Indeed, Appel and Cocroft (2014) reported that *Arabidopsis thaliana* can discriminate sound waves emitted by chewing of caterpillars from those caused by wind. It was proved that there was an increase in chemical defence responses of the endangered leaves. This reaction could suggest that vibrations travel through the plant, stimulating other leaves about the danger. To conclude, this is a demonstration of one of the several possibilities for “why” perception of sound would be a beneficial adaptive feature for plants. There have been other experiments that prove the adaptation of plants to sound waves. Jeong et al., (2014) found that it was possible to enhance drought tolerance in rice plants with sound waves. They have a significant effect on physiological changes. Sound frequencies enhanced relative water content in plant under drought stress compared to control. Therefore, it is presumed in this experiment that sound enhance relative water content in drought condition could be a reason of drought tolerance in rice. Other parameters as osmotic potential, H₂O₂ content, and stomatal conductance were measured. The increase of osmotic potential after treatment by sound are the indication of physiological adjustments of the plant in drought stress environment. In parallel, H₂O₂ content

gradually declined with sound treatment in drought environment. As there is a link between H₂O₂ and stomates because H₂O₂ has been proved to be a mediator of stomatal closure or inhibition of stomatal opening, observations showed that sound frequencies triggered the stomatal conductance in stress environment compared to control. All these reactions after sound treatment proved that rice plant can adapt physiological changes to enhance drought tolerance. Research on the adaptation of plants to sound waves inspired other scientists who made experiments to find other benefits generated by sound waves on plants, like in agronomy field or urban planning field.

III- PERSPECTIVE & APPLICATIONS

As said above, metabolism of plants and gene transfer are impacted by sound waves. With a better comprehension of plant mechanostimuli perception and their response, it could be possible now to manipulate plant growth to our advantage.

Agronomy applications

Applications of the sound wave stimuli on plants are abundant in agronomy sector. Productivity, seasonality of the crop, quality, maturation delay, disease prevention, alarm signal activation... all those problematics are currently treated by chemical agents than can potentially be replaced by acoustic treatment (Jung et al., 2018).

The PAFT produces an intermittent pulse of sound wave frequencies (Hassanien et al., 2014). The effects of PAFT on strawberries and cucumbers characteristics were studied in greenhouse (figure 5A). 42 days of greenhouse sound treatments allowed to increase the number of flowers and fruits, the

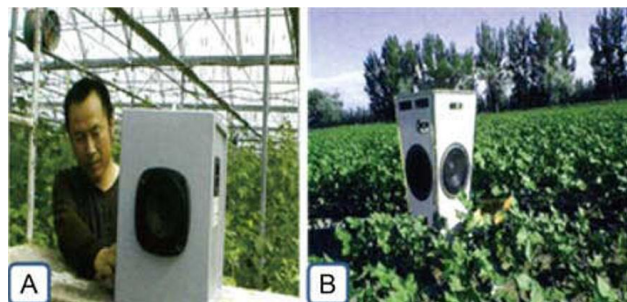


Figure 5 : (A) Treatment of PAFT in greenhouse. (B) Treatment of PAFT on field crops (Hassanien et al., 2014).

content of chlorophyll, the net photosynthetic rate and other characteristics. Moreover, there were effects in the “intern” system. The production of endogenous hormones such as IAA were stimulated and IAA content increased in tomato, muskmelon, cowpea, cucumber as well (Hassanien et al., 2014). PAFT effects have also been studied in outdoor cotton plants (*figure 5B*). Results showed that the yield of treated cotton increased in average of 12,7% compared to control. The distance between the sound source and plants was decisive in this experiment. Indeed, according to the distance transmitter-receiver, the rate of return was not the same (Hassanien et al., 2014). However, the limit of the experiment can be found in the disturbance of other species nearby.

Unlike chemical molecules, sound response seems to have specificities which can bring us to use sound to help some plant without affecting other species (Milburn and Johnson, 1966; Gagliano et al., 2012a; Rodrigo-Moreno et al., 2017; Jung et al., 2018). On the contrary, sound emission device is easily adaptable. Using the same machine, we can produce several frequencies. The chemical fabrication is not that simple. It is important to avoid the use of chemical pesticides for ecological concerned and human health. Using sound waves on plants would replace pesticides while improving the plants resistance (Mohanta, 2018). Indeed, sound waves enhanced behavior changes in the cell, regulate gene expression and these allow plants to face the biotic and abiotic factors which damage them (Hassanien et al., 2014). For instance, (Qi et al., 2010) studied the sound effects on strawberries in the leaf area. Results showed that sound stimulation allowed strawberries to grow stronger than control. In addition, resistance against disease and insect pest has been enhanced.

Most of the time, the emission of sound by plants is caused by morphological phenomenon. Consequently, sound emissions can be used to follow the state of plants health. Acoustics tools have been improved since the firsts studies and we can now use them as usual indicators for stress. Ultrasound technique can already evaluate the occurrence of freezing and dehydration stress in woody plant (Raschi et al., 1989; Rosner et al., 2006). Daily monitoring in woody plantations or in forest can be a concrete application of sound perception in plants to prevent frost and embolisms. However, for the moment our technical supplies do not allow us to apply this technic in open field because of the “noisy pollution” of the environment (Mohanta, 2018). Maybe future technics will fix this problem?

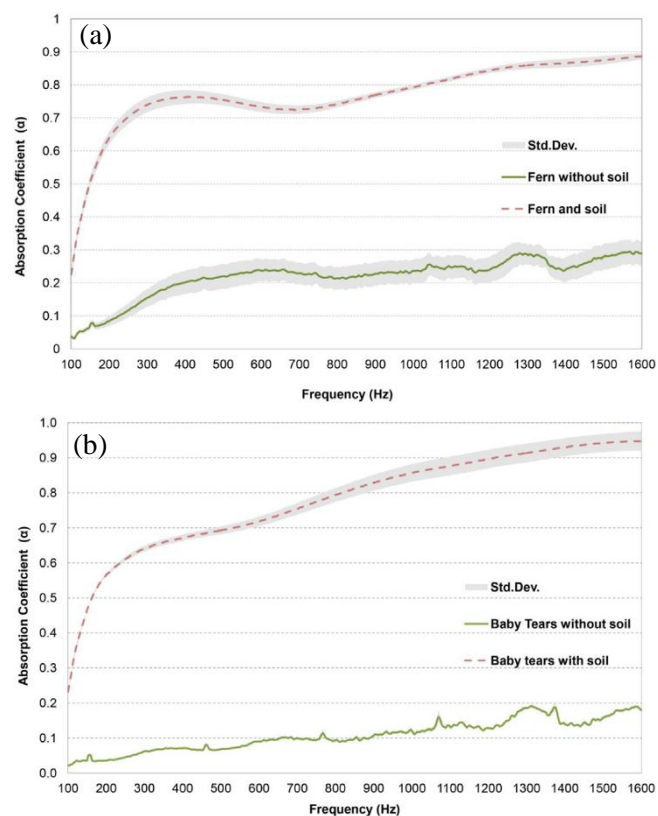


Figure 6 : Sound absorption of Fern (a) and Baby Tears (b) samples depending on the frequency and the presence of soil or not (D'Alessandro et al., 2015)

Urban applications

The « isolant » effect of plant to noise is well known and more and more used in architectural design, indoor as outdoor. This ability is not due to the perception of sound but to the physical particularities of sound waves. The mechanical waves, such as the sound ones, are well dispersed and absorbed by branches and foliage. The ground effect absorbs also a great part of the sound waves but much more in the lower frequencies (200-1000 Hz) (D'Alessandro et al., 2015; Huisman and Attenborough, 1991; Price et al., 1988). Once again, plant environment influences experiment results, indeed plants are only one of the sound absorption parameters. On the other hand, it has been noticed that high frequencies are better reduced by plant and that seems to be correlated with the leaves and spines. The species play a role in the sound effect, especially regarding the leaf density and the dominant angle of the species's leaves.

Leaves are able to absorb almost 50% of emitted waves and to convert their energy into heat through vibrations. This capacity is linked with the kind of soil, the humidity neighbouring, and of course the vegetation coverage. We saw in *figure 6*, from the experiment of D'Alessandro et al. (2015) that even if

the plant alone is able to absorb the sound wave, the ground is primordial to have a notice effect.

However, the noise reduction is not the only effect of plants on walls. They participate on the quality of the environment, depolluting the air, reducing the heat island effect on cities and even acting on work performance and stress for indoor installations (D'Alessandro et al., 2015). On a larger scale, woody barriers can minimize the negative impression of road sound. The sound of wind in leaves is relevant and induce a better perception of the area (Van Renterghem, 2018; Martens and Michelsen, 1981). All those sound barrier properties can lead to an improvement of urban life qualities. Vegetal walls are part of an interesting multidisciplinary approach for cities of tomorrow.

The manipulation of the growth of plant through sound wave is a new field and can be complicated to manage. However, the current interest of the subject and the researches published promise to do some interesting applications in the future for modern urban and agronomical fields.

CONCLUSION

Compared to other plant researches, studies about sound waves perception by plants is a really new field. Actual technologies allow us to glimpse all the plant sensitivity we barely know. Indeed, a lot of studies now shows how plants responded to sound wave stimulations from seed to fruit maturation.

Ecological field can enjoy perspectives offered by sound wave applications, as much in research as in agricultural sector. Plant protection and production could be developed with apparently limited risks.

However, we haven't much step back to physical and genetical modifications due to sound wave treatments and potential side effects. The emission of specific sound waves in open field can and will most of the time disrupt other species, especially the ones we know sensible to ultrasound. Indeed, specific frequency of the waves used by scientific are almost never hear in natural environment or on species without human benefits. Among others difficulties to further investigations, experiments in closed laboratory, isolating the studying plants, do not take into account some important bioclimatic parameters. Research about this part of sound wave emission treatment is still needed.

Furthermore, results from research still can be interpreted according to the theory of the author. If

further research identifies sound wave perception organ such as a kind of eardrums or a specific gene, it will be a great advance in mechanical stimuli perception in plants. In this way, the mechanism will be clear and without ambiguity.

To conclude, sound wave perception in plants is a new but already promoted field. The plant is more intelligent and sensible (touch, sound, memory...) to it surrounding that we thought. A large amount of possibility is at hand and future research could completely change our vision of plants and natural areas.

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Advances in relationship between plants and sound waves: Possible innovative applications

Résumé :

Tous les organismes vivants ont par nature un besoin vital de connaître leur environnement. Que ce soit par le toucher, la vue, l'odorat ou l'ouïe, ils ont tous développé des organes ou des sensibilités à un niveau plus cellulaire, spécialisés dans la perception des forces physiques et chimiques qui les entourent. Bien que faisant parti des organismes vivants, les plantes étaient considérées comme insensibles et muettes en raison de leur incapacité à se déplacer. Pourtant, Milburn et Johnson ont découvert dans les années 1960 que les plantes émettaient des sons. Depuis, de nombreux scientifiques se sont intéressés au rapport des plantes aux ondes audibles (20-20.000Hz) mais également aux ultrasons et infrasons. Il a en effet été prouvé que les plantes étaient capables d'émettre des vibrations sonores à différentes fréquences. Plusieurs théories (Ritman and Milburn, 1988; Laschimke et al., 2006; Gagliano et al., 2012a) ont tenté d'expliquer les raisons de cette production de sons et s'ils étaient intentionnels ou non de la part de la plante. D'autres études ont également montré qu'une exposition prolongée à des fréquences sonores spécifiques pouvait avoir des effets (positifs ou négatifs) sur le développement des plantes, et ce, à plusieurs échelles. Ces révélations peuvent remettre en perspective l'utilisation des produits chimiques sur les plantes, mais ouvrent également des applications potentielles dans d'autres domaines. De nombreuses études s'intéressent à l'environnement sonore des plantes mais des questions demeurent : quel organe ou molécule permet à la plante d'appréhender les ondes sonores ? Leurs émissions et réactions sont-elles seulement dues à un mécanisme physique ou sont-elles intentionnelles ? L'utilisation des ondes à grande échelle est-elle sans conséquence ? Malgré l'intérêt récent porté sur la bioacoustique appliquée aux plantes, les recherches dans ce domaine nécessitent d'être approfondies.

Mots Clés : Plantes, ondes sonores, stimuli mécaniques, perception, audio, bioacoustique.