Lab Notebook

Move Your Plant

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Objectives of the project

We want to observe the impact of different sound frequencies on lentils' growth and compare it with how a microphone receives frequencies.

Scientific questions :

Are the lentil seed growth sensitive to different sound frequencies ? [1][2][3][4][5][6]

(What is the impact of sound frequencies on lentil seeds growth ?)

Could we compare the precision and accuracy of this biological sound detector to an electronic sound detector ?

Material :

- Lentil seeds
- Water
- Coton
- Soundproofed room
- 12 wood boxes
- 24 leds
- 8 piezo buzzers
- 474-BOB-12758 sound detector
- 3 Arduino platforms

[1] « The Effect of Music on Plant Growth ». *Dengarden*. Consulté le 25 janvier 2017. https://dengarden.com/gardening/the-effect-of-music-on-plant-growth

[2] Silva, Jaime A. Teixeira da, et Judit Dobránszki. « Sonication and Ultrasound: Impact on Plant Growth and Development ». *Plant Cell, Tissue and Organ Culture (PCTOC)* 117, nº 2 (1 mai 2014): 131‑43. doi:10.1007/s11240-014-0429-0

[3] Jeong, Mi-Jeong, Chang-Ki Shim, Jin-Ohk Lee, Hawk-Bin Kwon, Yang-Han Kim, Seong-Kon Lee, Myeong-Ok Byun, et Soo-Chul Park. « Plant Gene Responses to Frequency-Specific Sound Signals ». *Molecular Breeding* 21, nº 2 (1 février 2008): 217‑26.doi:10.1007/s11032-007-9122-x.

[4] « Effects of sound field on the growth of Chrysanthemum callus ». Consulté le 1 février 2017. http://www.sciencedirect.com/science/article/pii/S0927776501002752.

[5] « Effects of sound stimulation on energy metabolism of Actinidia chinensis callus ». Consulté le 1 février 2017. http://www.sciencedirect.com/science/article/pii/S0927776503000274.

[6] « Effects of sound stimulation on protective enzyme activities and peroxidase isoenzymes of chrysanthemum ». Consulté le 1 février 2017. http://www.sciencedirect.com/science/article/pii/S0927776502000383

- 2 Proto-shield Arduino
- 12 grid, created by laser cutter
- Sound-proofing ribbon
- Sartorius TE212 scientific field weighing scale

25/01/17

Preparation of lentils.

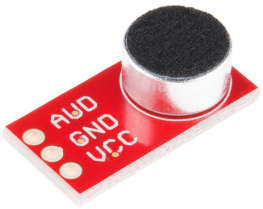
At 3pm we started to germinate the seed of green lentils.

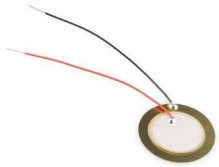
We took 3 plastic boxes where we put a layer of cotton at the bottom of each one and moisturized them with tap water. Then, we put a layer of seeds, being careful that seeds are not touching each others. At the top, we added a new layer of cotton moisturized.

We placed the plastic boxes under light.

Test of the sensors :

Test of piezo elements and 474-BOB-12758 sound detector [7][8] using library (Exemples) [9][10][11] on Arduino. We managed to understand the link between notes and frequencies, how to change and choose them using file, to change note duration.





[7] « Electret Mic Breakout Board Hookup Guide - learn.sparkfun.com ». Consulté le 25 janvier 2017. https://learn.sparkfun.com/tutorials/electret-mic-breakout-board-hookup-guide?_ga=1.249487495.1266180026.1485364000.

[8] « Microsoft Word - CEM-C9745JAD462P2.54R - CEM-C9745JAD462P2.54R-1022832.pdf ». Consulté le 25 janvier 2017. http://www.mouser.com/ds/2/813/CEM-C9745JAD462P2.54R-1022832.pdf

[9] « Arduino - Tone ». Consulté le 25 janvier 2017. https://www.arduino.cc/en/Reference/Tone

[10] « Arduino - ToneMelody ». Consulté le 25 janvier 2017. https://www.arduino.cc/en/Tutorial/ToneMelody?from=Tutorial.Tone

[11] « Use tone() with Arduino for an Easy Way to Make Noise ». *Electronics Academy* , 21 juillet 2015. https://programmingelectronics.com/an-easy-way-to-make-noise-with-arduino-using-tone/.

Buzzers | pitches.h

```
 http://www.arduino.cc/en/Tutorial/Tone

 */
#include "pitches.h"
int dt = 50;

void setup() {


}

void loop() {
  //for (int thisNote = 0; thisNote < 8; thisNote++)

    // to calculate the note duration, take one second
    // divided by the note type.
    //e.g. quarter note = 1000 / 4, eighth note = 1000/8, etc.
    //int noteDuration = 1000; // noteDurations[thisNote];
    tone(7, NOTE_B0);  //Box4
    delay(dt);
    noTone(7);
    tone(9, NOTE_GS6);  //Box3
    delay(dt);
    noTone(9);
    tone(10, NOTE_A7);  //Box2
    delay(dt);
    noTone(10);
    tone(11, NOTE_DS8);  //Box1
    delay(dt);
    noTone(11);

    //

}
```

Buzzers | pitches.h

```
/*************************************************
 * Public Constants
 *************************************************/

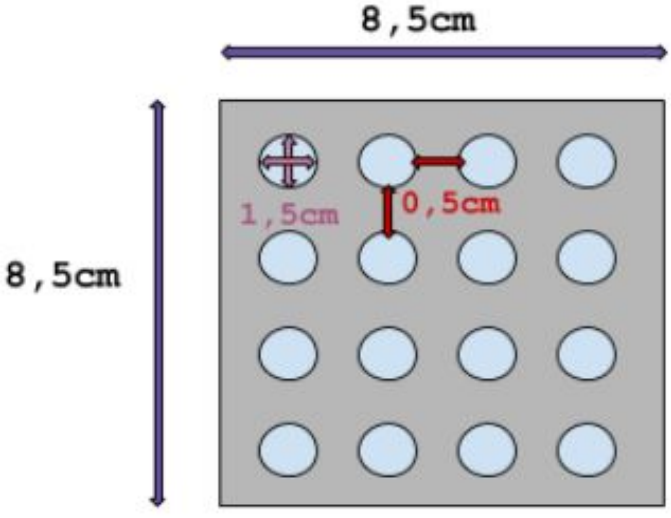
#define NOTE_B0  31
#define NOTE_C1  33
#define NOTE_CS1 35
#define NOTE_D1  37
#define NOTE_DS1 39
#define NOTE_E1  41
#define NOTE_F1  44
#define NOTE_FS1 46
#define NOTE_G1  49
#define NOTE_GS1 52
#define NOTE_A1  55
#define NOTE_AS1 58
#define NOTE_B1  62
#define NOTE_C2  65
#define NOTE_CS2 69
#define NOTE_D2  73
#define NOTE_DS2 78
#define NOTE_E2  82
#define NOTE_F2  87
#define NOTE_FS2 93
#define NOTE_G2  98
#define NOTE_GS2 104
#define NOTE_A2  110
#define NOTE_AS2 117
#define NOTE_B2  123
#define NOTE_C3  131
#define NOTE_CS3 139
#define NOTE_D3  147
#define NOTE_DS3 156
#define NOTE_E3  165
```

26/01/17

Set-up design beginning

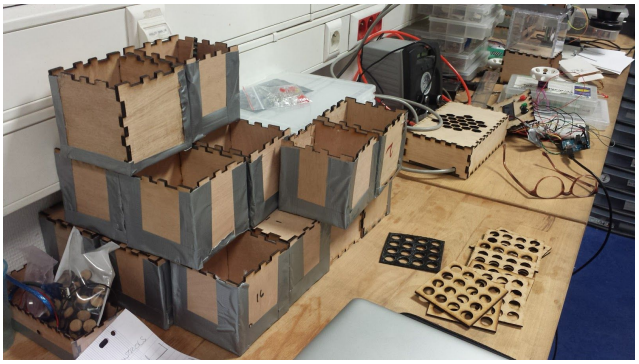
Preparation of grids for lentils cultures so that each seed will be separated in special designed-boxes using laser cutter machine.

12 wood boxes were taken from the Biosensor week 1 group : Threshold Falcons (Nina Guérin, Adrien Vergès, Lara Narbona-Sabaté).



Design of the grid (thickness = 3mm)

See more about their project below :
https://github.com/lnarbona/LightProject_Biosensors/tree/master/Threshold_Falcons)



We pierced two holes (diameter = 6 mm) in the top of the boxes with the laser cutter where we will put red LEDs that will allow lentils seeds to maximize photosynthesis and grow for our experience.

We sticked the outside of 12 boxes with a soundproofing tape ( ) in order to avoid sound to spread around the box and influence other boxes with lentils.

We tested the piezo buzzer that arrived in the afternoon [12] ( ) with the code Arduino Tone. It sounds !

At 3 pm we started to germinate new seeds of green lentils. This lentils are our biological replicate. Same protocol than the 25/01/2017 : moisturized layer of cotton, a layer of separated lentil seeds and another layer of cotton at the top.

We also labelled plastic boxes with time and day of cultures for each sample.

[12] « Fiche produit : Buzzer Continu 83dB, pilotage Interne, 2400 ? 3400 Hz ». Consulté le 25 janvier 2017. https://www.rs-particuliers.com/WebCatalog/Buzzer_Continu_83dB__pilotage_Interne__2400__34Hz-6221405.aspx.,

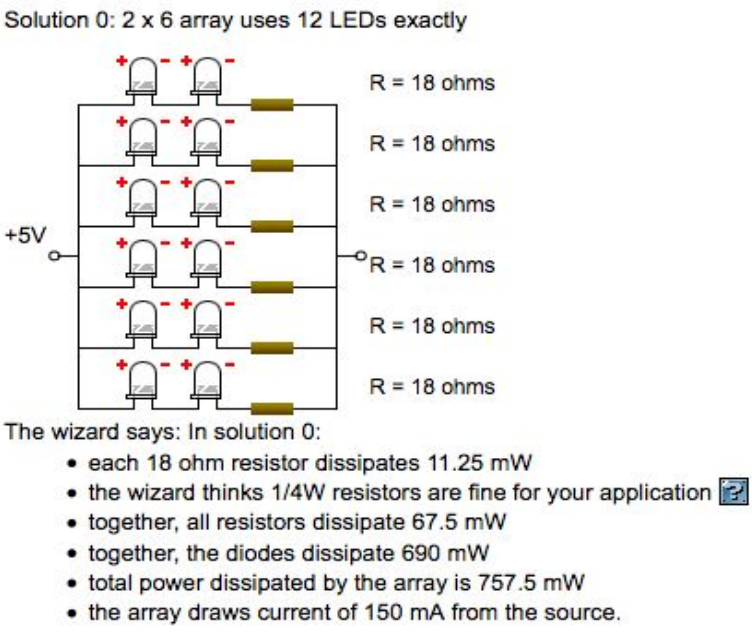
27/01/17

Welding day

⚠ The cotton was dried very rapidly because of the light, so we wet again the lentils with the same amount of water.

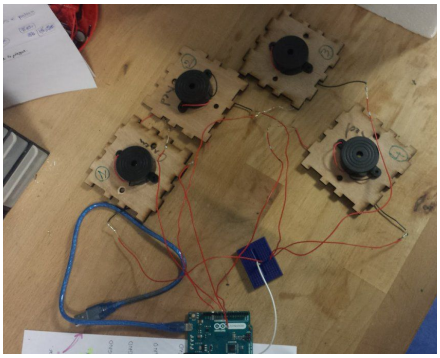
We welded the set-up for 12 LEDS for one replicate as following.
This design was taken from the " " (http://led.linear1.org/led.wiz) [13] and using some additional informations given by Threshold Falcons team to do this tipe of installation.

LED	Voltage	Intensity
infrarouge	1.5 V	50 mA
red	2.3 V	25 mA
green	2.2 - 2.5V	20 mA
blue	4 V	20 mA
uv	3.8 V	20 mA



As described in the schematics, we used 18 ohm resistors in order to regulate electricity and components. We welded the circuits on a proto-shield platform[14] in order to have more 5V and GND pins.

During the other part of the day, we focused on the inclusion of piezo buzzers in the set-up that will help to spread sound frequencies in boxes. We began to add them to the setup and managed to create a code that diffuse continuously all different frequencies that we wanted to work with using that we already talked before. We fixed them under the lid of the boxes between the two holes of LEDs.



[13] « LED series parallel array wizard ». Consulté le 27 janvier 2017. http://led.linear1.org/led.wiz.
[14] « Arduino - ArduinoProtoShield ». Consulté le 27 janvier 2017. https://www.arduino.cc/en/Main/ArduinoProtoShield.

To implement the buzzers we used 33k ohm resistors in order to regulate electricity flow and decrease sound power so that the other boxes will not be influenced by each other frequencies.

28/01/17 :

Beginning of the biological experiment

- We welded the 4 piezo buzzer to the Proto-shield (This Proto-shield was already linked to the 12 LED).
  - ❏ The Piezo buzzer of the box " 1" was linked to the pin 11 (Positive side) and the GND (Negative side).
  - ❏ The Piezo buzzer of the box " 2" was linked to the pin 10 (Positive side) and the GND (Negative side).
  - ❏ The Piezo buzzer of the box " 3" was linked to the pin 9 (Positive side) and the GND (Negative side).
  - ❏ The Piezo buzzer of the box " 4" was linked to the pin 7 (Positive side) and the GND (Negative side).

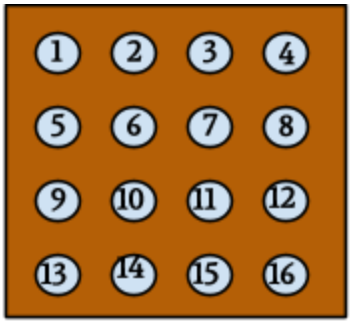
INCLURE UN SCHÉMA

- We prepared the inside of the box. In each box, we put :

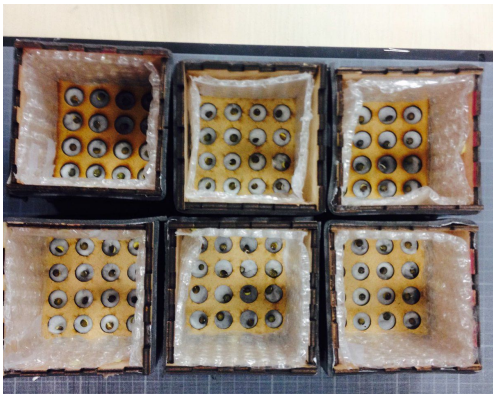
__ ___ _____ _ a layer of waterproof tape (prevent the wood to decompose) + the wood grid realised with the laser cutter the 26/01/17 to separate each seed lentil.

_____: A layer of bubble pack to diffract sound waves and keep them inside the box .

- We weighed the germinated seeds with the
. We also determined the germ length in millimeters. The ruler used was not precise so the length values are not really accurate. We put the 16 seeds in each box in the same order and we colored in pink a side of each box in order to know the box orientation. Finally, each seed is labeled with its own weight and length.



Positioning the lentils seeds



Lentil label : boxnumber_positionnumber.
Example : Lentil in the 1st box at the position 15 → 1_15

- We put 0,5 ml of water for each seed.
- We taped the top of the box with the sound-proof tape. It allows to soundproof but also to fix the piezo to the boxes.

⚠ Not all the seeds were germinated. We didn't have enough germinated seeds to fill all the boxes so we took both germinated and ungerminated seeds in each boxes. The problem is that we didn't do it really randomly so there is maybe more germinated seeds in the boxes 1 and 2 than in the box 5 and 6.

- The arduino is powered by a plug socket.
- All the boxes were recovered by a blanket for a sound-proofed.

Global scheme of the experiment :

Note	Equivalent frequency
note_DS8	4978 Hz
note_A7	3520 Hz
note_GS6	1661 Hz
note_B0	31 Hz

Each lentil is lighted by two red LED (see above).
Controls consisted in lentils not exposed to any sound frequency.

29/01/17

Beginning of electronic sensor experiment

During the morning, we brainstormed about the electronic sensor part and finally decided to work with mobile microphone instead of 474-BOB-12758 sound detector due to setup installation and data collecting facilities.

The goal of the experiment was to expose the electronic sensor (mobile microphone) to the piezo speaker at the different frequencies.
By that, we would like to analyse the precision and reaction time of mobile microphone using data analysis.
Protocol :

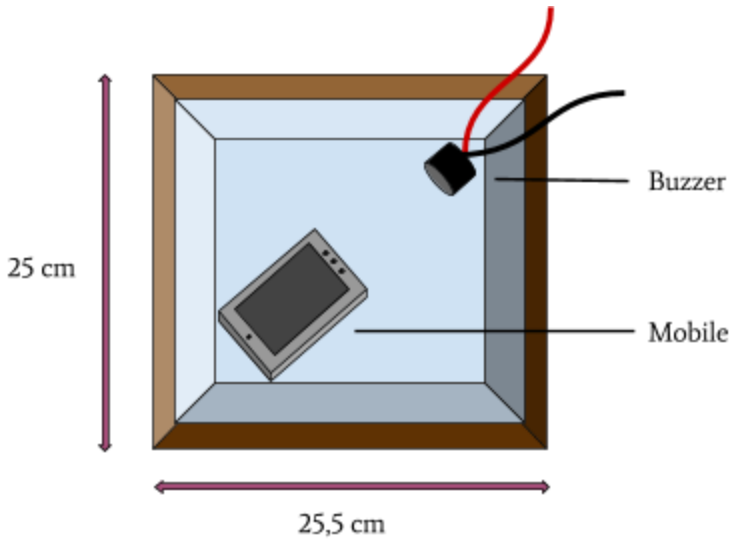
We recovered a big box from @HertzSSystem (25,5x25cm) similar to our small experiment boxes. The outside was already sticked with soundproofing tape and we had a layer of bubble pack inside.

Realize the following Arduino montage using a piezo buzzer, a 33k ohm resistor, a breadboard and a Leonardo card.

Connections :

- Put the positive terminal of the piezo buzzer on pin 7.
- Put the resistor just after negative terminal of the piezo buzzer on the breadboard.
- Close the montage putting a cable on GND pin.

Once done, we placed the latter installation on the big experiment box with a Huawei P8 mobile as following :



We realized the experiment exposing the phone with the four different frequencies that we used for the biosensor (**note_DS8, note_A7, note_GS6, note_BO**) during 30 seconds and repeated it 10 times for each of them. We did 3 replicates so that we can have 30 data per frequency.

In order to make the piezo buzzer spread sound we used exactly the same code as before with the spreading only one one frequency of sound from only one pin of the Leonardo card.

We recorded sound all along the experiment using the built-in microphone of the mobile. Finally we got a lot of mp4 files that we will use for data analysis.

Buzzers § pitches.h

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 http://www.arduino.cc/en/Tutorial/Tone

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#include "pitches.h"
int dt = 50;

void setup() {


}

void loop() {
  //for (int thisNote = 0; thisNote < 8; thisNote++)

    // to calculate the note duration, take one second
    // divided by the note type.
    //e.g. quarter note = 1000 / 4, eighth note = 1000/8, etc.
    //int noteDuration = 1000; // noteDurations[thisNote];

    tone(7, NOTE_B0);  //connect to pin 7, choose the sound frequency here
    delay(dt);

    //noTone(7);
    //tone(9, NOTE_GS6);
    //delay(dt);
    //noTone(9);
    //tone(10, NOTE_A7);
    //delay(dt);
    //noTone(10);
    //tone(11, NOTE_DS8);
    //delay(dt);
    //noTone(11);
}
```

Data analysis of the first sample of lentils

At the end of the afternoon we stopped the first set of lentils. We continued quantitative and qualitative observations measuring weights of seeds and germ length in millimeters … data began to take shape !

# of replicate	# of box	# of lentil in the box	Lentil ID	Weight before	Germ length before	Weight after 48h	Germ length after 48h

01/02/2017:

<u>Statistical analysis :</u>
We did a student test, using the t.test function in R.

⚠ We didn't find a way to control if our datas were following a normal law before doing this statistical test.
All our samples were < 30.

#t-test lenght box4, B0 vs box 5 control :

- p-value = 0.08774 > 0,05

→ NOT significative

#t-test lenght box4, B0 vs box 6 control :

- p-value = 0.06955

→ NOT Significative

AU DODO ARMELLE :) ON VERRA ÇA DEMAIN VITE FAIT ENSEMBLE C'EST PRESQUE FINI ! HAHA YES MERCI POUR CETTE POLICE INCROYABLE.