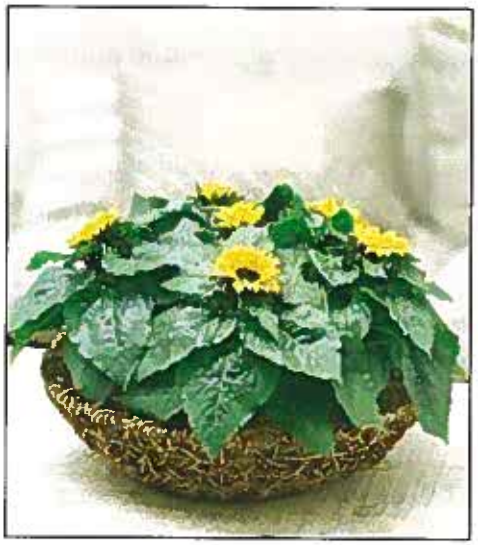
"From a seed to a nice plant"



"From a seed to a nice plant"

Abstract

In this study I wanted to examine the growth of selected seeds in different conditions. In particular, the focus was to find out which factors give the longest sprouts in a limited amount of time.
I chose the following variables: seeds ("broccoli Decicco" vs."sunflowers"), "watering" fluid (coffee vs. water), growth medium (soil vs. cotton), and additional nutrients (without vs. with). A full 2^4 factorial design was performed, for a total of 16 experiments.
The interaction between growth medium and additional nutrients turned out to be very important. The use of soil without nutrients is the best choice for the longest sprouts.
Regarding the type of seeds, the results show that sunflowers germinate longer than broccoli if inserted in soil.
No significant differences resulted between the employment of water and coffee.

Introduction

Most of us have tried in life to seed some lentils and see that something amazing happens afterwards.
It is interesting to consider the seeding procedure as a possible background in which to perform a design of experiments. For this type of analysis it is necessary to choose some factors which are supposed to influence the response we want to examine. However, we are sometimes not able to predict if the variables can be significant at a certain level. Eventual interactions between the factors could be even harder to predict if the operator is not an expert in the field. Thus it is important to use a program like MINITAB to investigate the problem and draw reasonable conclusions.

1 Seeding procedure

Many years ago I used lentils seeded in cotton for similar experiments. At that time my teacher recommended keeping the seeds in a warm and moist place in order to get sprouts. She also suggested watering them daily. This is what I remember from previous experience when I started the experiments for this study.
The general procedure I have repeated for all the experiments is the following:

- I took plastic glasses where I first inserted the growth medium. The amount of the medium was such that it filled 2/3 glass. I used soil (for flowers) and cotton.
- Additional nutrients were added to some glasses. The nutrients are specific for plants. Regarding the dose, I prepared a solution diluting one spoon of liquid nutrient in one glass of water; then one spoon of this solution was given to the medium of the glasses which were supposed to receive nutrients.
- I proceeded positioning some seeds (7 for each glass) inside the growth medium.
- All the containers were put in a box (Figure 1.1); the box was covered with a plastic film on the top to guarantee humidity for the seeds. The box had some small holes on the sides, thus oxygen could still come inside it.

Figure 1.1 Box and 16 containers with the seeds: during the experience all the glasses were put inside the green box which was covered with a plastic film on the top to guarantee proper humidity conditions.

- I went on watering the seeds daily using two watering-cans, one filled with water and one filled with coffee.
- I kept all the containers in the warmest place of the flat (bathroom, 21° C). As mentioned before, the seeds always need warm environment and humidity to start sprouting.

In eight days it was possible to notice sprouts grown from small seeds which did not seem could have generated any life. Some sprouts grew a lot (up to 23.3 cm), some others grew just few centimeters and others did not grow at all.
Afterwards the statistical analysis was carried out to see which factors and which interactions were significant.

2 Factors and response variable

The following four factors were considered to be interesting for the problem:

- Regarding the seeds, broccoli Decicco (Brassica oleracea) and sunflowers (Helianthus anuus) were chosen. Since they belong to two different species, I thought that one of them would have probably grown faster.
- Two kinds of "watering" fluids (coffee vs. water) were employed. Water is the most typical used, while coffee is not usual. I wanted to know if the use of coffee would have compromised the growth of sprouts.
- Concerning the type of growth medium, the most natural one is soil. When someone wants to perform a seeding at home, cotton is often used. Cotton is an excellent absorbent material, where the roots of the sprouts can absorb water (and eventually nutrients) from. I believed that using soil or cotton would not have affected the experience.
- Regarding the presence (or absence) of nutrients, generally the nutrients are used for grown plants and not for seeds. Anyhow I thought that the presence of nutrients may have facilitated the growth phase.

There are some factors that were not included but which could be important to investigate in a further experience.

- In the present study all glasses stayed in an environment where light and darkness alternated. Instead, one could try to keep some containers in a room with light on and others in a room with light off constantly.
- The use of additional nutrients could be introduced when the sprouts are already born. Similarly to the present study, one may check an eventual effect in the response.

Concerning the interactions, it was difficult to say a priori which ones would have been significant since I am not expert in this kind of simple cultivation. This is why it was interesting to analyze the data.
In Table 2.1 it is shown the scheme of the factors used in the present study.

Factor	-	+
Seeds (A)	Broccoli Decicco	Sunflowers
Watering fluid (B)	Coffee	Water
Growth medium (C)	Soil	Cotton
Additional nutrients (D)	Without	With

Table 2.1 Factors used in the experience.

The response variable was measured in the following way. Eight days after the experiments started, in each glass, the longest sprout was selected. A ruler of 30 cm was used to measure its length from the level of the growth medium up to the extremity of the sprout (Figure 2.2).

Figure 2.2 The response variable was measured from the level of the growth medium up to the extremity of the longest sprout. The sprouts in figure are from broccoli seeds.

This length was chosen as response variable.
The accuracy of my measurements is approximately 1 mm.
As response variable it could also be possible to choose the weight of the sprouts. Thus one could evaluate which seeds are more productive. However, due to the lack of high-precision scales this response was not investigated.

3 Choice of the design and execution of the experiment

Four factors were chosen. I had the possibility to perform a full factorial design (the costs related to experiments were not high). All 16 experiments were done the same day thus no block effect occurred.
The experiments are performed following the randomized order from MINITAB. Randomizing the runs helps to avoid systematic error due to the order.
The matrix from MINITAB, as well as the measured responses (Length), is reported in Table 3.1.

StdOrder	RunOrder	CenterPt	Blocks	Seeds	Watering fluid	Growth medium	Additional nutrients	Length (response variable)
5	1	1	1	-1	-1	1	-1	0.1
2	2	1	1	1	-1	-1	-1	20.3
16	3	1	1	1	1	1	1	0.9
9	4	1	1	-1	-1	-1	1	0.2
15	5	1	1	-1	1	1	1	0.0
12	6	1	1	1	1	-1	1	6.9
6	7	1	1	1	-1	1	-1	1.1
1	8	1	1	-1	-1	-1	-1	11.7
10	9	1	1	1	-1	-1	1	5.9
13	10	1	1	-1	-1	1	1	0.0
4	11	1	1	1	1	-1	-1	23.3
8	12	1	1	1	1	1	-1	4.5
7	13	1	1	-1	1	1	-1	9.1
3	14	1	1	-1	1	-1	-1	12.2
14	15	1	1	1	-1	1	1	1.5
11	16	1	1	-1	1	-1	1	2.9

Table 3.1 Matrix of the design of experiments.

The unit measure of the response variable length is cm.

4 Problems related to the seeding procedure

It is not necessary to be an expert in cultivation to reproduce the experiment and, if someone wants to try it, I will now share some of the experience gathered during this work.
In the beginning I tried to use some seeds I already had at home, i.e. lentils and beans. I did not have any idea of how deep to insert the seeds, so I decided to position them quite deeply (almost at the bottom) either in soil or cotton. After seven days I saw that very few seeds were sprouting. I was quite surprised because I was sure that I would have got some sprouts.
I decided then to call Leuthens Frø AS to ask for some advice. The expert on the phone recommended changing type of seeds since I wanted a fast growth process. He sent me some broccoli and sunflower's seeds. He also suggested positioning the seeds in the soil (or cotton) at a depth equal to the diameter of the seed and not as deep as I did before.
Crossing my fingers, I started the experiments once more, and this second time they worked a lot better.

5 Analysis and results

I used MINITAB to perform the analysis. In the beginning I included all terms up to the 4th order in the analysis. When there is no error term, MINITAB uses Lenth's method to find important effects [1]. The results are displayed in Figure 5.1, Figure 5.2 and Figure 5.3.

```
Estimated Effects and Coefficients for length (coded units)

Term       Effect    Coef
Constant             6,287
A           3,525    1,763
B           2,375    1,187
C          -8,275   -4,138
D          -8,000   -4,000
A*B        -0,675   -0,337
A*C        -3,825   -1,913
A*D        -0,500   -0,250
B*C         0,575    0,287
B*D        -1,600   -0,800
C*D         4,900    2,450
A*B*C      -0,875   -0,438
A*B*D       0,100    0,050
A*C*D       2,000    1,000
B*C*D      -1,650   -0,825
A*B*C*D     1,150    0,575


S = *    PRESS = *

Analysis of Variance for C9 (coded units)

Source              DF   Seq SS   Adj SS   Adj MS   F   P
Main Effects         4  602,168  602,168  150,542   *   *
2-Way Interactions   6  168,947  168,947   28,158   *   *
3-Way Interactions   4   29,993   29,993    7,498   *   *
4-Way Interactions   1    5,290    5,290    5,290   *   *
Residual Error       0        *        *        *
Total               15  806,398

* NOTE * Could not graph the specified residual type because MSE = 0 or the
         degrees of freedom for error = 0.
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Figure 5.1 Results from MINITAB analysis with terms up to 4th order.

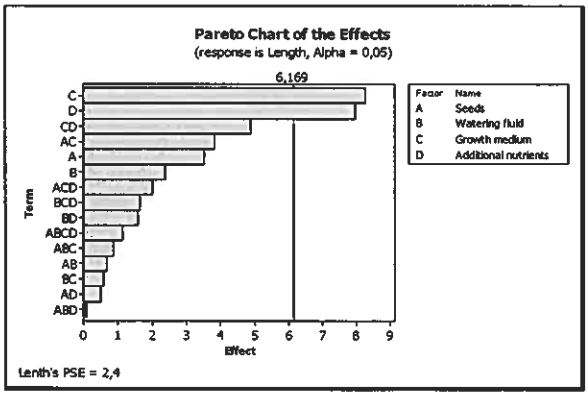

Figure 5.2 Pareto-chart of the effects with terms up to 4th order.

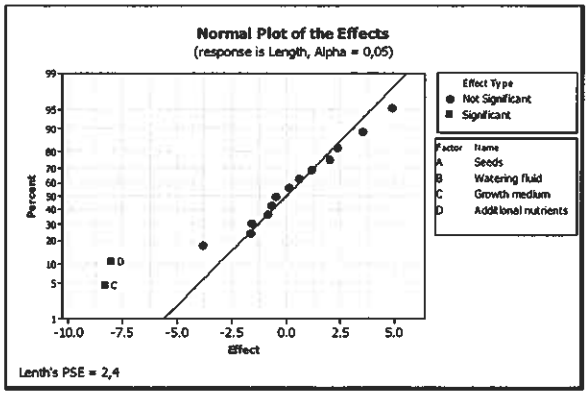

Figure 5.3 Normal plot of the effects with terms up to 4th order.

Any effect that exceeds the red line is significant. It follows that the main effect C (growth medium) and D (additional nutrients) are significant.
Afterwards a second analysis was performed: no replicates were done but the higher order interactions (3rd and 4th) were set equal to zero (now there are 5 degrees of freedom for the residual error). In this case MINITAB uses p-values to identify important effects [1]. The analysis was performed with $\alpha = 0.05$, by default. Figure 5.4 shows the results from MINITAB.

```
Estimated Effects and Coefficients for length (coded units)

Term      Effect    Coef  SE Coef      T      P
Constant           6.287   0.6641   9.47  0.000
A          3.525   1.763   0.6641   2.65  0.045
B          2.375   1.187   0.6641   1.79  0.134
C         -8.275  -4.138   0.6641  -6.23  0.002
D         -8.000  -4.000   0.6641  -6.02  0.002
A*B       -0.675  -0.338   0.6641  -0.51  0.633
A*C       -3.825  -1.913   0.6641  -2.88  0.035
A*D       -0.500  -0.250   0.6641  -0.38  0.722
B*C        0.575   0.287   0.6641   0.43  0.683
B*D       -1.600  -0.800   0.6641  -1.20  0.282
C*D        4.900   2.450   0.6641   3.69  0.014


S = 2.65641     PRESS = 361.293
R-Sq = 95.62%   R-Sq(pred) = 55.20%   R-Sq(adj) = 86.87%


Analysis of Variance for Y (coded units)

Source              DF  Seq SS  Adj SS   Adj MS      F      P
Main Effects         4  602.17  602.17  150.542  21.33  0.002
2-Way Interactions   6  168.95  168.95   28.158   3.99  0.075
Residual Error       5   35.28   35.28    7.057
Total               15  806.40
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Figure 5.4 Results from MINITAB analysis with terms up to 2nd order.

The residuals are now available, see Figure 5.5.

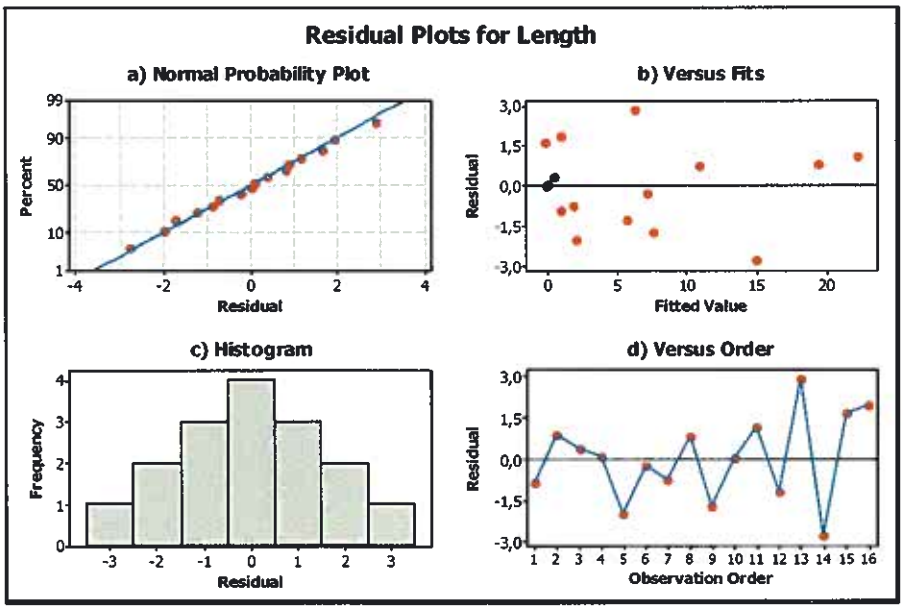

Figure 5.5 Four residual plots for the measured response, length.

The plots (Figure 5.5) show the behavior of the residuals. The assumption is that the errors are independent N (0, σ^2) random variables. The errors are not observed, but however we can measure the residuals that "reconstruct" the errors and verify the assumption [2]. The four plots in Figure 5.5 are the following:

a) Normal probability plot of residuals: the points follow a straight line, meaning that the residuals are normally distributed and thus the normality assumption is verified.
b) Residuals vs. fitted values: this plot shows a random pattern of residuals on both sides of zero. Hence the constant variance assumption is valid.
c) Histogram of the residuals: the plot shows the typical values of the residuals (from -3 to 3) and their spread. The histogram is bell-shaped and symmetric and this confirms the normal distribution of the residuals.
d) Residuals vs. order of data: this is a plot of all residuals in the order that the data was collected and can be used to find non-random error, especially of time-related effects [1]. The residuals at observation number 13 and 14 are slightly larger than the others. However, this is not enough to indicate a trend in the data since the observations were only sixteen. Moreover, we have to consider that the seeds were planted within some minutes, left growing for some days, and finally the sprouts were measured within some minutes: in this context it seems very unlikely that the order of the measurements can influence the results. Thus assumption that the residuals are uncorrelated with each other can be considered valid.

The new pareto-chart and the normal plot of the standard effects is the following (Figure 5.6 and Figure 5.7):

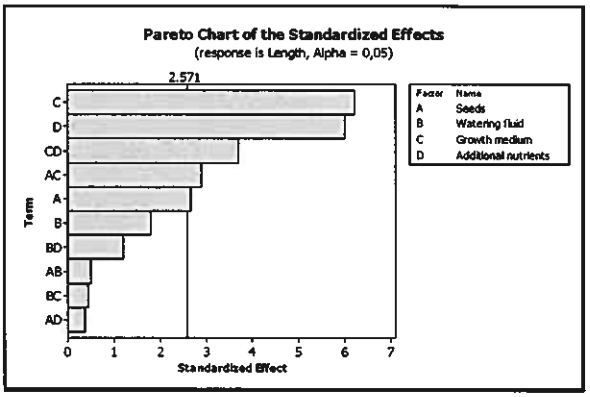

Figure 5.6 Pareto-chart of the effects with terms up to 2nd order.

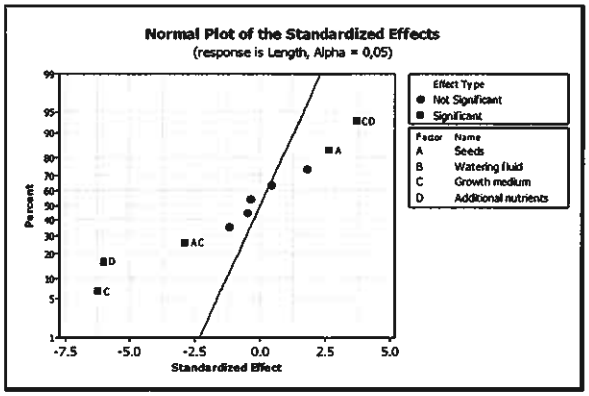

Figure 5.7 Normal plot of the effects with terms up to 2nd order.

As before, C (growth medium) and D (additional nutrients) are the most significant, but now their interaction is also significant, hence it follows that the main effects should be analyzed jointly.
Another difference respect to the previous analysis is that the main effect A (seeds) and the interaction AC turns out to be significant. A discussion regarding the interaction CD and AC follows.

6 Discussion

The plots of the interactions CD and AC are the following:

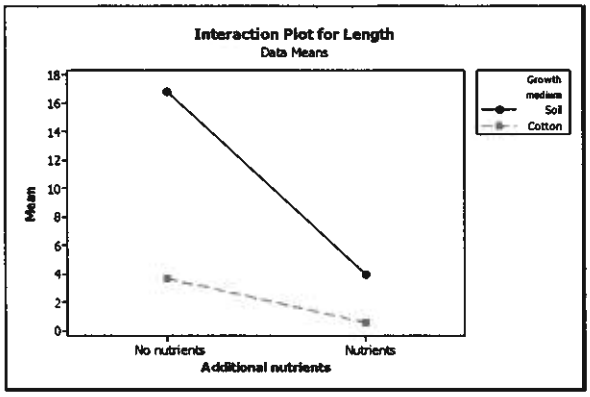

Figure 6.1 Interaction plot between growth medium and additional nutrients (CD).

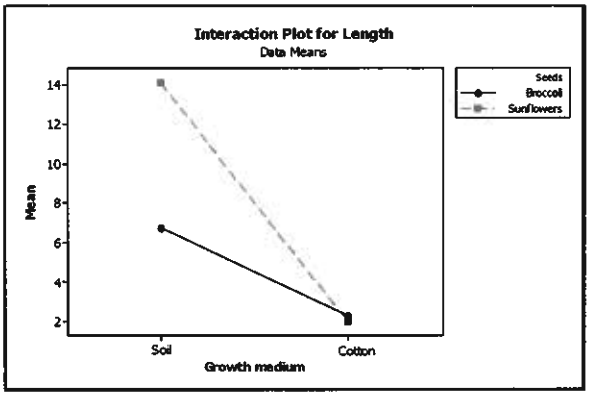

Figure 6.2 Interaction plot between seeds and growth medium (AC).

- In Figure 6.1 there is the plot of the interaction CD (growth medium vs. additional nutrients). The plot shows that using nutrients and cotton produces short sprout (less than 2 cm); the seeds inserted in soil without the addition of nutrients produced very long sprouts (about 17 cm). Regarding the slope of the two lines, the line for the soil is steeper; it follows that the effect D "additional nutrients" has greater effect when the soil is used instead of cotton. The results suggest that soil without additional nutrients produces the tallest sprouts regardless the type of seed used.
  In the beginning of the experiments I thought that the nutrients could have helped the sprouts to grow faster. After the experiment I can say that the nutrients are probably more useful in phase of maintenance of the plant and not in phase of growth. When the seeds are sprouting, it seems that the nutrients work as a "poison" in the growth medium, so the sprouts can not grow up easily.
- Figure 6.2 shows the interaction AC (seeds vs. growth medium). When cotton is used, the obtained length is not that high (about 2.5 cm) for both types of seeds. The results show that cotton is not a good medium for an experiment whose response is evaluated within eight days (short time). Nevertheless, I remember that some sprouts could germinate from lentils seeded on cotton. But probably, when I performed the experiment years ago, it took more than eight days before I could notice sprouts as long as some centimeters.
  The employment of soil together with sunflower seeds gives a better response (about 14 cm) respect to the use of soil with broccoli (about 7 cm). The sunflower seeds are represented by a steeper line, meaning that the growth medium has more effect when sunflowers are used.

An example of sunflowers seeded in soil is reported in Figure 6.3.

Figure 6.3 Sunflowers seeded in soil.

- The first analysis (performed with Lenth's method) shows that 3rd order interaction between A, C and D is not significant. But from the experiments, it seemed the tallest sprouts are generated when sunflowers are seeded in soil with no nutrients. In spite of this, it is very important to remark that the interaction ACD is not significant, and no conclusion can be drawn regarding it.
- From the experiments it is also possible to state that the effect of watering fluid (factor B) is not significant. This is a surprising result because I thought that watering with coffee could have inhibited the growth of the seeds. The results show that it is possible to use either water or coffee without any significant difference in the response.

7 Conclusions and further developments

The focus of the present experiment was to study which factors give tallest sprouts in 8 days. The results say that it is important to use soil without nutrients to gain the wanted response for both types of seeds.
If one wants to choose a type of seed, then the results show that sunflower seeds should germinate more efficiently than broccoli if inserted in soil.
Regarding the fluid, watering the seeds either with water or with coffee does not have any significant result.
About eventual developments, it is possible to perform a DOE study by introducing another factor (i.e. full light vs. full darkness). With 5 variables, 2^5 experiments are performed. Then it would be very interesting to assess which are the most significant effects in this more complex background.

References

[1] Minitab version 15.1.20, Documentation.
[2] Walpole, Myers, Myers and Ye, "Probability & Statistics for engineers and scientists", Pearson Education, 8th edition.