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Roxane Trabattoni

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Study of the origin of the heterogeneity of the germinative quality in fennel seed production (*Foeniculum vulgare* Mill. var. *azoricum* Tell.)

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Confidentiel

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Les analyses et les conclusions de ce travail d'étudiant n'engagent que la responsabilité de son auteur et non celle d'AGROCAMPUS OUEST

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Je tiens à remercier toutes les personnes qui ont contribué au succès de mon stage et qui m'ont aidée lors de la rédaction de ce mémoire.

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Table of content

List of abbreviation

List of figures

List of tables

List of appendix

Introduction	1
I - State of the art	3
1. Fennel: from flowering to seed setting	3
1.1. Flowering dynamic	3
1.2. Seed characteristics and ripening	3
2. The various sources of heterogeneity in the germination quality	4
2. 1. The possible sources of variability of the germination quality	4
2. 2. The sources of variability related to the cultural itinerary of fennel seed-bearing	5
2. 2. a. Fennel seed production at Bejo	5
2. 2. b. The sources of variability related to the technical practices	6
3. Seed ripening indicators	8
4. Conclusion	9
II - Material and Method	11
1. Material	11
1.1. Plant material	11
1.2. Observation sites and technical practices	11
2. Methodology	11
2.1. Environmental monitoring	11
2.2. The flowering dynamic observation	12
2.3. The seed ripening monitoring	12
2.4 Data processing	14
III – Results	15
1. Environmental monitoring	15
2. The flowering dynamic	15
3. The seed ripening	15
4. Statistical analysis	17
IV – Discussion	19
1. Discussion of the results	19
2. Discussion about the later acquisition of the germination at Delabarre site	20
3. Critical analysis of the study	22
4. Perspectives	23
Conclusion	25
References	
Appendix	

List of abbreviation

EU	European Union
GLM	Generalized linear model
DAF	number of days after the end of flowering
MC	moisture content
DW	dry weight
GF	Germination faculty

List of figures

Figure 1	Schematic diagram of Fennel flowering stems showing the general umbel arrangement	3
Figure 2	Fennel umbel (<i>Foeniculum vulgare</i> Mill.)	3
Figure 3	Schematic fennel flower	3
Figure 4	Fennel umbelets	3
Figure 5	Evolution of the fennel fruit (schema and pictures)	3
Figure 6	Planting pattern and observation sites according to the type of tunnel	11
Figure 7	Bejo cultural practices for fennel seed crop production from the young plant reception to harvest	11
Figure 8	CIELAB model of color space	13
Figure 9	Minolta CM-700d spectrophotometer	13
Figure 10	Color measurement of a fennel sample made with the spectrophotometer	10
Figure 11	Mean daily temperatures (°C) in tunnels from the respective planting date to the respective day of cutting	15
Figure 12	Flowering dynamics according to the accumulation of degree days (°D) for each tunnel	15
Figure 13	Evolution of the physical characteristics of secondary and tertiary umbel order per tunnel	16
Figure 14	Seed color changes of the secondary umbels (left) and tertiary umbels (right), represented by the color variables a* and b*	18

List of tables

Table 1	The characteristics of the production sites	11
Table 2	Sampling calendar	12
Table 3	Degree-days cumulated during the physiological stages	15
Table 4	Beauchêne, Physical and Physiological characteristics of the secondary umbel seeds (A) and tertiary umbel seeds (B)	16
Table 5	Auxère, Physical and Physiological characteristics of the secondary umbel seeds (A) and tertiary umbel seeds (B)	16
Table 6	Delabarre, Physical and Physiological characteristics of the secondary umbel seeds (A) and tertiary umbel seeds (B)	16
Table 7	Values of the color variables, CIELab color space for the secondary umbel seeds	17
Table 8	Values of the color variables, CIELab color space for the tertiary umbel seeds	17
Table 9	Significance of the factors effects on the germination faculty when run in the different GLM model	18

List of appendix

Appendix 1	Additional pictures
Appendix 2	Illustrations of the moisture content and the germination faculty per tunnel
Appendix 3	Multiple linear regression for the moisture content and color variables
Appendix 4	Example of script and results of data analysis (samples of the harvest day)
Appendix 5	Technical practices of each tunnel (1/2) and (2/2)

Introduction

Fennel (*Foeniculum vulgare* Mill. *Apiaceae*) is used since the Antiquity for culinary and medicinal purposes (Collin and Fourc, 2014) and is still worldwide cultivated for its numerous properties. The most well-known is the use of fennel as remedy against digestive disorders (El-Awadi and Hassan, 2010). Fennel production extends in temperate to sub-tropical climatic zones, and particularly in the Mediterranean basin, where fennel comes from (Raj and Thakral, 2008). The whole plant can be widely used (Heidari *et al.*, 2014), from its enlarged edible base as vegetable, to the seeds as spice and for essential oil, especially in India, Pakistan, Iran, Egypt (Simandi *et al.* 1999, Choi and Hwang 2004, Anand *et al.* 2008, El-Awadi and Hassan, 2010).

Depending on the final product required, different botanical varieties are used, all coming from the species *vulgare*. These varieties are var. *vulgare*, var. *dulce* and var. *azoricum* (Collin and Fourc, 2014). It is the latter, var. *azoricum*, the “bulbous” fennel which is used for the production and thus, consumption of vegetable. *Foeniculum vulgare* var. *azoricum* is, in the European Union (EU), mainly produced in western countries and particularly in Italy, which is the leading producer (542 000 tonnes per year) and exporter (80 % of EU exports) of “bulbous” or also named Florence’s fennel (Serrurier and Ottens, 2010). In France, fennel volume production is not big enough to be recorded but it is admitted that fennel production is mostly localized in the south-east, in Brittany and in Loire-Atlantique (Serrurier and Ottens, 2010).

Several seed companies offer fennel varieties in the market, and the Bejo Company is one of them. Bejo France, subsidiary of Bejo Zaden, the Netherlands, is established at Beaufort –en – Vallée (Maine et Loire) since 1987. The company experiments, multiplies (own production and production from farmers) and commercializes conventional and organic vegetable seeds for professionals. The varieties of fennel sold by Bejo are exclusively F1 hybrids to reach as much as possible the market criteria for Florence’s fennel: a round and white (as much as possible) fennel “bulb” and low sensitivity to bursting. Some of the conventional and organic (9 and 5 varieties obtained from Bejo, respectively) ones like Solaris, Rondo, Orion, are internationally recognized (GAB, 2010; Mazollier, 2009; Neacsu *et al.*, 2015). Fennel seeds sale contributes to 3% of the Bejo France turnover (€280,000, with 23% for the organic part in 2015). Even though it represents a few part, compared to the 20% for onions, carrots, cabbages each, this proportion is increasing (€160000 in 2010). According to Bejo, fennel production is still developing in the Britain and Nantes regions in France, and in Italy as well, due to the recognition of the beneficial properties for health of this vegetable.

Good quality seed lots are required to establish good crops and develop the production. National standards for the seed market are high and company standards are even higher. For fennel seeds, national standards are 80% of emergence (Directive 2002/55/CE) and Bejo standards are 90% of emergence. Moreover, Bejo fennel seeds are mainly sold with post-harvest treatments (simple coating or chemicals added) and the required standards rather reached 95% of germination, especially for the Italian market.

However, during the last years of production of fennel seeds, the results, both in germinating quality and in yield, are highly heterogeneous. From 2006 to 2014, germinating percentages within years and especially within producers (for Bejo production or farmer production) greatly vary, without any notable trend that could explain these variations (organic or conventional management, indoor or outdoor, commercial varieties). This is well illustrated with the year 2014 for which the average of the germination percentage was 84% but varies between 35% and 98% with 22 lots of 44 not reaching the 90% germination.

Given the market standards, the required quality and the means employed by the company and the farmers, with this heterogeneity problem, fennel seed lots do not properly meet the demand. It rises beyond the production side, an economic issue for Bejo.

Although poor seed germination is a well-known problem in plants belonging to the *Apiaceae*, with most of the studies specific to carrots (*Daucus carota* L.) (Robinson, 1954; Dean *et al.*, 1989; Geard *et al.*, 2006), there is a lack of information about fennel seed production (for vegetable crops and not oil production) under temperate climate. The Bejo Company needs to better understand the fennel plant, and specifically the mechanisms involved in the germination quality during periods of flowering and seed ripening which are key stages in seed production. It is indeed the morphological characteristics of flower and the flowering dynamics (plant scale) of fennel, specific to the *Apiaceae* (*Umbelliferae*) family, which mostly determine the formation of the future fruits and their maturation until harvest. This is, furthermore, in understanding the seed maturation dynamic that it will be possible to optimize the date of harvest and consequently, the germination percentage.

That is why we are now proposing a study of the origin of the heterogeneity of germination quality in fennel seed production (*Foeniculum vulgare* Mill. var. *azoricum* Thell.) through the observation of the flowering and seed ripening phenomena. Thus we are interested in these two issues: What is the influence of umbel orders on the germination quality? How does the seed maturation dynamic take place and when is the germination faculty acquired?

To do this, after explaining the characteristics of flowering and fructification of fennel over time we will broach the various sources of variability in germination quality, in general, or related to the fennel cultural itinerary at Bejo. Finally, we will see if it is possible to establish relationships between the germination percentage and maturity indicators, particularly the water content, the maturation period after flowering and the seed colors.



Figure 1. Fennel umbel (*Foeniculum vulgare* Mill.) Umbel without involucre and involucl.



Figure 2. Fennel umbels. The corolla of a flower is formed of five equal petals, free, rounded end, bented.

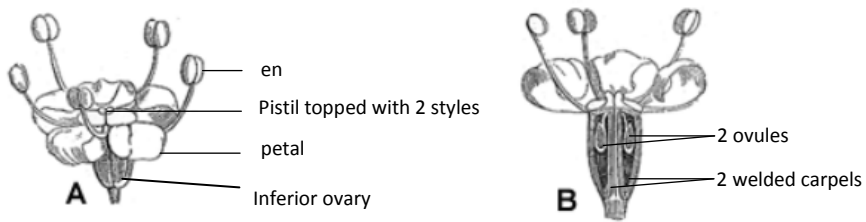


Figure 3 Schematic fennel flower. **A.** Global view. **B.** Longitudinal section

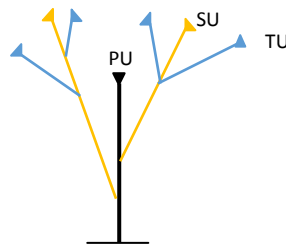


Figure 4. Schematic diagram of Fennel flowering stems showing the general umbel arrangement. PU (black) = primary umbel, SU (blue) = secondary umbel, TU (yellow) = tertiary umbel. Inspired by Bianco *et al.* 1994.

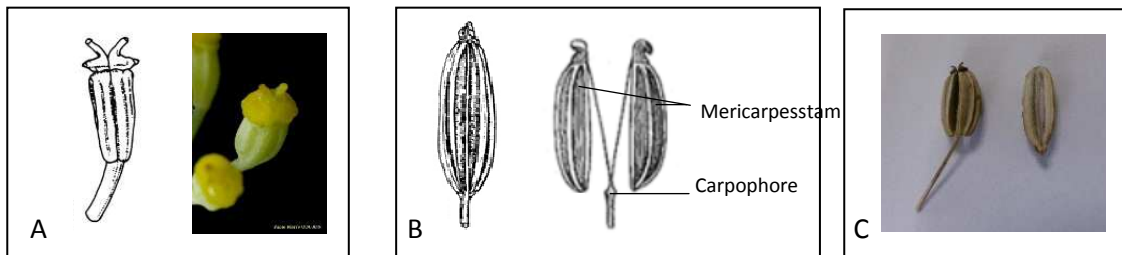


Figure 5. Evolution of the fennel fruit (schema and pictures) Immature fennel fruit (A); schizocarpe (B) which divide into two mericarps (B and C).

I - State of the art

1. Fennel: from flowering to seed setting

Fennel is a perennial herbaceous plant, mostly cultivated as an annual plant. Leaf fennel (*Foeniculum vulgare* var. *dulce* or var. *vulgare*) can be 250cm tall whereas bulbous fennel (*Foeniculum vulgare* var. *azoricum*) leaf length will not exceed 60cm. One main stem, erected and divided, constitute the fennel plant. Leaves are pinnatisected, 3 to 4 times divided, linear, thin and elongated (Collin and Fourc, 2014). The edible portion of fennel that is constituted by a compact overlapping arrangement of the wide and thick sheath leaves is called “crown” or “bulb” (Damato *et al.*, 1994).

1.1. Flowering dynamic

As a member of the *Apiaceae*, fennel produces flowers grouped in compound umbels, each carrying 15-30 rays (Renaud and Delaunay, 2011), terminated with umbellet (figure 1). Flowering takes place from July to late August, depending on varieties, in the temperate zone (Chung *et al.*, 2003). The yellow flower (figure 2) has a pentaradial symmetry, with 5 stamens, 5 petals, 2 styles and 2 carpels (figure 3A). The reduced sepals are not observable. The flowering is centripetal in umbellets and umbels (Collin and Fourc, 2014). Fennel is protandrous. That is to say, within each flower, pollen is shed before the stigma becomes receptive (Sundararaj *et al.*, 1963). Within each umbel and within each umbellet, flowers at the periphery reach anthesis first and those in the centre last. (Falzari *et al.*, 2005).

Different umbel orders are commonly described. Each plant produces a central leading stem with one primary umbel. Several secondary umbels are produced terminally on lateral branches, and on branches arising from the secondary branches, tertiary umbels are produced (figure 4). Plants may produce quaternary and even fifth umbel orders (Peterson, 1990, Falzari *et al.*, 2005). A characteristic of fennel species is that the total number of the lower rank umbels increases continuously, while their diameter, number of umbellets and number of individual flowers decrease (Szekely *et al.*, 2002).

An interesting point of the flowering dynamic at the whole plant scale is that the development of the different umbel orders is time shifted and frequently when the elder umbels end flowering, the next umbel order only start (Németh *et al.*, 1999). Furthermore, Szekely *et al.* (2002) described that the total flowering period of the 1st umbel range takes about 27-28 days, and is reduced to 22-23 days for the 3rd umbel range.

1.2 Seed characteristics and ripening

Fennel is self-fertile and can be self-pollinated (Németh *et al.*, 1999, Falzari *et al.*, 2005) but is considered as a cross-pollinated plant because of time difference in the development of different umbel ranges and protandry characteristics with pollen shed before the stigma becomes receptive (Sundararaj, *et al.*, 1963; Bell, 1971; Falzari *et al.*, 2005).

As for all the *Apiaceae* family members, the ovary is inferior (figure 3B), compound of 2 welded carpels according to a commissural plan (Richard and Drapiez, 1837). Each lodge contains an ovule. The fruit is composed of two akenes (diakene), welded together and

covered externally by the calyx (Agrobio47, 2011; Collin and Fourc, 2014). As illustrated by the figure 5, these two akenes detach from one another from the bottom to the top at maturity, and remain suspended by the summit at the end of a filiform axis called carpophore (Bautier, 1832). This fruit can be called more precisely a schizocarp, due to its bicarpellary gynoecium origin and the division at maturity in two segments (called mericarp) each containing a seed. Each mericarp has 2 sides, one convex, it is the dorsal side; and the one by which the two akenes apply one against the other is planar or concave, it is the commissural side (Beautier, 1832).

Fennel seeds dress various colours during their development, from green (early stage of development) to brown. Seeds are oblong, with 5 prominent ribs. Seed length is about 6 to 7mm and the thousand seeds weight is between 4 to 8 grams according to different authors (Chung *et al.*, 2003, Collin and Fourc, 2014;).

2. The various sources of heterogeneity in the germination quality

2. 1. The possible sources of variability of the germination quality

Germination is the transition for the seed from latent to active life under favorable conditions (Khanfouci, 2005). According to Mazliak (1982), it is the physiological process whose limits are the early hydration of the seed and the beginning of growth of the radicle. A seed is germinated when the radicle has pierced the envelopes (Come, 1970).

The causes of the variability of seed germination capacity are multiple and not always well known. They mostly depend on the genetic background and the added environmental factors can alter the expression of the genetic properties (Come, 1970).

That heterogeneity is due to (Come, 1970; Mazliak, 1982):

- The genetic factors
- The factors before harvest, consisting in a combination of the microenvironment experienced by the seed due to its position on the parent plant and the abiotic environment of the plant. Gutterman (2000) takes an inventory of the maternal factors and the environmental effects which contribute to the variability in seed germinability in many species. These maternal factors are the position of the inflorescence on the mother plants, the position on the seeds in the inflorescence or in the fruit and the age of the mother plant during flower induction or seed maturation. In addition, environmental factors operating during development and maturation which can modify seed germination are day length, temperature, parental photothermal environment, light quality, water availability and even altitude, during seed development and maturation.
- The harvest factors, in particular at the seed maturity stage at harvest time, determined by the date of harvest.
- The factors after harvest, including all the cleaning, drying, sorting and other treatments.
- The germination factors (temperature, light, gases).

In addition to these factors, some seeds can be considered as dormant, meaning that even if they are viable and placed under good conditions of germination, they will not germinate. Different kinds of dormancy exist, and two are commonly admitted as references: seed coat dormancy and embryo dormancy.

2. 2. The sources of variability related to the cultural itinerary of fennel seed-bearing

The sources of variability that can be considered in a context of production at Bejo France are those that occur from the period "pre-harvest" (from the mini plugs planting) to seed sampling before the germination tests. We are not concerned here with the variability due to genetic factors or post-harvest treatment. Therefore, in order to understand where the sources of variability related to the production come from, we will firstly explain how fennel crop production is conducted at Bejo.

2. 2. a. Fennel seed production at Bejo

- Hybrid varieties production

All the fennel varieties commercialized by Bejo are hybrid varieties, coming from cross-breeding between lines with cytoplasmic male sterility used as "female" plants and lines used as "male" for pollination and destroyed after flowering. The most important thing in hybrid production is the consistency of the flowering period for male and female lines. Otherwise the planting scheme is 2 male lines for 4 female lines.

- Fennel production under shelters

Fennel seed-bearing production can be established in open field or in tunnels. At Bejo France it is mostly cultivated in tunnel in order to have a better control and expect to achieve higher production goals (germination quality or yield). In the same vein, fennel can be sown directly or planted from trays. Transplanting interest results in getting a better recovery and crop uniformity for the market gardener. It requires high quality seeds to optimise and make profit with the nursery stage (Collin and Fourc, 2014). An internal nursery of Bejo is responsible for producing the trays and send them to the other production sites and seed growers in order to be planted out in March/April.

- Water requirement

Fennel root system is able to ensure efficient water supply (Collin and Fourc, 2014), however in greenhouses or under shelters, water inputs from outside are limited to the ambient humidity. Irrigation is made by sprinkling or dripping from the flowering period. It is important that the plant does not suffer from water stress during seed setting and ripening because a water deficit could reduce seed formation and grain yield (Meena *et al.*, 2013; Parthasarathy, 2008).

- A better seed-setting with pollinators

Pollination is a crucial element in seed production. The most important impact will not be on the germination quality but on the seed yield. Fennel is self- fertile and can be self-pollinated (Németh *et al.*, 1999, Falzari *et al.*, 2005) but is considered as a cross-pollinated plant because of time difference in the development of different umbel ranges and protandry characteristics

with pollen shed before the stigma becomes receptive (Sundararaj, *et al.*, 1963; Falzari *et al.*, 2005). Artificial pollination and especially pollinators work is a significant benefit to increase seed yield (Ramanujam *et al.*, 1964; Pillai and Nambiar, 1982, Chaudhary, 2006). Németh *et al.* (1999) observed the possibility of autogamy inside the same umbel (between umbellules or among umbels of the same range) with a lower seed setting, 50% in average (but from 25 to 69%) compared to the 92% rate of seed setting with free pollination. In most production system farmers bring pollinators to insure good pollination work. Honey bees (*Apis cerana* F., *A. florea* F. and *A. mellifera* L.) and syrphid flies (*E. balteatus*, *Eristalis arvorum*, *Eristalis* spp.) are the most common pollinators contributing to fennel seed production (Youngken 1956; Narayana *et al.* 1960; Sagar 1981; Baswana 1984; CCS HAU 2000, 2001; Chaudhary *et al.* 2002). Bejo works on pollination with its own beekeeping team and installs its own hives in the greenhouses. Bumblebees and flies are also used.

- Male destruction after flowering

Fennel production at Bejo is exclusively hybrid production, thereby fennel male plants have to be destroyed at the end of flowering to avoid crop mix at harvest.

- Pest management

Fennel is not very sensitive to diseases and pests (Collin and Fourc, 2014). The sanitary management differs according to conventional or organic crops but the diseases and pests to monitor remain the same: sclerotinia (*Sclerotinia sclerotiorum* or *Sclerotinia minor*), oïdium (*Erysiphe umbelliferarum*, syn *Erysiphe heraclei*) and recently observed *Diaporthe angelicae* (anamorph *Phomopsis foeniculi*) (Rodeva and Gabler, 2011) for the diseases; aphids (*Myzus persicae* (polyphagous) and *Hyadaphis foeniculi* (more specific to the Apiaceae family hosts) and bedbugs, *Graphomposita italicum*, *Lygus* spp., for the pests (Collin and Fourc, 2014).

- Seed harvest

The harvest takes place from September to October. Maturity stage, in order to optimise the germination quality of all umbel orders, is not fully known. Assessment is visual and is based on the seeds colour. Bejo technicians recommend to harvest when seeds of the secondary umbel orders turn brown. Generally, the threshing is done only once and there are therefore all the umbel orders in the lot, whatever the seed maturity stage. At Bejo, the lots are simply micro-cleaned to remove impurities, and not sorted, before sampling for the germination tests. Lots are then sent to The Netherlands (Bejo Zaden) for the eventual post-harvest treatments and conditioning.

2. 2. b. The sources of variability related to the technical practices

As previously explained, the factors mostly influencing the seed germination are those related to the development of the seed on the mother plant, and those related to the environment. In this study our interest is in the first ones and we will see the sources of variability that may arise directly from the technical practices. According to the literature and available information (given the lack of information about the culture of fennel, research has

sometimes been extended to Umbellifereae, or carrot culture), these sources may have more or less influence on the germination quality.

- The sources of variability considered as having few impact on the germination quality are:

- The planting date

Few studies have been conducted to evaluate the impact of the planting date on seed yield and quality, however the few ones reveal that the planting period may influence the number of primary branches, secondary branches and seed yield in open field under semi-arid conditions (Ahmad *et al.*, 2004; Ayub *et al.*, 2008; Lofti *et al.*, 2013). No data have been found on this same kind of experiment under occidental (temperate) conditions and with the variety *azoricum*.

- The plant density

The plant density recommended to Bejo producers is about four plants per linear meter. Several studies have been conducted on density and row spacing effect on fennel crop, however the aim of most of these studies is to improve essential oil production (Falzari *et al.*, 2005, Khorshidi *et al.*, 2009). Few authors take into account the germination quality and when it is done the results show there are not differences among different plant density in seed quality characteristics (speed of germination, germination value, thousand seed weight) (Damato *et al.*, 1994). Plant density will have a major role in the plant architecture (number of umbel per order) and on subsequent potential for pollination and yield (Falzari *et al.*, 2005).

- The fertilisation input

Nitrogen fertilization is considered as one of the most important factor for a good establishment in most of crops (Damato *et al.*, 1994). Though, in the literature, results of experiments suggest that the quality of fennel seeds cannot be improved by higher nitrogen rate (it only improves vegetative biomass). Nitrogen rate has a poor influence on seed yield and yield components (number of umbellets per primary umbel, thousand seed weight) and in addition may possibly have a negative impact on seed quality in increasing the time to obtain 95% of final germination (Damato *et al.*, 1994).

- The sources of variability known for their impact on umbelliferae seeds quality are:

The studies about the establishment of seed and low germination rates in umbellifer crops (Sehgal, 1966) reported several recurring factors that could be related to the heterogeneity of the germination quality of fennel seeds:

- Seed abortion because of lack of pollination or fertilization.
- Insect attack after fertilization of the ovaries which prevents the development of the embryo: Chalcid fly (*Systole albipennis*) and bedbugs (*Orthops* sp, *Lygus* spp.) are known to be responsible of low seed-set in umbellifers (Gupta, 1964; Sehgal, 1966, Flemion, 1949) and it is quite studied in carrot seed production (Spurr, 2003, Morel *et al.*, 2014, Fnams, 2014). *Systole* lays eggs in the developing ovaries, then adults

develop at the cost of the endosperm, embryo or both, and escape by boring a hole in the fruit wall (Seghal, 1966). Flemion and Olson (1950) observed that ungerminated seeds in several genera of the Umbelliferae had a normal seed coat and endosperm but lacked the embryo. Therefore they conclude that *Lygus* and *Orthops* bugs cause damage by consuming the young embryo in maturing seeds, causing embryolethality. To ensure an ideal crop protection against these bugs it is recommended to do insect detections with shaking umbels, sticky traps or pheromone (Fname, 2014, Morel *et al.*, 2014; Blackmer *et al.*, 2008; Holopainen *et al.*, 2001). At Bejo this problem is emergent and studies are in progress on fennel (Italy) even if the presence of these insects was not the object of observations at Bejo France yet.

- The embryonic problems, bad development or dormancy for which reason is unknown.

However, we know that the harvest period is a key element since it results from the flowering dynamic and the ripening stages, and that we will find all the umbel orders in the seed lots.

Now, the contribution of the seed position on the mother plant to the yield and quality is not well known in fennel. Bianco *et al.* (1994) described the influence of years, cultivars and umbel orders of Florence fennel on yield, yield component and germination. However the study shows opposite results between years, due probably to weather conditions. Even in carrot production studies show divergent results in seed qualities coming from different umbel positions. Authors reported that seeds from the primary umbels had higher quality and higher thousand seeds weight than those from the secondary ones (Muniz, 1999; Gray, 1979; Szafiriowska, 1994, Panayotov, 2010) and globally that carrot seeds from primary and secondary umbels had higher percentages of germination capacity and developed larger seeds than those from the tertiary and higher order umbels (Corbinea *et al.*, 1995. Satyaveer *et al.*, 1994, Shanta *et al.*, 1999) whereas others substantiated that they found the best quality in carrot seeds from secondary, and not primary, umbels (Elballe and Cantliffe, 1997). We can just be sure that in the *Apiaceae*, seeds from different positions on the umbel vary greatly in size and degree of dormancy (Gutterman, 2000).

That is the reason why it is highly interesting to know good indicators of fennel seeds maturity to optimize the harvest date and then the germination rate.

3. Seed ripening indicators

An important quality property of seeds is the maturity status. Seeds which are immature or not fully mature are generally speaking of lower quality as mature seeds (Jalink *et al.*, 1998). Maturation is a set of successive stages in preparation for successful seed germination. During the seed development there is initially the formation of the different tissue types within embryo, endosperm and surrounding seed as a result of extensive cell division; this is the histodifferentiation stage. A large increase in fresh weight is achieved by the time this stage is completed. Then follows the stage of cell enlargement or expansion (phase 2), when the major reserves within the embryo and storage tissues are laid down. As reserve deposition occurs, water is displaced from the seed and is replaced by the insoluble polymeric resources. Hence, the dry weight of the seed increases, and its proportional water content declines. During the third phase dry matter accumulation slows and stops at physiological maturity (Bewley *et al.*, 2006).

For many seed crops, it is important to know when physiological maturity has been reached across the crop as a whole prior to starting the harvest because the timing of harvesting a seed crop can directly influence the quality of the seed.

Visual indicators or physiological parameters are very helpful since quick scheduling decisions often must be made. In research as well as in production practices indicators are used to identify seed maturation: physical appearance (colour, size, shape, texture ...), seed moisture content, dry weight, chlorophyll content, visual indicators specific to the species (black layer or milk line in maize for example).

The seed moisture content is an helpful indicator for estimating the seed maturity (Bewley *et al.*, 2006) In carrot crops it has been concluded that to obtain the highest quality of seed, harvest should be delayed until seed moisture contents have fallen to approximately 20% or below (Joyce *et al.*, 1989).

Furthermore estimations of the optimum time to harvest in carrot is based currently on the appearance of the seeds and the time elapsed from flowering (Hawthorn & Pollard, 1954; Joyce *et al.*, 1989; Bewley and Halmer, 2006). Joyce *et al.* (1989) found that the maximum seed dry weight occurred between 40 and 45 days after flowering as the percentage germination after 14 days in carrot seeds.

Another indicator could be to characterize the colour of the seed lots and study whether there is a link between the colour of the lot and the quality of germination. As we have seen previously, fennel seeds will go through different colours according to their maturity. This approach by colorimetry can help determine whether there is a colour level before the adequate maturity stage. It has been demonstrated that a relationship exists between seed coat colour and seed quality in watermelon (Mavi, 2010). The author used a Minolta colorimeter, as we will do, to characterise seed colour values in the CIELAB reference. The L* value indicates the changes in colour brightness, a* value indicates colour change from green (negative values) to red (positive values). The b* value indicates colour change from yellow (positive values) to blue (negative values). H parameter indicates hue angle value and C* (chroma) indicates colour density.

4. Conclusion

Fennel, as a member of the Apiaceae family, has a particular flowering dynamic. Not only the flowers, grouped in umbels, are protandrous; but also the formation and the flowering of the different umbel orders is time shifted. This time interval inevitably follows the same pattern for the fruit formation, which, according to the umbel orders, will not be mature at the same time. Added to this, the architecture of the plant suggests an internal variability with factors contributing to the good seed development on each order umbel.

Otherwise, the production of fennel seed at Bejo is exclusively an hybrid seed production, partly under tunnel for a better control of the environment. It is admitted that the technical practices influence the germination quality of seeds in general and can be the cause of this variability in quality, as well as genetic and environmental factors. In fennel, these potential factors of variability are poorly studied and then not well known. It is reported that the Apiaceae's seeds are subject to bad embryo development (flat seeds but with well-formed

envelops) or by insects like bed bugs that feed on embryos. Moreover, we know that the harvest date is a key stage for the germination quality of the seed lot, and that harvest date is determined empirically without being assured of the full seed maturity. Several indicators exist to measure seed maturity, including the water content, the time of development after flowering and the seed color measurement.

We propose in this work to test the following hypotheses:

- Is there a link between the umbel orders and the germination seed quality? We assume that the seeds of the primary and secondary umbel orders have a better germination rate than the other orders, at any stage of their development.
- Is it possible to determine when the germination faculty will be acquired by following the seed maturation dynamics with indicators?
 - During maturation the seed water content decreases. Considering the fresh weight graph it would be possible to determine in days after flowering a stage of maturity
 - There is a link between the seeds color and the germination quality. In theory, the more the seed is brown, the more the germination percentage increases (up to a certain threshold).

Table 1. The characteristics of the production sites

Site	Tunnel characteristic	Area (m ²)	Insect-proof	Orientation	Agronomic management	Previous crop	Soil	Planting date
Beauchêne (Bejo production 49250 Beaufort en Vallée)	Bi-chapel SR 9,6	1000	Yes	West-East	Conventional	Leek	Silty	24/03/16
Auxère (Bejo production 49250 Beaufort en Vallée)	Bi-chapel SR 9,6	1000	Yes	West-East	Organic (soil covered with black plastic)	Leek	Silty	14/03/16
Delabarre (Farmer, 49350 Les Rosiers sur Loire)	Plastic covered tunnel	300	No	North-South	Conventional (soil covered with black plastic)	Leek	Clay- loam	21/03/16

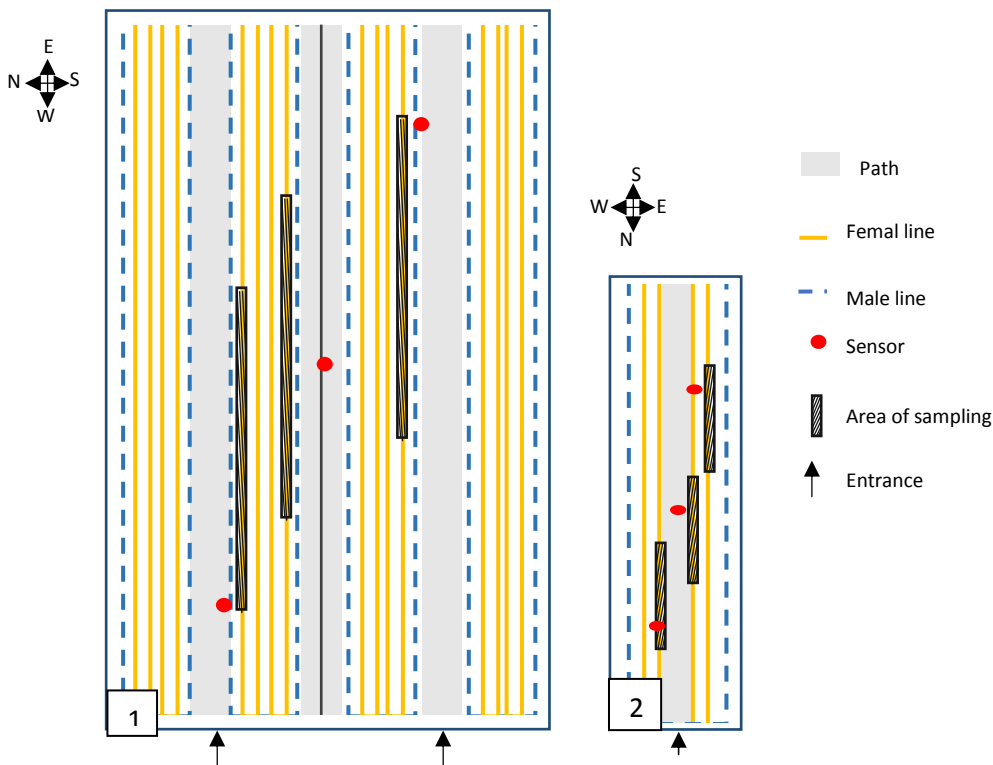


Figure 6. Planting pattern and observation sites according to the type of tunnel (1) Auxère and Beauchêne types, (2) Delabarre type.

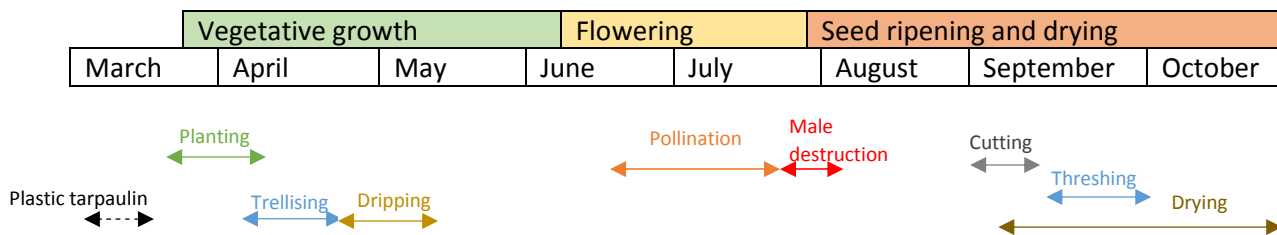


Figure 7. Bejo cultural practices for fennel seed crop production from the young plant reception to harvest (theoretical periods, adjusted throughout the climatic conditions of the year). Plastic tarpaulin = placed on the floor (if needed), Planting = period for planting, Trellising = installation of posts and strings, Dripping = placed on the floor, ready to be plugged, Pollination = hives and flies are present in the tunnel during the full period of flowering, Male destruction = rows of male plants can be remove, Cutting = “harvest day”, umbels are cut and let on the floor to dry, Threshing = possible period for threshing, Drying = drying period.

II - Material and Methods

This study intends to collect details, especially during periods of flowering and seed maturation in different production sites. This information will then be analyzed in order to understand the relationships existing between the characteristics of these different phases and the germination seed quality as stipulated with the above hypotheses.

1. Material

1.1. Plant material

Observations are realized on one F1 hybrid, the Victorio. This hybrid was selected according to its precocity consistent with the period of study and its past results which are fluctuating and make it interesting to study.

1.2. Observation sites and technical practices

The variety was grown in three sites in order to observe the plant behavior under different conditions (environment, organic or conventional) and any differences in the cultural practices between Bejo production and a farmer production (introduced pollinators, differences in the technical practices...). The production sites were exclusively tunnels and could be characterized as shown in the table 1.

At the farmer site, the total area grown with the Victorio crop was 1000m² divided in 3 tunnels. The choice was made to observe the fennel crop in only one tunnel, considered as a production unit. Given the difference between the production areas, the planting protocol is adapted (figure 6). For the Bejo's tunnels the planting pattern is four sterile male (female) lines between two fertile male (male) lines with a planting density of 9 plant per m² (0.30cm spacing between plants on the row and 0.50cm spacing between rows). Four units can be repeated in the tunnel. The smaller farmer tunnel allows only a 2 female lines between 2 male lines planting pattern. The planting density is then 7 plants per m² (0.30cm spacing between plants on the row and 0.75cm spacing between rows).

The current technical practices are described in the part I- 2.2.b. and illustrated with the figure 7.

2. Methodology

2. 1. Environmental monitoring

It is important to monitor the environment in which the crop is growing because of its direct effect on the plant behavior and the final product development. We are here distinguishing two key factors: the temperature and the sanitary condition.

The first one is recorded with sensors. The temperature data may have an explanatory value for a physiological phenomenon and are also used to characterize the plant growth with the degree days and the sum of degree days required to achieve a given phenological stage. Three sensors were placed in each tunnel (one in the middle and the two others at each sides near the tunnel end), at an hand height (about 1m40), what is equivalent to the umbels height. Temperature data were recorded each hour in order to have the minimum (Tmin) and maximum (Tmax) of the day and calculate the degree-day (°D).

Table 2. Sampling calendar (Umbel order 1= primary umbel, 2= secondary umbel, 3= tertiary umbel, Sampling site A= Auxère, B=Beauchêne, D= Delabarre site)

Sampling date	25/07	08/08	22/08	29/08-30/08	05/08	12/08
Umbel order sampled	1	1	1	1	1	1
	2	2	2	2	2	2
		3	3	3	3	3
Sampling site	A	A	A	A (30/08)	D	D
	B	B	B	B (29/08)		
	D	D	D			
				Harvest day*		Harvest day*

* The harvest day means the day when umbels are cut and left on the ground (or directly in the hairdryer) to dry before threshing.

Degree-days (DD) were calculated with the following formula:

$$DD (^{\circ}C) = \frac{T_{max}+T_{min}}{2} - T_{base} \quad \text{with } T_{base}=4^{\circ}C \text{ (Collin and Fourc, 2014)}$$

The disease and pest monitoring was conducted and managed by the technical teams. However in order to know if bedbugs were present in the tunnels this year, specific censuses were made. These censuses were made in shaking 50 umbels per tunnels during the flowering period and the seed ripening period. Yellow sticky traps were also placed in tunnels when there were not pollinators anymore. Although pest monitoring is not the subject of this study, it is highly important not to miss any cause in case of bad germination rate.

2. The flowering dynamic observation

The flowering dynamic was observed at the level of the tunnel and also at the level of the umbel orders. Regular visits (1 or 2 times per week) were carried out to observe the flowering stages and record the dates. The selected stages are the start of flowering, the full flowering and the end of flowering.

At the tunnel scale, the start of flowering means 10% of opened-flowers (it starts with the primary umbels), the full flowering stage is when 50% of the flowers are opened (flowering of the secondary umbels), and the end of flowering when the tertiary umbels petals begin to be dry.

At the umbel order scale, the start of flowering corresponds to the flower opening of the umbellets at the umbel periphery, the full flowering when all the flowers of the umbels' umbellets are opened and the end of flowering when all the umbels' umbellets are without petals (dried or fallen).

When flowering was finished, the fertilization rate was visually assessed for each umbel orders (only for information) and determined in three levels: good fertilization rate (80-100% of the flowers of an umbel are giving seeds), intermediate fertilization rate (40-80% of the flowers of an umbel are giving seeds), and bad fertilization rate (0-40%, more than half of the umbel flowers are not giving seeds).

3. The seed ripening monitoring

Monitoring the maturation of the seed was made from analysis of samples taken successively in a regular time interval. Sampling began about 15 days after the end of flowering of primary umbels and stop at harvest day. Flowering stages for primary, secondary and tertiary umbels are time shifted, following this pattern, samples of secondary and tertiary umbels must be shifted. For the first sampling day, only the primary umbels were ready to be sampled. Secondary and tertiary ones should wait the second day of sampling. The sampling planning is illustrated with the table 2.

Three sampling areas were marked out in different part of each tunnel (excluding borders) as illustrated with the figure 2. These areas correspond to a group of 25 plants on a female line row. Each day of sampling, 5 umbels of each order per area were taken off, always coming from new plants per sampling days (and not plants that have already been sampled). This number was adjusted according to the fertilization rate.

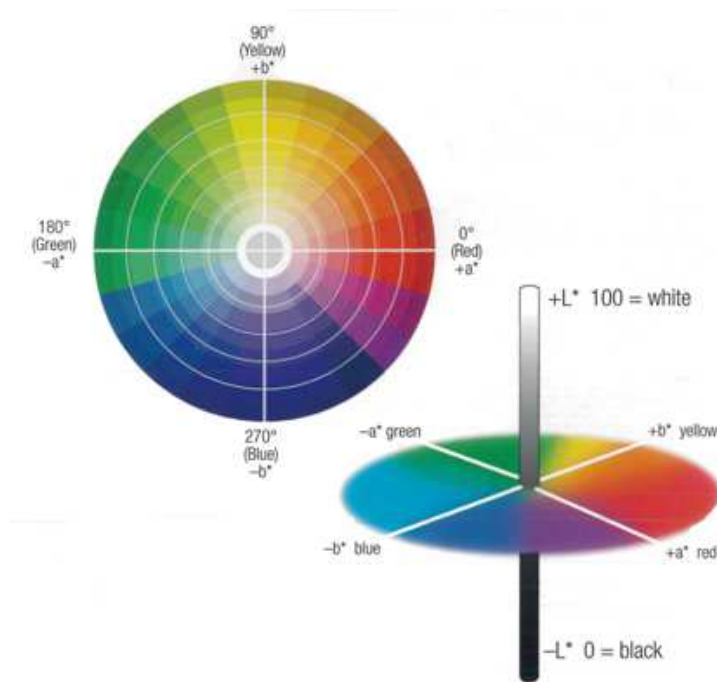


Figure 8. CIELab model of color space



Figure 9. Minolta CM-700d spectrophotometer



Figure 10. Color measurement of a fennel sample made with the spectrophotometer

One umbel order per area per tunnel is considered as a batch. That means there were three replications (three batches) of an umbel order per tunnel for a sampling day.

Batches were characterized (photographed, colour described and quantified) and divided for the measures. One part was used for being weighed and the moisture content, the other part was used for the germination test.

- The colorimetry measure

Color values (L*, a*, b*, C*, H°, figure 8) were determined using a Minolta CM-700d spectrophotometer (figure 9 and 10). Six measures were repeated per batch under SCI (Specular Component Included) measurement conditions, 10° observer and D65 illuminant1, 3mm aperture. Data were analysed with the SpectraMagicTMNX software.

- The physical characteristics – Fresh weight - Dry weight – Moisture content

Two groups of 100 seeds per each batch were used for measuring the fresh weight and the dry weight in order to calculate the seed moisture content (mean of the two groups per batch). Seeds were weighed (precision scale 0.001g) and then dried at 103°C during 17h in a thermal chamber. After drying, seed groups were weighed again.

The following formula is used to calculate the moisture content:

$$\text{Moisture content (\%)} = \frac{\text{Fresh weight (g)} - \text{Dry weight (g)}}{\text{Fresh weight (g)}} \times 100$$

A data change was necessary to correct the fresh and dry weight of the samples taken before the 22/08 (secondary umbels) and the 29/08 (tertiary umbels). Indeed, in previous sample dates, mericarps of each diakene were inseparable from each other. Weighing was performed on 100 diakenes. After these dates, mericarps were separating when touched and it was impossible to gather 100 diakenes (inseparable mericarps). Fresh and dry weight data were therefore divided in half for the first samples. Although this biological constraint brings a bias to the data, it remained better to weigh the same number of "unit" of seeds throughout the study.

- The germination test

The germination tests were delegated to the laboratory Labosem (49800 Brain sur l'Authion) to be made under the ISTA standards (pleated paper, 20°C night/30 °C day, reading at 14 days). 200 seeds per batch were used for the germination test. The batches were stored at 5°C (±1°C) between the time after preparation and before delivery to the laboratory.

4. Data processing

Graphs are made with the Excel 2013 software.

The analysis of the variables influence on the germination rate is carried out with the construction of a generalized linear model (GLM). Indeed, the dependent variable '% germination' (called GF) being a percentage, the residuals will not follow the Normal Law and the same deviance. Furthermore, the variable distribution is not symmetrically distributed around the mean, values are only positive or null and within the range [0, 1] or [0, 100]. A percentage rather belongs to the binomial distribution. Parameters are not estimated by the least-squared method (in ANOVA or linear regression for example) but by the maximum likelihood method. The mathematical used function is called the link function and in the case of the binomial distribution the link function is the logit function : $f(y)=\log(y/(1-y))$. The statistical model is then: $\text{Logit } p_{\beta(x)} = \beta_0 + \beta_{1x1} + \dots + \beta_{pxp}$ (Wajnberg, 2011; Fermin, 2015).

In this study the qualitative variates are:

- The production site (Tunnel)
- The plot (Area)
- The umbel order (Order)

The quantitative variates are:

- The number of days after the end of flowering (DAF)
- The moisture content (MC)
- The dry weight (DW)
- The degree-days accumulated after the end of flowering (DD)
- The color variables (L, a, b, h, C)

The model can be written:

$$\text{Logit } p(\text{GF}) = \beta_0 + \beta_1 * \text{Tunnel} + \beta_2 * \text{Area} + \beta_3 * \text{Order} + \beta_4 * \text{DAF} + \beta_5 * \text{MC} + \beta_6 * \text{DW} + \beta_7 * \text{DD} + \beta_8 * \text{A} + \beta_9 * \text{b} + \beta_{10} * \text{L} + \beta_{11} * \text{C} + \beta_{12} * \text{h} + \text{residuals}$$

In order to choose the best model (variables order) the choice is made with the stepAIC() function and based on the criterion of the lowest value of AIC (Akaike criterion).

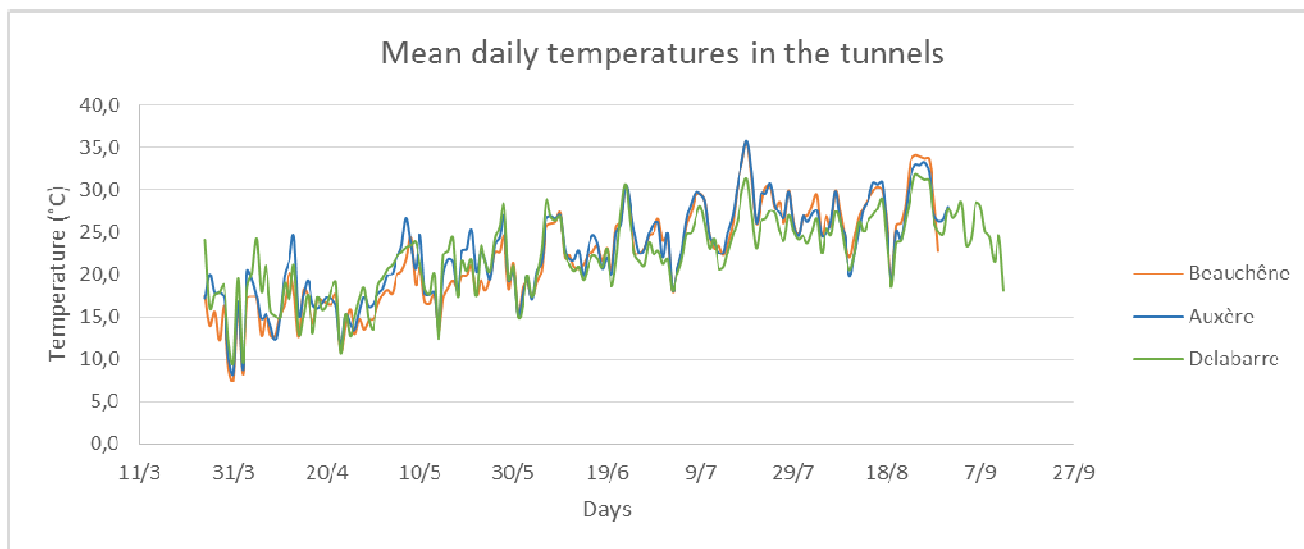


Figure 11. Mean daily temperatures (°C) in tunnels from the respective planting date to the respective harvest

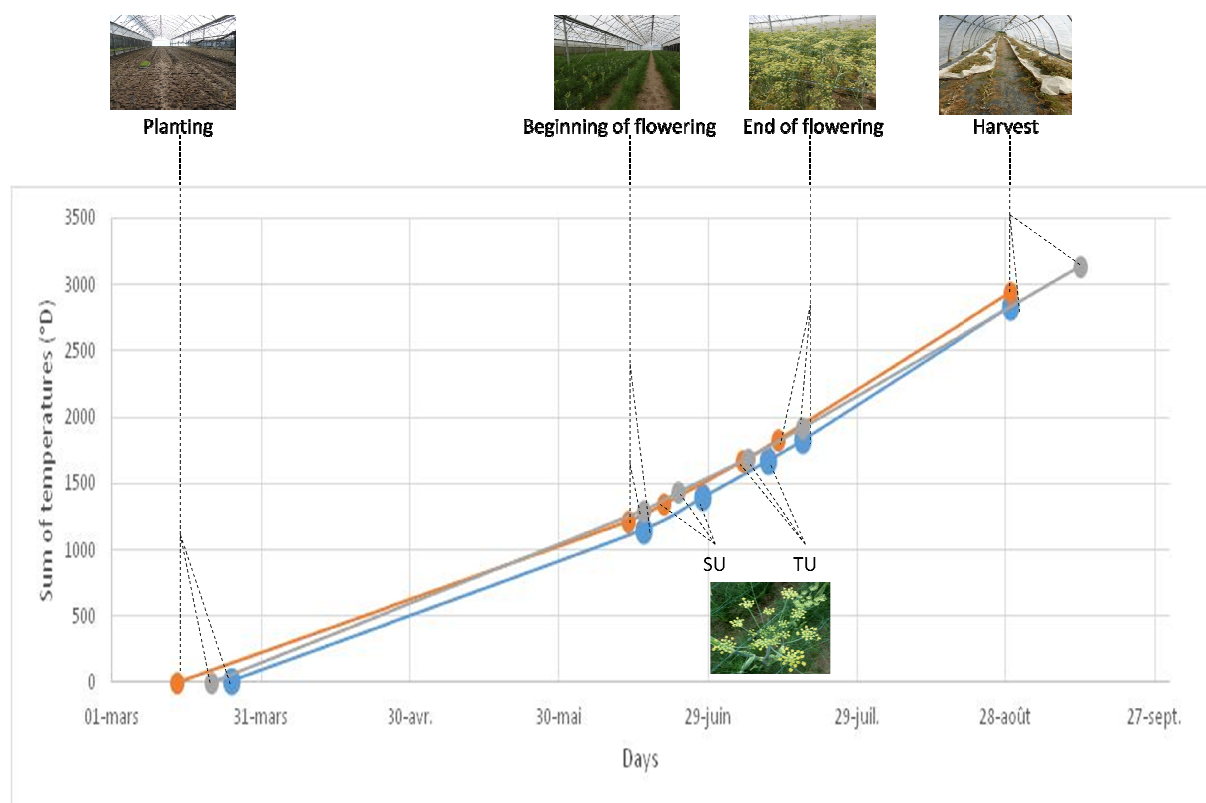


Figure 12. Flowering dynamics according to the accumulation of degree days (°D) for each tunnel. SU= secondary umbels (beginning of flowering) and TU = tertiary umbels (beginning of flowering).

Tableau 3. Degree-days (°D) cumulated during the physiological stages, from 25/03 (sensors placed in tunnels) until the harvest day of each tunnel. The number of corresponding day is indicated between parentheses. Before flowering means until the beginning of primary umbels flowering. After flowering means from the end of the tertiary umbels flowering.

Physiological stage	Beauchêne	Auxère	Delabarre
Before flowering	1156 (85)	1216 (80)	1300 (85)
During flowering	671 (33)	612 (31)	622 (33)
After flowering	1013 (42)	1112 (47)	1217 (56)
Total	2840 (160)	2940 (158)	3139 (174)

III - Results

1. Environmental monitoring

As we can see with the figure 11, globally there is no great difference between the mean daily temperatures between the three sites. Temperatures seem to be slightly higher at the Auxère tunnel and slightly lower at the Delabarre tunnel.

Concerning the pest and disease monitoring, there is no serious pest problem to indicate and no Orthops were detected on any of the three sites, whereas few bedbugs *Graphosoma italicum* (AppendixI, fig. 16) have been observed at the Delabarre site, tunnels were then treated accordingly.

2. The flowering dynamic

As we can see with the figure 12, the flowering dynamic is rather similar for the three tunnels and the flowering period begins at about the same date, in mid-June (beginning of primary umbels flowering) and ends mid-July (end of the tertiary umbels flowering). The flowering is very spread out (one month) due to the different umbel orders.

The primary and secondary umbel orders' duration of flowering was in average 15 days and it was a bit shorter for the tertiary umbel order, about 10 days.

A remarkable point is the shift in flowering period between the male and the female lines as we can see with the (AppendixI, fig.17). The flowering of the female line effectively starts one week before the flowering of the male line. It is a sufficiently significant time interval to have an impact on the possible fertilization of the primary umbels. In fact, the primary umbel order of the female line is flowering (with receptive stigmas) when there is no umbel carrying pollen at the opposite. So there is no pollination of primary umbels, and therefore no primary umbels to be considered for analysis (AppendixI, fig. 18).

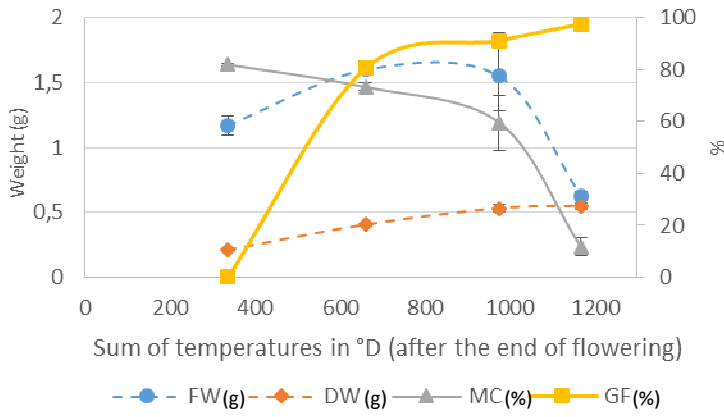
That shifted flowering issue plays a role on the fertilization rate for each tunnel (only for the primary umbels), and there are other undetermined factors added, specific to each crop site.

The fertilization rate at Beauchêne is good, in the range of 80-100%, whatever the umbel order is (secondary or tertiary). The results are different for the two other sites. At Auxère, the fertilization rate is very low for the secondary umbels (between 0 and 40% on average) and intermediate (about 50%) for the tertiaries. The Delabarre site follows the same pattern as the Auxère site with a bad fertilization rate for the secondaries and a good one for the tertiaries (about 80%). Fennel plants at the Delabarre site show the specificity to produce quaternary umbels (only three umbel orders for Beauchêne and Auxère sites), with a good fertilization rate (80-100%). Fertilization rate are illustrated with the (AppendixI, fig. 19).

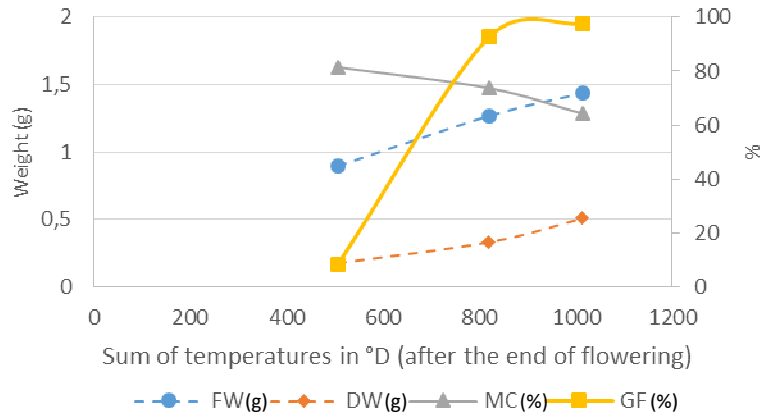
3. The seed ripening

- The physical characteristics per umbel order: Moisture content - Fresh weight - Dry weight

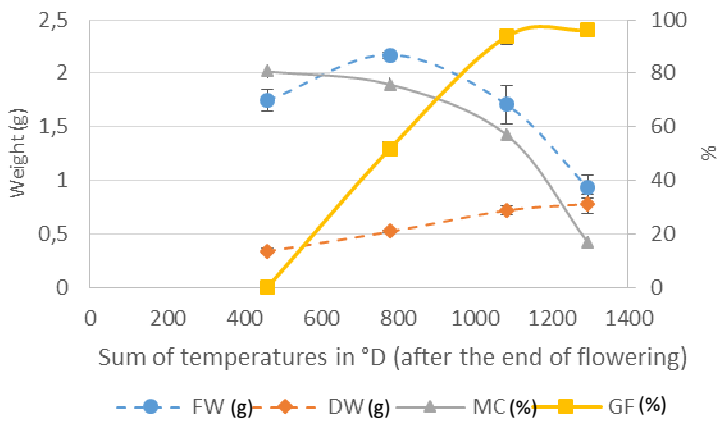
For the first day of sampling, 15 days after the end of flowering of the secondary umbels, the seed moisture content is 82%. Then the seed maturation for Beauchêne and Auxère follows the same pattern with a progressive decrease of the water content (about 20 points are lost) until 45 days after the end of flowering to reach about 60% of water content. This corresponds to the beginning of the desiccation phase. In fact, we noticed an abrupt water loss of 40 points



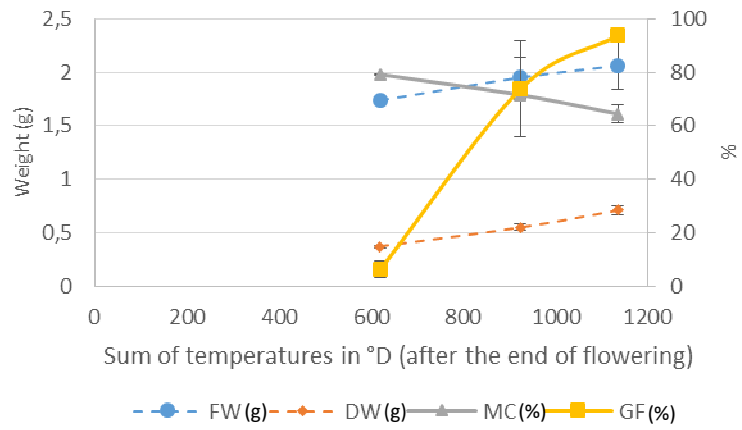
A. (Beauchêne, secondary umbel seeds)



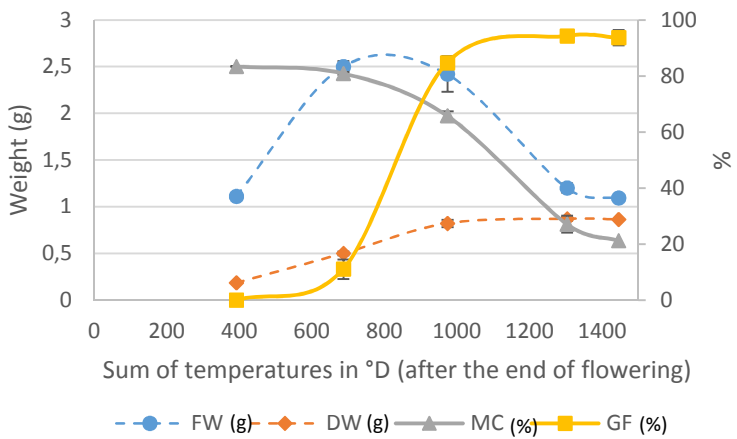
D. (Beauchêne, tertiary umbel seeds)



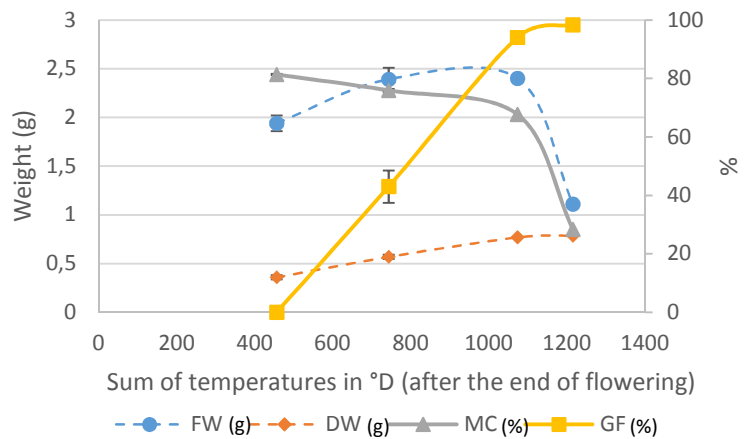
B. (Auxère, secondary umbel seeds)



E. (Auxère, tertiary umbel seeds)



C. (Delabarre, secondary umbel seeds)



F. (Delabarre, tertiary umbel seeds)

Figure 13. Evolution of the physical characteristics of secondary and tertiary umbel order per tunnel.

Secondary umbels: A (Beauchêne), B (Auxère), C (Delabarre). Tertiary umbel: D (Beauchêne), E (Auxère), F (Delabarre). FW = Fresh Weight, DW = Dry Weight, MC = moisture content, GF = Germination faculty.

or more and a moisture content around 15% the harvest day about 50 days after the secondary umbels end flowering. The harvest date is the time when umbels are cut and put on the ground to dry for about 2 weeks before threshing. In the same time the seeds dry weight stabilizes. The reserve accumulation phase is normally completed at this time (45 days after the end of flowering) and seeds must have reached their physiological maturity. If we look at the germination rate at this point, germination rates for the Beauchêne and Auxère sites are almost at their maximum and are greater than 90% (figure 13, A and B).

About the Delabarre site, even if the desiccation phase begins also 45 days after the end of flowering (about 66% of moisture content) it takes twice as long to lose the same water quantity (about 40 points). The harvest day was done 67 days after the end of flowering and the water content was 21%. At the presume beginning of the desiccation phase (about 60% moisture content and dry weight stabilization), the germination was also close to 90% (figure 13, C). We can notice the superior dry weight of the Delabarre 100 seeds than the two others (0,55g; 0,70g and 0,86g for Beauchêne, table 4A, Auxère, table 5A and Delabarre table 6A, respectively).

About the tertiary umbels, graphs (figure 13 D, E) show that for the three sites the desiccation phase did not begin, or has just begun, when umbels were cut to be dried. The first samples indicate the water content was about 80% 22 days after the end of flowering, that is quite similar to the secondary umbels; but the day of cutting happened 30 days after the end of flowering and the moisture content was still about 65% for Beauchêne and Auxère sites. For the Delabarre site tertiary umbels were cut off 60 days after the end of flowering and the moisture content decreased up to 28% (figure 13, F).

With the graphs we cannot see the dry weight stabilization of the tertiary umbel seeds (they were cut before it appears). However we can notice that the dry weight at the harvest day is almost identical from an umbel order to another per tunnel (table 4, 5 and 6), meaning tertiary umbels have probably reach their maximum dry weight, filled by the embryo and reserves substances.

For a water content around 70%, and less than 40 days after the end of flowering, the germination rates are contrasted. The germination rate of Beauchêne tertiary umbels is already 92%, while the Auxere one is 74% and the Delabarre is only 43% (table 4B, 5B and 6B, respectively). Maturation over the time for the tertiary umbels is less homogeneous than for the secondary ones.

- The germination faculty

Generally, the 3 production tunnels have very satisfying results in terms of germination quality this year. The last samples (corresponding to the harvest days) reported a germination faculty from fresh seeds of 95% (Auxère tunnel) and 97% (Beauchêne tunnel) and thus exceeded the 90% threshold (these results are the mean between the fresh seeds germination of secondary and tertiary umbels and do not correspond to the official germination rate of the seed lots).

Germination is acquired from 28 days after the end of flowering for Beauchene. 80% of the secondary umbel seeds are mature and germinate. 42 days after the end of flowering it is over 90% of germinating seeds. Similarly, the germination faculty for the tertiary umbels is more than 90% from 36 days after the end of flowering.

Table 4. Beauchêne, Physical and Physiological characteristics of the secondary umbel seeds (A) and tertiary umbel seeds (B). DAF=number of day after the end of flowering, DD = degree-days accumulated after the end of flowering, GF= Germination faculty for 200 seeds, MC= Moisture content for 100 seeds, FW= Fresh weight for 100 seeds, DW=Dry weight for 100 seeds. Mean values \pm standard error (n=3).

A	Sampling date	DAF	DD (°D)	GF (%)	MC (%)	FW (g)	DW (g)
	25/07	14	336	0,00 \pm 0,00	82,1 \pm 0,48	1,17 \pm 0,07	0,21 \pm 0,01
	08/08	28	660	80,6 \pm 2,60	73,4 \pm 1,56	1,60 \pm 0,03	0,40 \pm 0,01
	22/08	42	974	91,3 \pm 2,96	59,7 \pm 10,6	1,55 \pm 0,27	0,53 \pm 0,03
	29/08	49	1167	97,7 \pm 0,33	11,8 \pm 3,58	0,62 \pm 0,03	0,55 \pm 0,02

B	Sampling date	DAF	DD (°D)	GF (%)	MC (%)	FW (g)	DW (g)
	08/08	22	506	8,33 \pm 5,61	81,5 \pm 0,46	0,90 \pm 0,07	0,17 \pm 0,01
	22/08	36	820	92,7 \pm 1,76	73,7 \pm 2,02	1,27 \pm 0,07	0,33 \pm 0,01
	29/08	43	1013	97,7 \pm 0,33	64,3 \pm 1,70	1,44 \pm 0,13	0,51 \pm 0,03

Table 5 Auxère, Physical and Physiological characteristics of the secondary umbel seeds (A) and tertiary umbel seeds (B). DAF=number of day after the end of flowering, DD = degree-days accumulated after the end of flowering, GF= Germination faculty for 200 seeds, MC= Moisture content for 100 seeds, FW= Fresh weight for 100 seeds, DW=Dry weight for 100 seeds. Mean values \pm standard error (n=3).

A	Sampling date	DAF	DD (°D)	GF (%)	MC (%)	FW (g)	DW (g)
	25/07	19	461	0,00 \pm 0,00	80,7 \pm 0,10	1,74 \pm 0,10	0,34 \pm 0,02
	08/08	33	779	51,7 \pm 2,41	75,8 \pm 0,47	2,17 \pm 0,03	0,52 \pm 0,01
	22/08	47	1083	93,7 \pm 2,85	57,0 \pm 2,69	1,71 \pm 0,18	0,72 \pm 0,04
	30/08	55	1294	96,0 \pm 0,58	16,8 \pm 0,30	0,94 \pm 0,11	0,78 \pm 0,09

B	Sampling date	DAF	DD (°D)	GF (%)	MC (%)	FW (g)	DW (g)
	08/08	27	620	6,33 \pm 2,96	79,1 \pm 0,12	1,74 \pm 0,03	0,37 \pm 0,01
	22/08	41	924	73,7 \pm 17,9	71,5 \pm 1,82	1,95 \pm 0,19	0,55 \pm 0,03
	30/08	49	1135	93,7 \pm 0,67	64,5 \pm 3,17	2,06 \pm 0,22	0,78 \pm 0,04

Table 6. Delabarre, Physical and Physiological characteristics of the secondary umbel seeds (A) and tertiary umbel seeds (B). DAF=number of day after the end of flowering, DD degree-days accumulated after the end of flowering, GF= Germination faculty (n=200), MC= Moisture content (n=100), FW= Fresh weight (n=100), DW=Dry weight (n=100). Mean values \pm standard error (n=3).

A	Sampling date	DAF	DD (°D)	GF (%)	MC (%)	FW (g)	DW (g)
	25/07	18	391	0,00 \pm 0,00	83,4 \pm 0,05	1,11 \pm 0,02	0,18 \pm 0,00
	08/08	32	686	11,0 \pm 3,47	80,9 \pm 0,46	2,50 \pm 0,06	0,50 \pm 0,02
	22/08	46	973	84,7 \pm 2,19	65,8 \pm 1,61	2,42 \pm 0,19	0,82 \pm 0,04
	05/09	60	1303	94,3 \pm 10,6	27,1 \pm 3,04	1,20 \pm 0,05	0,87 \pm 0,02
	12/09	67	1445	93,7 \pm 2,73	21,2 \pm 0,49	1,09 \pm 0,03	0,86 \pm 0,02

B	Sampling date	DAF	DD (°D)	GF (%)	MC (%)	FW (g)	DW (g)
	08/08	22	456	0,00 \pm 0,00	81,3 \pm 0,19	1,94 \pm 0,08	0,36 \pm 0,02
	22/08	36	743	43,0 \pm 5,51	75,9 \pm 0,48	2,39 \pm 0,12	0,57 \pm 0,02
	05/09	50	1073	94,0 \pm 1,66	67,7 \pm 1,93	2,40 \pm 0,09	0,77 \pm 0,02
	12/09	57	1215	98,3 \pm 0,67	28,4 \pm 1,88	1,11 \pm 0,06	0,79 \pm 0,03

For the Auxère site, the germination faculty arrives a bit later compared to Beauchêne, because it is necessary to wait 47 days to have more than 90 % of the secondary umbel seeds to germinate, and 49 days to have the same rate for the tertiary umbels.

As for the evolution of the physical characteristics in Delabarre seeds, germination is acquired more slowly compared to the two other tunnels (AppendixII, fig. 23 and 24).

We have seen that the daily average temperature of the 3 tunnels is relatively the same, even if the minimum is lower at the Delabarre site. A focus on the sum of degree-days received until a certain phase (before flowering, during flowering and after flowering) confirms that it does not seem to be any real difference between the sites (table 3). The differences may be explained rather by the number of days necessary to complete a stage, in particular for seed maturation phase (after flowering) for the Delabarre tunnel, which was longer than for the other tunnels.

- The color changes

The color change is visually noticeable over time by looking to the seeds in tunnels. The color changes from green to gray-green to brown, each time with a different hue depending on the tunnel (AppendixI, fig. 20, 21, 22). In practice, this is the criterion that determines the harvest date (brown and dry appearance for secondary umbels, and at least grey for the tertiary ones). The color change is gradual but we see the transition from green to grey-green happening at the same moment of the beginning of the desiccation phase, about 45 days after the end of flowering for secondary umbels and between 35 to 50 days for the tertiary ones. If we represent these colors by the color variables quantified by the spectrophotometer (figure 14), we can then observed the movement along the a^* axis (green to red).

The color does not seem to be directly related to the acquisition of germination as it continues to evolve as germination is being acquired. However, it seems to be extremely dependent on the water content as the seeds appear green when they are at 80% moisture content and brown below 20% for the harvest day, and as previously said, we can observe the movement from -4.5 / -6.5 to 3.5 / 4.2 on the a^* axis (table 7 and 8). In addition, once the brown color is reached, it does not change even after drying (visible after removal from the thermal chamber to measure the water content). The link between the moisture content and the color variables is effectively revealed by statistical analyses. Results are showed in the AppendixIII.

4. Statistical analysis

Analysis of the maximum likelihood on the 12 variables that may explain the germination faculty variations reveals that almost all the variables influence the germination rate when samples are considered all together (table 9). All together mean at different sampling date interpreted by the DAF variable and to some extent the DD variable, which can explain their significance. Furthermore, even if the tunnel variable is not included in the selected model, it is indirectly nested in the Area variable. The significance of all the variables makes interpretations difficult and analysis for key sample dates may perhaps reveal more details. It seems interesting to look at the influence of these variables at one particular date when all the sites were sampled, and particularly the time when the desiccation phase begin. Then we can analyze the effect of factors on the samples taken at the harvest date, when the development cycle is theoretically completed.

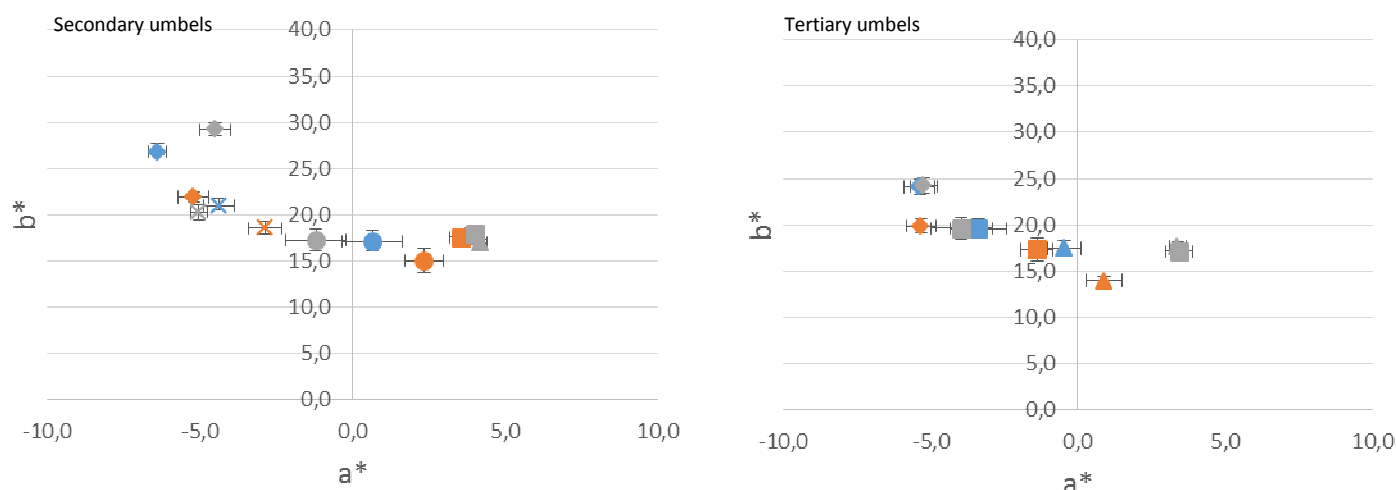


Figure 14. Seed color changes of the secondary umbels (left) and tertiary umbels (right), represented by the color variables a^* ($-a^*=180^\circ$ (red)/ $+a^*=0^\circ$ (green) and b^* ($-b^*=90^\circ$ (yellow)/ $-b^*=270^\circ$ (blue)). In blue= Beauchêne, orange = Auxère, grey = Delabarre. The different shape represent a sampling date Diamond=25/07, Cross=08/08, Circle=22/08 and rectangle=harvest date for the secondary umbels. Diamond=08/08, Rectangle=22/08, Triangle=harvest day for the tertiary umbels.

Table 7. Mean values \pm standard error (n=6) of the color variables, CIELab color space. Secondary umbel seeds

		a^*	b^*	L^*	C^*	H°
Beauchêne	25/07	$-6,4 \pm 0,27$	$26,8 \pm 1,00$	$49,9 \pm 1,57$	$27,6 \pm 0,98$	$103,7 \pm 0,73$
Beauchêne	08/08	$-4,38 \pm 0,50$	$20,9 \pm 0,73$	$46,3 \pm 1,88$	$21,5 \pm 0,74$	$101,9 \pm 1,33$
Beauchêne	22/08	$0,65 \pm 1,00$	$17,2 \pm 1,18$	$45,9 \pm 1,66$	$17,3 \pm 1,18$	$88,4 \pm 3,48$
Beauchêne	29/08	$3,99 \pm 0,23$	$17,8 \pm 0,37$	$51,6 \pm 1,49$	$18,3 \pm 0,37$	$77,3 \pm 0,69$
Auxère	25/07	$-5,2 \pm 0,53$	$21,9 \pm 0,63$	$45,9 \pm 2,32$	$22,5 \pm 0,67$	$103,0 \pm 1,23$
Auxère	08/08	$-2,87 \pm 0,55$	$18,6 \pm 0,67$	$46,2 \pm 1,42$	$18,9 \pm 0,70$	$98,7 \pm 1,52$
Auxère	22/08	$2,34 \pm 0,63$	$15,1 \pm 1,26$	$46,8 \pm 2,29$	$15,3 \pm 1,31$	$81,4 \pm 1,97$
Auxère	30/08	$3,54 \pm 0,34$	$17,6 \pm 0,39$	$55,8 \pm 1,60$	$17,9 \pm 0,40$	$78,6 \pm 1,04$
Delabarre	25/07	$-4,5 \pm 0,54$	$29,2 \pm 0,72$	$51,0 \pm 1,89$	$29,6 \pm 0,73$	$98,7 \pm 1,01$
Delabarre	08/08	$-5,03 \pm 0,27$	$20,3 \pm 0,80$	$47,6 \pm 1,97$	$20,9 \pm 0,79$	$104,1 \pm 0,82$
Delabarre	22/08	$-1,20 \pm 1,00$	$17,3 \pm 1,24$	$47,4 \pm 3,55$	$17,5 \pm 1,24$	$93,8 \pm 3,17$
Delabarre	05/09	$4,01 \pm 0,29$	$17,9 \pm 0,55$	$53,2 \pm 2,05$	$18,5 \pm 0,56$	$77,4 \pm 0,86$
Delabarre	12/09	$4,2 \pm 0,19$	$17,0 \pm 0,44$	$54,8 \pm 1,78$	$17,5 \pm 0,44$	$75,9 \pm 0,65$

Table 8. Mean values \pm standard error (n=6) of the color variables, CIELab color space. Tertiary umbel seeds

		a^*	b^*	L^*	C^*	H°
Beauchêne	08/08	$-5,37 \pm 0,52$	$24,1 \pm 0,84$	$53,1 \pm 1,09$	$24,8 \pm 0,85$	$102,5 \pm 1,16$
Beauchêne	22/08	$-3,38 \pm 0,97$	$19,6 \pm 1,08$	$48,2 \pm 1,70$	$20,0 \pm 1,10$	$99,7 \pm 2,76$
Beauchêne	29/08	$-0,46 \pm 0,55$	$17,9 \pm 0,76$	$44,8 \pm 1,29$	$17,6 \pm 0,79$	$91,2 \pm 1,64$
Auxère	08/08	$-5,32 \pm 0,44$	$19,9 \pm 0,71$	$54,5 \pm 1,35$	$20,6 \pm 0,75$	$104,8 \pm 1,08$
Auxère	22/08	$-1,38 \pm 1,27$	$17,3 \pm 1,27$	$47,8 \pm 3,51$	$17,6 \pm 1,29$	$93,8 \pm 4,18$
Auxère	30/08	$0,89 \pm 0,54$	$14,0 \pm 0,49$	$48,4 \pm 1,84$	$14,2 \pm 0,49$	$86,1 \pm 2,17$
Delabarre	08/08	$-5,25 \pm 0,46$	$24,2 \pm 0,86$	$48,6 \pm 1,60$	$24,9 \pm 0,83$	$102,5 \pm 1,11$
Delabarre	22/08	$-3,97 \pm 1,05$	$19,6 \pm 1,19$	$51,3 \pm 2,84$	$20,2 \pm 1,22$	$101,4 \pm 2,85$
Delabarre	05/09	$3,45 \pm 0,44$	$17,2 \pm 0,58$	$39,9 \pm 1,89$	$17,6 \pm 0,60$	$78,8 \pm 1,38$
Delabarre	12/09	$3,40 \pm 0,30$	$17,6 \pm 0,50$	$52,6 \pm 2,20$	$18,0 \pm 0,50$	$79,2 \pm 0,90$

For the samples of 22/08, which correspond to the beginning of the drying phase for the Bejo tunnels, and a little before for Delabarre, many variables affect the germination faculty. There are the color variables, the physical characteristics (DW and TM), the tunnel (positive influence of Beauchene tunnel, which is easily identified by looking at the germination rates), the number of days after the end of flowering (DAF) and the sum of degree-days (DD) received. It is interesting to note that the umbel order does not significantly influence the germination, contrary to what we might think.

When we look at the factors influencing the germination faculty (GF) at the harvest date, it is unsurprisingly that we notice their number decreased. In fact, after the best model selection to identify the factors that significantly influence the germination and their predictors, these variables are finally the moisture content (MC) and the a* color variable. With this model, the probability to obtain germinated seeds can be written:

Logit $p(\text{GF}) = 1.45 + 2.67 * \text{Order} - 9.32 * \text{MC} - 0.30 * a$ (see appendixIV for the coefficients).

The residual deviance is very low (12,18) compared to the model including all samples (198,51).

Table 9. Significance of the factor effects on the germination faculty when run in the different GLM model.
 Significance codes for p-value: 0=***, 0.001=**, 0.01=*, 0.05=., 0.1 or more =/. ddf = degree of freedom

	DAF	Tunnel	Area	Order	DW	MC	DD	L	a	b	C	h	Residual deviance
Every samples and variables	***	/	***	*	**	/	***	/	***	*	*	***	198,51 (46 ddf)
22/09	**	*** (Beauchêne, influence positive)	/	/	*	*	/	***	**	**	***	**	6,84 (7 ddf)
Harvest day	/	/	/		/	***	/	/	*	/	/	/	12, 18 (14 ddf)

IV - Discussion

1. Discussion of the results

- The flowering dynamic

The flowering dynamic was spread such as described by Németh et al. (1999) for the flowering succession of the umbel orders. On the other hand the flowering duration per umbel order was shorter compared to the author results and it was the total blooming which lasts one month. Of course, we don't forget that environmental and cultural conditions and the studied genotypes are different.

- The seed ripening

Seeds can be mature from 30 days after the end of flowering (for Beauchene) but rather in average about 45 days after the end of flowering or from 970 °D. Studies on carrots like the Ravenel one (Ravenel et al., 2016) showed a shorter development with 30 days after flowering or 700°D for example.

At first sight, the umbel order does not affect the germination percentage as it is possible to obtain similar rates between secondary and tertiary ones when umbels are harvested. However, statistical results tend to show the contrary and umbel order has a significant effect on the germination rate. That is statistically different from the results obtained by Bianco *et al.* (1994) for these orders. On the other hand, the same study notes a clear difference between primary umbels and other levels, what we cannot confirm or deny here. It remains difficult to decide on this issue, given the contradictory results obtained in several studies, including in carrot studies (Thomas and Gray, 1978; Gray and Steckel, 1981; Jacobsohn and Globerson, 1981). In addition, the seed germination tests are performed under optimum conditions while differences might appear in adverse conditions. A study conducted by Pereira (Pereira *et al.*, 2008) shows that carrot seeds did not show differences under standard condition tests for the different umbel orders but reveal a difference when tested under adverse temperature. It pointed out the best seed vigor of the primary and secondary umbels compared to the tertiary ones.

It is necessary to pay attention to the harvest date for not harvesting immature seeds, as for all crops, however the harvest date is not a strict period. The results showed that waiting at least the seed color change of the tertiary umbels into "grey-green" (which corresponds simultaneously to the seed browning for secondary umbels) allows *a priori* to match the tertiary umbel seed maturity (although this differs from one production site to another), and certainly for the secondary ones. At this time, the secondary umbel seeds are mature and have acquired their desiccation tolerance. Apart from an important stress or a shock, the germination shows very little evolution waiting the maturation of the tertiary umbel seeds and therefore the harvest date. This point remains to be confirmed, as well as the duration limit of drying up on the mother plant (if any exists), in future studies. On the other hand, the scope of opportunity for increasing points from the percentage of germination (for maturing tertiary umbels but it is also true for the secondary umbels) is situated after the color change and according to the statistic results, is related to the decrease of the water content (prior to forced drying). Seeds of the tertiary umbels are harvested when they still have a high water content

and not started their drying phase. It is not known for this study if it can have an impact on the germination faculty (tests are running).

Drying and threshing are not *a priori* factors that alter germination (past and ongoing studies), but it remains to be confirmed for fennel. Ravenel *et al.* (2016) study shows a limited effect of drying conditions on the germination faculty.

Given the germination results achieved this year it would be inconsistent to propose a separate harvest date per umbel order, or a later one; and decisions taken for the harvest dates motivated by the expertise of technicians and farm manager are appropriate (visual appearance of seeds based on the color of tertiary umbels and the dry appearance of secondary umbels seeds).

- The seed coat color

The seed color decomposition into quantitative variables in the CIELab color space is not often used as a seed maturity indicator and there are no references found on fennel or carrot seeds. Studies have been conducted on watermelon seeds (Mavi, 2010) and showed the correlation between the germination faculty and some color variables. There is more work done on the chlorophyll content, and the loss of chlorophyll content as a maturity indicator for various species (Jalink 1998, Eyletters *et al.*, 2010, Ooms, 2012 for example). This is particularly the case with carrot seeds, which show that the germination percentage is negatively related to the chlorophyll a and b and the seed moisture content (Joyce, 1988).

Otherwise, the fennel seed colors are described and classified. The Székely *et al.* (2002) results indicate that the germination rate is low when seeds are true green (green) and is increasing from the stage "waxy", which matches the stage "grey- green "for us, to "brown". The results of our study correspond to those comments.

2 - Discussion about the later acquisition of the germination at Delabarre site

We can rule out an effect of different seed vigor or other problem related to the seeds used for the young plants production. Indeed the basic seeds used to produce them for Beauchêne and Delabarre come from the same lot (conventional), and as an organic production, those used for the Auxère production site come from another lot.

The hypotheses that could explain the differences in the speed of the seed maturation are related to the pedoclimatic context and the technical itinerary of each tunnel. These are summarized in appendixV.

Regarding the first item we have seen that the temperatures in tunnels, which are comparable with the degree days, are little different between the three production sites. The climatic context for the three sites was similar (given the close geographical locations). It was difficult for the crop sanitary quality with a cool, rainy spring (floods at the end of May) and little sunshine during the vegetative growth. The disease pressure was serious with an important Sclerotinia pressure at the Bejo's tunnel (Auxère and Beauchêne) during the vegetative growth (AppendixI, fig. 15). The female line was more sensitive than the male line and the disease

appeared earlier on female plant rows. There was a severe leaves removal, as well as plants removing (at least 250 plants at Beauchêne), what impacts the potential seed yield. Sclerotinia disease was also present in the Delabarre tunnel but to a lesser extent. On the contrary the summer was dry and hot, that are good conditions for flowering and seed ripening. This difficult beginning for the culture does not seem to have influenced (at least negatively) the germination faculty.

Furthermore, the soil can actually be a cause of the longer cycle for the maturation of seeds at Delabarre. The Bejo cultures were implanted on loamy soils while the soil at Delabarre is clay-loam type, “heavier” than the two others.

About the cultural practices, the basis are the same, as presented in the Material and Method part (figure 7). Points that can differ are those about the soil fertilization, the phytosanitary products (or biologic control at Auxère), irrigation (in water quantity) and pollination.

The soil fertilization:

For the three sites the units brought in N-P-K have been relatively similar.

At Beauchene, the fertilization inputs are fractionated with a content of organic matter and minerals before planting, and additional phosphate before the bolting stage.

Inputs at the Auxère site are all brought at the crop beginning. It consists in organic matter, minerals and growth stimulators. As for the Auxère site, at the Delabarre site (both soils are covered with black plastic tarpaulin) organic matter and nutrients were brought once at the beginning of the culture. However there was a boron input at the beginning, and during, flowering, to compensate for a boron lack in the soil, according to the farmer.

Boron has been found to play a vital role during the reproductive stage by participating to the pollen tube growth, pollen germination, plasma membrane stimulation, floret fertility, anther development and development of seeds (Herrera-Rodriguez *et al.*, 2010). However an excess of boron can leads to an inhibition in the germination percentage of germplasm as reported by Bañuelos *et al.* (1999). Pariari *et al.* (2009) reports from Sharangi *et al.*, (2002) that application of boron in fennel increased the seed yield. It was suggested that this positive effect may be due to an increased value of the number of diakenes per plant, then the number of seeds per plant. However, foliar application of boron was made during the vegetative growth and not the flowering period.

The phytosanitary products:

No remarkable element about the chemicals applications for the plant protection (preventive or curative insecticides and fungicides in conventional practices) and no organic practice appears to influence in one way or another.

Irrigation:

Technicians considered that fennel crops are globally over irrigated that could be a factor in susceptibility to disease. However data are missing for a possible comparison of the water input between each site and the possible differences on the fennel growth.

Pollination:

As for the practices related to pollination and fertilization which potentially explain the yield between the sites, we do not either find large differences between Beauchêne and Auxere with two hives per tunnel, and flies in supplements. While pollinators were present during the flowering period, there is a very low rate of fertilization for the secondary umbel seeds at Auxère. There are several hypothesis but which do not necessarily explain the selective character of the umbel order: the tunnel height and the material used, the black plastic tarpaulin on the soil can increase the surface temperature and modify the solar radiation, the soil components and the soil fertilizers can affect the nectar composition and therefore the attractiveness to pollinators, as well as irrigation, and finally the fly amount can affect the pollination activity of bees in changing their behavior to defend the hive rather than looking for pollen and nectar. For the Delabarre site the situation was different as there were no pollinators brought directly on the crop. We can suspect a preference for another culture, perhaps more attractive than fennel during the secondary umbels flowering.

3. Critical analysis of the study

Observations on a single variety:

The choice of having only one variety is restrictive for the results, and it seems not very probable that these results can be extended with confidence to all the fennel varieties grown by Bejo (genetic determinism, early variety, area production and field or tunnel production etc) However it facilitates the comparison between production sites by removing a factor (genetic/variety) otherwise always significant. Verma and Solanki (2015) found the existence of genotype x environment interactions and the effect of the genotypes on the yield and yield component. The germination faculty has not been tested.

The production sites:

The chosen sites finally have few differences. It would have been interesting to have had not only more observation sites for the same variety, but also more contrasting environments in soil types, temperatures, soil fertilization and irrigation management.

The sampling protocol:

There has been no experimental system set up or randomization of individuals because it is tricky in a production environment (planting protocol, limited circulation among the rows which prevent the random selection). However the aim was to have samples as representative as possible of the tunnel and when it was possible, only few seeds per umbel (but on several umbels) were taken in order to mix them and so has not to have a batch full of seeds coming from only some umbels.

We can raise the question of the time scale for the fixed sampling dates and not a number of days after the end of flowering equal for all the sites for example; as well as the time interval between two samples which may be too long. A weekly sampling would have followed the

evolution of the seed physical characteristics more faithfully to reality. The choices for this study come from compromise between physiological stages to follow, logistics, work capacity and cost.

Critique of the statistical method used:

The dependent variable was not modified using the $\text{Arcsin}\sqrt{y}$ transformation as it is often used when percentages are studied but it is becoming controversial (Warton and Hui, 2011). The reasons are linked to the difficulties of interpretation when using a transformed variable, and more simply, because it didn't give results (does not reveal any factor). Furthermore this kind of transformation does not satisfy statistical results as explained in the part II- 2.4.

Moreover, in this context of study we wanted to know the factors that influence the germination rate, regardless if it was a good one or not (it was counted as the probability that germination occurs, probability which increased when the germination rate is high). Or we might think about making an analysis by considering the desirable germination (90%) and (taking as a dependent variable a binary one: the lot germinates from 90% or more (1) or not (0)).

4. Perspectives

The results give little avenue to explore to find a factor to change in the cultural practices. The perspectives will then rather concern the seed analysis, on former seed lots or new ones at different ripening stages.

These analyses may concern:

- The seed components and their evolution during maturation, such as soluble sugars, saccharose and oligosaccharides which accumulate during the seed development (Casals, 2012). It is also possible to search for and quantify certain hormones like the abscisic acid. In the same vein we can think of an analysis of the essential oil content and its compounds, to determine if a trend exists between the presence, increase / decrease or absence of certain compounds and the acquisition of germination. Székely *et al.* (2012) began to explore this and suggests that the essential oil content of fruits is the highest in green stage and shows a decreasing tendency in case of waxy and ripen fruit stages. About the components, the anethole, and the methyl chavicol contents show an increasing tendency during the fruit development, while the fenchone, limonene, α -pinene and β - pinene contents decrease. The essential oil and its components could possibly be an idea of maturity indicator to explore. However, these contents must be variable according to the variety and modified according to the environment; and a practical application (in a seed production context) seems to be difficult to implement.
- The appearance of non-germinated seed embryos, if they are effectively present (or empty seeds) and alive (coloration test by the tetrazolium salt). These analyses can be combined with

an analysis of the pollen quality and viability (percentage of germinated pollen grain, variation during the flowering period and temperature effects (Casals, 2012)

- The former seed lots of low germination rate can be analyzed for the presence of insect damages (Orthops and Lygus).
- If lots are not distinguished by their germination rate in that study, maybe other characteristics of the germination quality such as the speed of germination or test of germination faculty under adverse conditions can reveal differences.

Other perspectives should be interesting in order to complete the database:

- To compare the germination rate of all the Victorio fennel productions under tunnel in the region (similar climate), or extended to the other varieties, to see if all the germination rates are above 90%. This could mean that for an agro-ecological area, the year was a « good year for fennel seed production" and to rule on an assumption of temperatures and sunshine received during the period of flowering and ripening (dry, sunny and hot). The comparison can also be made with other regions although the climate context was rather favorable over the country in July-August.
- If the color could be used as a reliable indicator of the fennel germination, which is not the case today, measures can be realized directly inside the tunnel or the field, (the spectrophotometer is portable) or after sampling, to attest of the germination quality compared to a confidence interval for the color data (considering that data obtained this year are not enough and should be completed).
- As previously said, it can be interesting to extend the period of sampling beyond the harvest and see how germination faculty evolves (if it stabilizes or if it falls).
- Further investigations about the micronutrients needed by the fennel seed crops (Bore for example) and research of deficiencies or excess in the soil analyses for bad rate germination seed lots (compared to good ones).

V - Conclusion

This study is part of a preliminary work conducting to a thesis. The aim was to collect data to learn more about the fennel seed culture and in particular the seed maturation phase. Monitoring of the seed maturation for certain production sites (under tunnel) reveals several things and enables us to give partially confirmation for the previous assumptions:

- The umbel order has an effect on the germination faculty but this effect evolves and seems to be due to the flowering time shift and then the maturation time shift. According to a statistical point of view, the umbel order (at the harvest day) doesn't have an effect on the germination rate. In practical terms, the germination of fresh seeds for both orders at the harvest day is more than 90%.

- Indicators like the number of day after the end of flowering, the moisture content and the seed coat colour provide good indications of the germination faculty.

The seed maturation duration of the secondary and tertiary umbels differs from a few days, the seed maturation period of the tertiary umbels is shorter than the secondary one. Seeds are immature before 45 days after the end of flowering for the secondary order and 38 (on average) for the tertiary one. It corresponds to the beginning of the desiccation phase and the germination rate is no longer changing.

The water content is a good indicator of maturity, knowing it is situated in average at 60-65% when germination is acquired, then the water loss is related to an increase in the germination percentage. With the current practices, secondary umbel seeds are harvested when the moisture content is around 10-20% while the moisture content of the tertiary umbel seeds is still around 60% (for Bejo tunnels). We do not know yet if a sudden drying could affect the seed quality of tertiary umbels.

The seed coat color is linked to the water content and thus indirectly to the germination faculty. The beginning of the drying phase seems to correspond to a color that can be called "grey-green". From a practical point of view, we know that we can harvest the seeds when they have exceeded this color change (from green to "gray-green") and then earn points on the germination rate when leaving the seed drying on the mother plant.

This year, the germination rates are up to 90% for the three observation sites. It is difficult to highlight a factor affecting the germination faculty related to the technical practices or the environment and unfortunately, it does not give more indications about the origin of the heterogeneity of the germination quality. Perspectives are rather towards going deeper in the analyses of the seed components, or a characterization of embryos.

To date we have been able to shed light on some answers among the many questions raised by this crop, even if it is only for a single variety. More work remains to be undertaken by Bejo France, but also in collaboration with other subsidiaries of Bejo Zaden, in order to cover all the fennel seed production sites and their respective issues. Work can be inspired to some extent from those carried out on carrot, a species also known for its unexplained variations in the germination rate.

It has been a great opportunity to participate in the productive, and indirectly economic, development of an internationally renowned company. This study reflects the benefit from knowing its crops and entering a process of continuous improvement and competitiveness. The research and development are integral part of the ways of economic development (strong presence in the seed industry) and it is structures as Bejo that allow to make the link between the optimization of the cultural practices, the market demand, and to move research forward.

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References of the figures

- Figure 1, 2, 5 (A) Pictures from Pierre Goujon,
http://abiris.snv.jussieu.fr/herbier/Fenouil_commun.html [15/06/2016]
- Schéma Figure 3, 5 (A et B) <http://www.lavisdesplantes.fr/fenouil-carte-identite/>
- Figure 8 https://www.bonlalum.com/education/the_truth_about_color.shtml [30/08/2016]
- All the other figures are personal illustrations

Appendix I Additional pictures



Figure 15. Sclerotinia on a fennel bulb



Figure 16. Graphosoma italicum on immature



Figure 17. Flowering of the primary umbels begins for the female line whereas the flowers of the male primary umbels are not opened (Auxère, 14/06/2016)



Figure 18. No fertilization for the primary umbels. Flowers are drying.



Figure 19. Different fertilization rates. A. Umbel without pollination and fertilization. B. Bad fertilization, the umbel is carrying few seeds. C. All the flowers were pollinated and fertilized, the umbel shows a great number of immature seeds.



Figure 20. Umbel sampled the 25/07/16. Seeds are green.



Figure 21. Umbel sampled the 22/08/16. Seeds have turned « grey ».



Figure 22. Umbel sampled the 29/08/16. Seeds are brown.

Appendix II Illustrations of the moisture content and the germination faculty per tunnel.

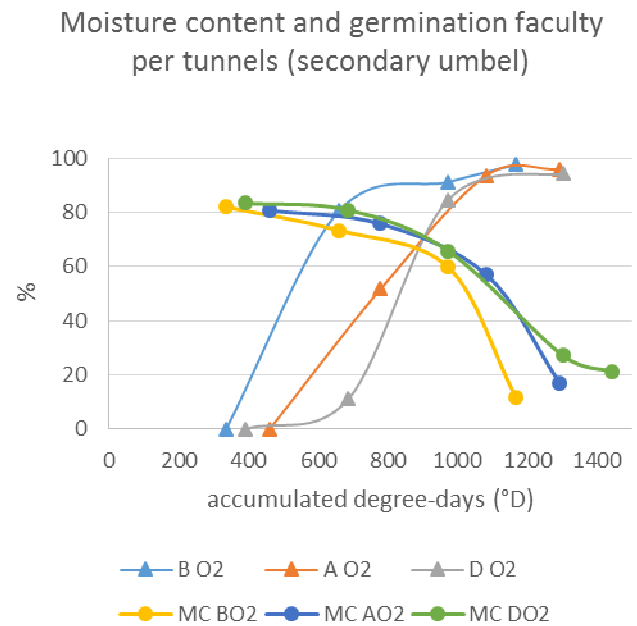
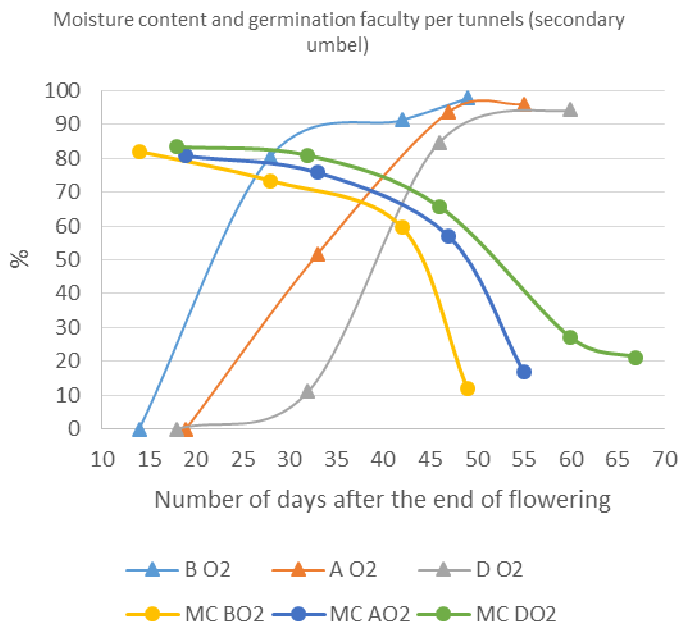


Figure 23. Moisture content and germination faculty per tunnel. Triangle = Germination faculty (%) and circle = Moisture content (%). Letters (A, B and D) characterize the production sites. A= Auxère, B = Beauchêne, D = Delabarre. O2 means secondary umbel order.

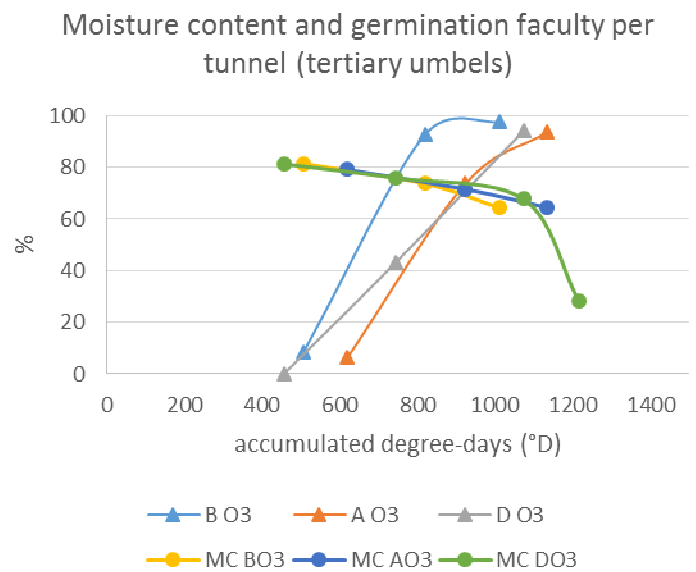
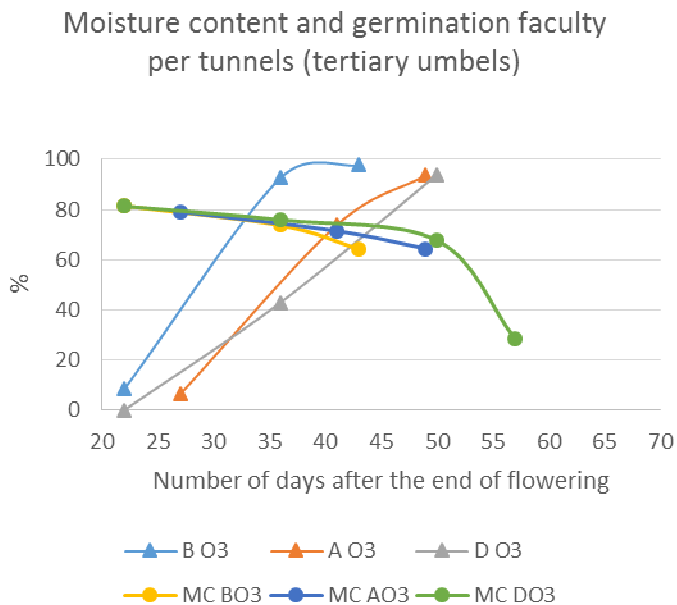


Figure 24. Moisture content and germination faculty per tunnel. Triangle = Germination faculty (%) and circle = Moisture content (%). Letters (A, B and D) characterize the production sites. A= Auxère, B = Beauchêne, D = Delabarre. O3 means tertiary umbel order.

Appendix III. Multiple linear regression for the moisture content and color variables.

Multiple linear regression to tests the dependency between the moisture content (dependant variable) and the color variable (a*,b*,L , H° and C°, explicative variables) made with XLSTAT Version 2016.05.35209.

n=69

Analyse Type III Sum of Squares (MC) :

Source	DDL	Somme des carrés	Moyenne des carrés	F	Pr > F	
L	1	0,349	0,349	49,146	< 0,0001	***
a	1	0,205	0,205	28,971	< 0,0001	***
b	1	0,076	0,076	10,666	0,002	**
C	1	0,084	0,084	11,902	0,001	**
h	1	0,097	0,097	13,727	0,000	**

Adjusted R²= 0,878

There is a strong link between the color variables (all) and the seed moisture content (87% of the moisture is explained by the model).

Appendix IV. Example of script and results of data analysis (samples of the harvest day).

Script:

```

donnee=read.table("fenouil_recolte.txt",header=T)
attach(donnee)
donnee
as.factor(donnee$Order)
# GLM model
res=glm(cbind(G,NG)~DAF+Tunnel+Area+Order+DW+MC+DD+L+a+b+C+h,
family=binomial(link="logit"))
# Selection of the best model
library(MASS)
# Writing candidate models
modC=glm(cbind(G,NG)~DAF+Tunnel+Area+Order+DW+MC+DD+L+a+b+C+h, family=binomial(link="logit"))
#selection of the final model
modF<-stepAIC(modC,direction=c("both"))
summary(modF)
anova(modF,test="Chi")
#residus
qqnorm(residuals(modF,type="pearson"))
qqline(residuals(modF,type="pearson"))

```

Results:

```
> summary(modF)
```

Call:

```
glm(formula = cbind(G, NG) ~ Order + MC + a, family = binomial(link = "logit"))
```

Deviance Residuals:

```

  Min      1Q   Median      3Q      Max
-1.4730 -0.3612  0.2733  0.7712  1.1524

```

Coefficients:

	Estimate	Std. Error	z value	Pr(> z)
(Intercept)	1.4578	2.0294	0.718	0.47254
Order	2.6705	1.1868	2.250	0.02444 *
MC	-9.3155	3.0086	-3.096	0.00196 **
a	-0.2932	0.1498	-1.957	0.05029 .

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

(Dispersion parameter for binomial family taken to be 1)

Null deviance: 28.833 on 17 degrees of freedom

Residual deviance: 12.178 on 14 degrees of freedom

AIC: 59.404

Number of Fisher Scoring iterations: 5

tests on the parameters. If a value emerges as significant it means that the parameter value is significantly different from 0 at the confidence threshold of 5%

```
> anova(modF,test="Chi")
```

Analysis of Deviance Table

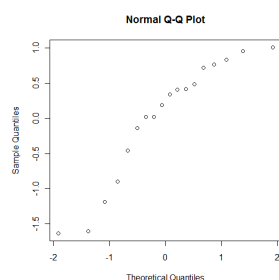
Model: binomial, link: logit

Response: cbind(G, NG)

Terms added sequentially (first to last)

	Df	Deviance	Resid. Df	Resid. Dev	P(> Chi)
NULL			17	28.833	
Order	1	0.6470	16	28.186	0.4211909
MC	1	11.9868	15	16.199	0.0005358 ***
a	1	4.0208	14	12.178	0.0449417 *

```
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```



Appendix V Technical practices of each tunnel (1/2)

Delabarre production site

Date	Treatment	Use
18/03/16	fumure (12/12/17 - 500kg/ha)	soil fertilization
	Orgaver (2T/ha)	soil fertilization
	Bio3G (1T/ha)	soil fertilization
	Microflore (500g/ha)	soil fertilization
Planting		
26/05/16	Decis Signum	Insecticide
		Fongicide
03/06/16	Amistar Bore	Fongicide
		nutrient
13/06/16	Karaté Zeon Bore	Insecticide
		nutrient
22/06/16	Decis protech Rovral Bore	Insecticide
		Fongicide
		nutrient
12/07/16	Switch	Fongicide
30/07/16	Suprême Teppeki Signum	Insecticide
		Insecticide
		Fongicide
10/08/16	Male destruction	
11/08/16	Suprême Teppeki Rovral	Insecticide
		Insecticide
		Fongicide
03/09/16	Karaté Zeon Topsin	Insecticide
		Fongicide
10/09/16	Rovral	Fongicide
12/09/16	Andainage	

Auxère production site

Date	Treatment	Use
23/02/16	TOURTEAU DE RICIN	soil fertilization
	OPTIMUS 4.8.8	soil fertilization
	OPTIMUS 13.5-0-0	soil fertilization
14/03/16	Panting	
24/03/16	APHIDALIA	Insects
30/03/16		Weeding
02/05/16	Trellising	
04/05/16	FRESAPROTECT	Insects
20/05/16	FRESAPROTECT	Insects
23/05/16	NORDOX 75 WG	Fungi
02/06/16	Eputation	
20/06/16		Weeding
20/06/16	Plant removal (disease)	
21/06/16	FLUIDOSOUFRE	Fungi
22/06/16	Pollination (brought flies)	
24/06/16	Pollination (brought hives)	
22/07/16	End of pollination	
26/07/16	Male Destruction	
29/08/16	Umbel cutting	

Appendix V Technical practices of each tunnel (2/2)

Beauchêne production site

Date		Treatment	Use
22/03/16		COMPOST BAUGEOIS	soil fertilization
		ALGO TONIC BIO	soil fertilization
		14-7-17 ENTEC	soil fertilization
24/03/16		FORCE 1,5 G	insecticide
	Planting		
25/03/16		PROWL 400	herbicide
		CHALLENGE 600	herbicide
12/04/16		ISO TONIC	growing stimulator
		PREVICUR ENERGY	fongicide
20/04/16		HUMIFIRST	fertilisant
22/04/16		AFALON 50 L	herbicide
03/05/16		DELTA BMo	insecticide
09/05/16		18-46-00	soil fertilization
10/05/16		KARATE K	insecticide
25/05/16		ROVRAL AQUA FLO	fongicide
		SCALA	fongicide
01/06/16			manual weeding
03/06/16	Trellising		
	Start of dripping		
14/06/16	Epuration		
15/06/16		TEPPEKI	insecticide
17/06/16		SWITCH	fongicide
21/06/16		SIGNUM	fongicide
24/06/16	Plant removing (250)		
05/07/16	Pollination (brought flies)		
08/07/16	Pollination (brought hives)		
10/08/16		SCORE	fongicide
18/08/16		PIRIMORE G	insecticide
23/08/16		SIGNUM	fongicide
		ROVRAL AQUA FLO	fongicide
29/08/16	Umbel cutting		

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Titre français : Etude sur l'origine de l'hétérogénéité de la qualité germinative des semences de fenouil (<i>Foeniculum vulgare</i> Mill. var. <i>azoricum</i> Tell.).		
Titre anglais : Study of the origin of the heterogeneity of the germinative quality in fennel seed production (<i>Foeniculum vulgare</i> Mill. var. <i>azoricum</i> Tell.).		
<p>Résumé : La production de semences hybrides de fenouil (<i>Foeniculum vulgare</i> var. <i>azoricum</i>) est soumise à des critères de qualité sanitaire et de germination élevée. Pour l'entreprise Bejo, la faculté germinative doit être au minimum de 90% pour qu'un lot puisse être commercialisé. Or, ces dernières années, les résultats de qualité germinative et de rendement ont été hétérogènes, sans qu'une tendance pouvant expliquer ces différences ne soit constatée. La maîtrise de la conduite de la culture porte-graine est nécessaire pour satisfaire à ces exigences de qualité et passe notamment par une date de récolte optimale pour assurer une bonne faculté germinative. Cependant, la compréhension des mécanismes et facteurs impliqués dans l'acquisition de la faculté germinative sont méconnus. Les objectifs de cette étude sont donc de mieux connaître la dynamique de maturation des semences de fenouil et de comprendre les causes de l'hétérogénéité de la qualité germinative. Trois sites de production de semences de la variété Victorio (hybride F1) ont fait l'objet d'observations pendant leurs phases de floraison et de maturation. Des ombelles secondaires et tertiaires ont été prélevées régulièrement au cours de la maturation, pour suivre l'évolution des semences à l'aide de trois indicateurs : le nombre de jours après la fin de floraison (peut être assimilé aux degrés-jours accumulés), la teneur en eau, la couleur (quantifiée avec un spectrophotomètre). Les résultats révèlent qu'avant 45 jours les ombelles secondaires sont immatures (38 jours pour les ombelles tertiaires). En ce qui concerne la teneur en eau et la couleur, elles sont effectivement liées au pourcentage de germination. On peut noter que pour cette étude, concernant cette année de production, la faculté germinative des semences fraîches pour chaque tunnel de production a dépassé les 90%. Ainsi, même si les objectifs de cette étude sont atteints en ce qui concerne la dynamique de maturation, les causes de l'hétérogénéité restent indéterminées et feront l'objet de travaux ultérieurs.</p>		
<p>Abstract: The production of fennel hybrid seeds (<i>Foeniculum vulgare</i> var. <i>azoricum</i>) is subjected to criteria of sanitary quality and high germination faculty. For the Bejo company, the germination faculty has to be at least 90% to allows the seed lot to be marketed. However, these last years, the results of germination quality and yield were heterogeneous, without any notable trend that can explain these differences. The control of the technical itinerary is needed to meet these quality requirements and include in particular an optimal date of harvest to ensure good germination. Nonetheless, the understanding of mechanisms and factors involved in the acquisition of the germination faculty are unknown. The aims of this study are therefore to bring knowledge about the dynamic of fennel seed maturation and to understand the causes of the heterogeneity of the germination quality. Three production sites of the variety Victorio seeds (F1 hybrid) were the subject of observations during their flowering and ripening phases. Secondary and tertiary umbels were taken regularly during maturation, in order to monitor the seed evolution using three indicators: the number of days after the end of flowering (it can be likened to the accumulated degree-days), the moisture content, the seed coat color (quantified by a spectrophotometer). The results reveal that before 45 days the secondary umbels are immature (38 days for the tertiary umbels). As regards the water content and color, they are actually related to the germination faculty. It is noticeable that in this study, for this production year, fresh seed germination for each production site exceeded 90%. Thus, even if the goals of this study are reached regarding the seed ripening dynamic, causes of heterogeneity remain undetermined and will be the subject of further work.</p>		
Mots-clés : fenouil, semences, faculté germinative, teneur en eau, couleur		
Key Words: fennel, seeds, germination faculty, moisture content, colour		