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# Evaluation of chicory seeds maturity by chlorophyll fluorescence imaging

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**Abstract**

Chicory (*Cichorium intybus* L.) seed production includes sorting to remove foreign materials and non-viable seeds. A machine vision system was developed to monitor the fluorescence in order to detect the immature chicory seeds. It comprised a monochromatic light source, a highpass filter and a monochromatic CCD camera sensitive to red and infrared. With this device, blue light reflected by the seeds was blocked whilst red fluorescence was measured by the camera. A segmentation algorithm was designed to estimate separately the fluorescence intensities of the pappus, a crown of scales, and the main body of the pericarp. Experiments were carried out on five clones of cross-pollinated chicory plants used for seed production. Two hundred flower heads were labelled at flowering and harvested at different times during the maturation process expressed in "days after flowering" (DAF). Germination tests were performed according to the recommendations of the International Seed Testing Association to measure the germination percentage (GP) and the germination rate (GR), an indicator of seed vigour. Seed chlorophyll content diminished during maturation following a different logistic trend for the pappus and the pericarp. The GP increased from 18 DAF to reach its maximum value at 21 DAF, but the GR remained low until 30 DAF and increased afterwards. The potential of chlorophyll fluorescence to be used as an indicator of chicory seed vigour was the greatest between 21 and 36 DAF.

**Nomenclature**

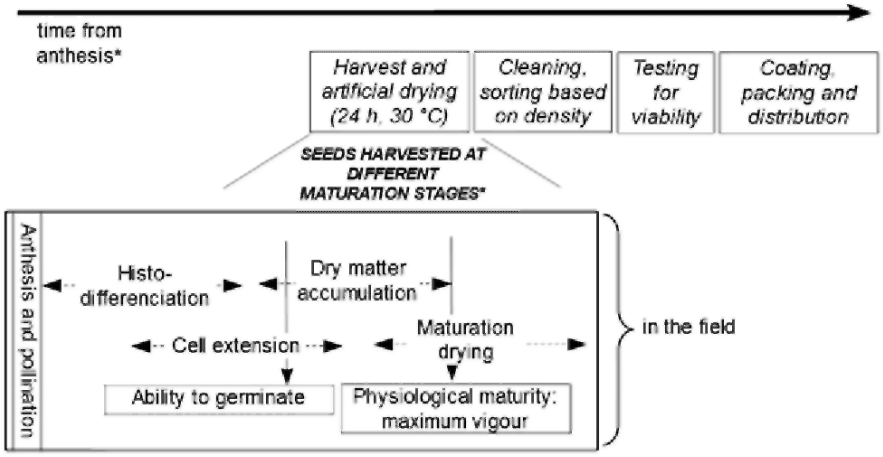
| | |
|---|---|
| CCD | charge-coupled device |
| CF | chlorophyll fluorescence[a] |
| CI | confidence interval ($p$ = 0.05) |
| DAF | maturation time expressed in days after flowering, d |
| FPAP | *pappus* fluorescence[a] |
| FPER | pericarp fluorescence[a] |
| GP | germination percentage, % |
| GR | germination rate, $d^{-1}$ |
| LED | light-emitting diode |
| LF, RF | fluorescence level of the left and right parts of a seed image[a] |
| LW, RW | width of the left and right parts of a seed image, pixels |
| NTSC | National Television System Committee standard |
| Pa, Pe | *pappus* and pericarp zones of a seed image |
| PAM | pulse-amplitude modulated |
| $r$ | correlation coefficient |
| a | The fluorescence is relative to the reflectance signal of a reference paper and multiplied by 60. |

## 1. Introduction

Industrial chicory is an important crop as a source of inulin, a naturally occurring oligosaccharide composed of fructose and, typically, a terminal glucose. The seeds are uniformly sown in the field. Ungerminated seeds cause a loss of yield. For the seed producer, one of the challenges is to provide seed lots having a sufficient germination percentage and vigour to ensure an even coverage of viable seeds across the field. Producers may acquire a certificate reporting the germination performance of their seeds provided by laboratories approved by the International Seed Testing Association (ISTA, Bassersdorf, Switzerland). Also, the producer may aim at a higher germination percentage than the legally required (80%). Causes of low germination percentage and weak vigour include intrinsic characteristics of seeds such as immaturity (full development not reached), low storage potential (maturation drying not attained), external damage caused by harvest and handling machines, ageing and pathogens.

Artificial drying improves the storage potential of seeds not achieving maturation drying in the field. Pre-cleaning removes most of the empty shells and foreign materials from the seed lots. Seeds are mechanically sorted on the basis of density and size. Immature seeds that have not fully achieved cell expansion and filling are lighter, and gravimetric devices have been used to select the heaviest seeds which generally present better germination performance. Size sorting improves size homogeneity which is necessary for coating operations, which include specific treatments aimed at reducing seedborne infections which affect the growing plant (Cockerell, 2006). Ageing can be avoided by observing a sufficiently short delay between harvest and sale. Fig. 1 summarises the scheme of chicory seeds development, harvest, and processing.

***Fig. 1*** *- The development, harvest and processing of chicory seeds intended for sale. Physiological aspects from Bewley and Black (1994).* **Seeds intended for sale are usually all harvested at the same time, but the anthesis (flowering) does not occur at the same time for all flowers. For the experiment this was reversed; only a portion of the flowers were labelled, all flowering the same day, and the harvest date was different to obtain seeds at different maturation levels.*




However, even after several sorting operations, some seed batches can contain a large proportion of viable seeds but still not sufficient for commercial use. These batches are lost because the viable and non-viable seeds cannot be separated using the methods described. The proportion of immature seeds in these lost batches is unknown. It would therefore be useful to provide a new, non-destructive method of distinguishing immature seeds from mature seeds in order to improve sorting processes.

In this context, the potential of fluorescence imaging (Chen, Chao, & Kim, 2002; Nedbal & Whitmarsh, 2004) has been examined. Fluorescence occurs when some of the light absorbed by leaves and the other green organs of the plant (including seeds) is re-emitted at longer wavelength, typically between 650 and 750 nm. On the assumption that the amount of chlorophyll tends to diminish during the maturation of the seeds, the fluorescent properties of chlorophylls have been used to evaluate the maturity of cabbage seeds (*Brassica oleracea* L.) (Jalink, Frandas, Van der Schoor, & Bino, 1998; Jalink, Van der Schoor, Birnbaum, & Bino, 1999). A red LED (light-emitting diode, wavelength 650 nm) was used as a light source. The seed was illuminated through an interference filter at 656 nm. The emitted fluorescence was filtered at 730 nm and focussed by a lens onto a photodiode. The results showed that the magnitude of the chlorophyll fluorescence (CF) signal was inversely related to the quality of seeds. The relationship between the CF and germination performance was studied for tomato (*Solanum lycopersicum* L.) by Jalink et al. (1999) as in their previous study (Jalink et al., 1998) but using a laser light source with 670 nm wavelength. They concluded that seeds with an intermediate CF level were of the best quality, followed by seeds having a low CF signal. Seeds having a high CF signal were the worst. Konstantinova, Van der Schoor, Van den Bulk, and Jalink (2002) measured the CF of barley grains (*Hordeum vulgare* L.) using the SeedScan I Laser Sorter (Satake, Stafford, TX. USA), using the principle developed by Jalink et al. (1998) but using a laser light source instead of a LED. They concluded that sorting a barley seed lot into six subsamples varying in CF values resulted in an optimal quality for the subsamples with low and intermediate CF signals. Suhartanto (2002) thoroughly described the relationships between the fruit CF, seeds CF and germination performance of tomato. A Pulse-Amplitude Modulated method (XEPAM fluorimeter, Heinz

Walz GmbH, Germany; method described by Schreiber, 2004) was used to measure the dark fluorescence (CF after adaptation to darkness) of fresh seeds, fruit fluorescence and their photosynthetic activity. The maximum germination percentage and maximum percentage of normal seedlings occurred at 51-54 days after flowering (DAF) when the CF of fresh and dried seeds, as well as seed chlorophyll content, reached a minimum. From these studies, it can be concluded that the performance of CF as a marker for seed performance is dependent upon the species, with a general tendency to a negative correlation between CF and germination performance.

Zude-Sasse, Truppel, and Herold (2002) estimated the relationships between the ripening state of apples assessed by the Streif index (firmness/(soluble solids × starch index)) and the spectral characteristics of the fruit surface. The chlorophyll content was measured destructively by spectrophotometry after acetone/diethyl ether extraction and non-destructively from the spectral characteristics. It was concluded that the estimation of the chlorophyll content improved the precision of the prediction of fruit ripeness; i.e. 6 days instead of 7 for the 'Jonagold' variety. Bron, Ribeiro, Azzolini, Jacomino, and Machado (2004) measured CF parameters on papaya fruits using a PAM fluorimeter (FMS1, Hansatech, King's Lynn, UK) and found significant correlations between fruit ripeness parameters (firmness, hue and days of ripening) and CF parameters (fluorescence yield of dark-adapted material ($F_0$), increase of fluorescence yield during saturation pulse ($F_v$) and maximum fluorescence yield ($F_m$) as described by Schreiber, 2004).

Both applications (seed and fruit) are relevant since the chicory seed sold on the market is in fact a fruit. Chicory belongs to the Asteraceae family. The inflorescence is a *capitulum* containing between 15 and 25 flowers (Rabau, Detry, & Bochkoltz, 1987). Fruits are *cypselae*, a kind of achene, which is an entire fruit containing a single seed. The seed embryo is enclosed within the testa and pericarp without significant endosperm. From this point onwards, the "seed" referred to in this paper will refer to the entire achene. In the process of commercial seed production, the seed is not removed from the achene and only the fruit pericarp and *pappus* are observable. The seeds are about 2.5-3 mm long and their mass is about 1.5 mg. Except for hybrids, their colour is highly variable. The pericarp may be entirely clear (i.e. cream) and plain, dark brown, spotted and any combination of brown colours. The *pappus* is always light-coloured when present. Means and confidence intervals for individuals (95%) were 81 ± 56, 72 ± 48 and 56 ± 24 respectively for R, G and B. The seeds were of the Nausica variety and tested after gravimetric sorting (the heaviest fraction, intended for sale). The light sources were fluorescent tubes (F36W125-ST, Sylvania - GTE, Irving, TX, USA) and the camera was a Donpisha 3CCD RGB (Sony, Tokyo, Japan) white-balanced using white paper. Ooms and Destain (2010) showed that the visual aspect of these seeds (360 features of colour, shape and texture were tested) was only weakly correlated with their germination performance: only the empty shells could be visually detected.

A device is needed to measure the CF of chicory seeds. In preliminary tests (spectrometry after extraction following the method of Bellomo & Fallico, 2007), it was found that the chlorophyll level was only about 1 mg $kg^{-1}$ in seeds harvested and artificially dried, which corresponds to 0.05 µg $mm^{-2}$, whilst the concentration of chlorophyll-a in leaves of root chicory growing at 16 °C is about 30 µg $mm^{-2}$ (Devacht et al., 2009). The device must be sensitive enough to detect such low levels of CF. During development, the *pappus* is exposed to sunlight, while the rest of the pericarp is hidden. It would be interesting to assess the fluorescence of both parts separately. Hence, it was decided to build a non-destructive highly sensitive CF imaging device (Ooms & Destain, 2010). A preliminary experiment was conducted with this device to simulate the sorting of a batch of chicory seeds on the basis of CF. It was shown, with the help of a sorting optimisation curve (Ooms, Palm, Leemans, & Destain, 2010), that the germination percentage of the seed batch could be improved from 85% to 89.5% with a quantitative loss of 40%. However, there was no attempt to link this result with the seeds maturity.

The objectives of this study were:

- to obtain images of the distribution of CF on chicory achenes, harvested at different dates to control their maturation time;
- to develop a specific algorithm for the segmentation of the different parts of the chicory achenes;
- to analyse the correlations between the fluorescence distribution on chicory seeds, the germination performance and the seeds maturity.

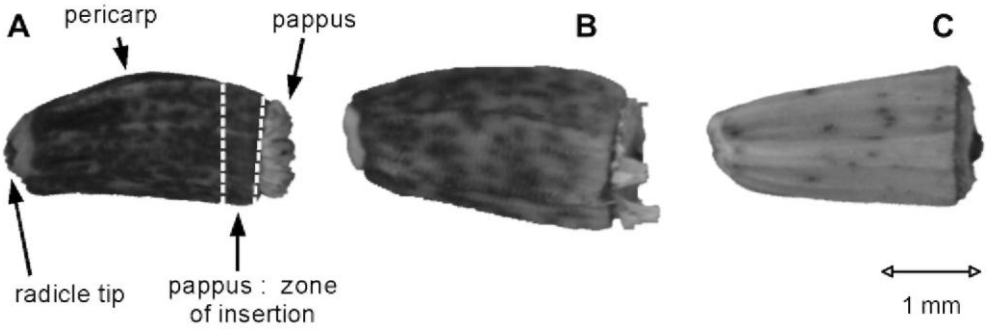
## 2. Material and method

### *2.1. Plant material*

Chicory seed is contained within the pericarp of the achenes. Husking of the achenes is not possible. The structure of chicory seeds is presented in Fig. 2. The *pappus* is composed of scales inserted at the top of the

achene (to the right of A in Fig. 2). The *pappus* and its zone of insertion onto the pericarp (A, Fig. 2) may present a higher chlorophyll content than the main body of the pericarp. The *pappus* is sometimes absent (C, Fig. 2). In such a case, the "*pappus*" refers exclusively to its zone of insertion.

***Fig. 2*** *- Structure and aspect of chicory cypselae. Images taken with a Donpisha 3CCD RGB camera (Sony, Tokyo, Japan; light source: fluorescent tubes (F36W125-ST, Sylvania - GTE, Irving, USA)). A: with pappus, B: with damaged pappus, C: without pappus.*




Two hundred and fifty-one chicory plants were grown outdoors in Warcoing, Belgium during the summer of 2006. The plants originated from cultivars selected for inulin production. Among these, five clones placed at different locations in the field were marked for our experiment. Pollination was carried out mostly by hoverflies (*Syrphidae* sp.) and possibly bumblebees (*Bombus* sp.) and bees (*Apoidea* sp.). The pollen came from neighbouring chicory plants which were genetically different (i.e. polycross). Each plant produced *capitula*, of which 40 were labelled. Each *capitulum* was composed of about 18 flowers which each produced one *cypsela*. For a particular *capitulum*, the date of flowering (bud initialisation) can be determined accurately since the duration of the anthesis is only a few hours (Pecaut, 1962). The *Capitula* were labelled on 26th July 2006 during anthesis and harvested between 13th August and 3rd September 2006 following the scheme of Table 1 and Fig. 3. The *capitula* were dried at 30 °C for 24 h on the day of harvest.

***Fig. 3*** *- Sequence of events and measurements. Labelling, harvest and drying refer to whole capitula. Chlorophyll fluorescence measurements and germination tests were performed on seeds.*

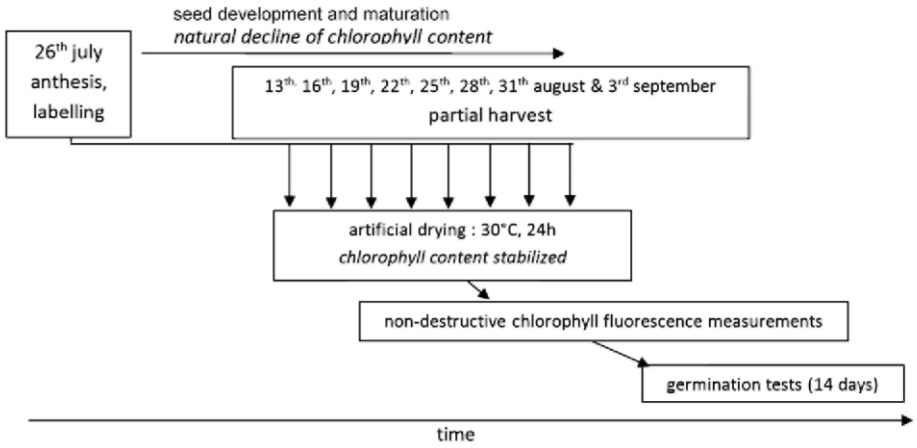
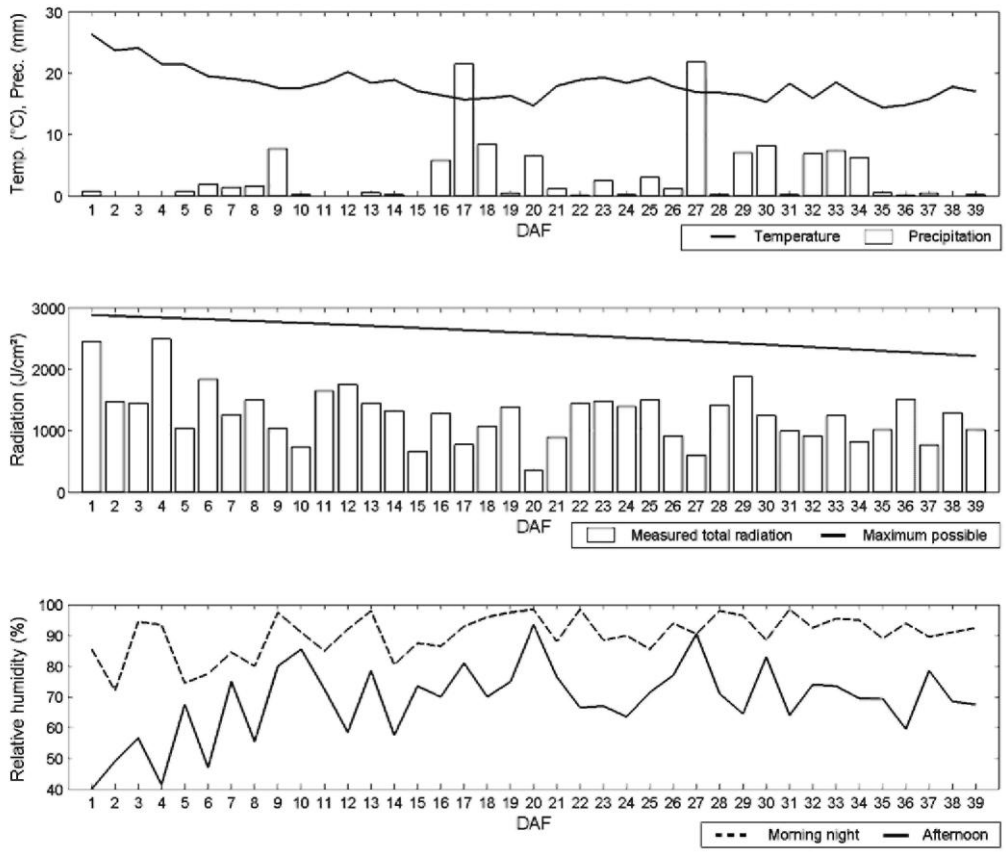


***Table 1** - Number of capitula (flower heads, bold) for each plant and harvest date expressed in days after flowering (DAF).*

| | Plant number | | | | | Seeds, total | Seeds/*capitulum* |
|---|---|---|---|---|---|---|---|
| DAF | 1 | 2 | 3 | 4 | 5 | | |
| 18 | 5 | 5 | 5 | 5 | 5 | 434 | 17.36 |
| 21 | 5 | 5 | 5 | 5 | 5 | 462 | 18.48 |
| 24 | 5 | 5 | 5 | 5 | 5 | 454 | 18.16 |
| 27 | 5 | 5 | 5 | 5 | 5 | 446 | 17.84 |
| 30 | 3 | 3 | 3 | 3 | 3 | 283 | 18.87 |
| 33 | 3 | 3 | 3 | 3 | 3 | 294 | 19.6 |
| 36 | 3 | 3 | 4 | 3 | 3 | 284 | 17.75 |
| 39 | 6 | 2 | 6 | 7 | 7 | 516 | 18.43 |
| Total | 35 | 31 | 36 | 36 | 36 | 3173 | 18.24 |
| Losses | 5 | 9 | 4 | 4 | 4 | lost due to wind | |

***Fig. 4** - Weather conditions during the chicory seeds development and maturation in Warcoing, Belgium, from anthesis (26th July 2006, 0 days after flowering or DAF) to last harvest (39 DAF, 3rd September).*




The evolution of the CF of a batch of seeds was monitored during storage (using the device described below) in order to ensure that there was no further degradation of the chlorophyll after artificial drying.

The weather conditions during the experiment are described in Fig. 4. Data were retrieved from the nearest weather stations of the Royal Meteorological Institute of Belgium (Institut Météorologique Royal de Belgique, 2006a, 2006b, 2007); Uccle (90 km to the east) for total radiation, Semmerzake (40 km to the northeast) for relative humidity and Néchin (8 km to the west) for temperature and precipitation. The weather was hot and dry during the 5 first days after anthesis, but the temperature remained stable afterwards. Two episodes of heavy rainfall occurred; the first 17 days after anthesis (i.e. the day before the first harvest), the second on the 27th day.

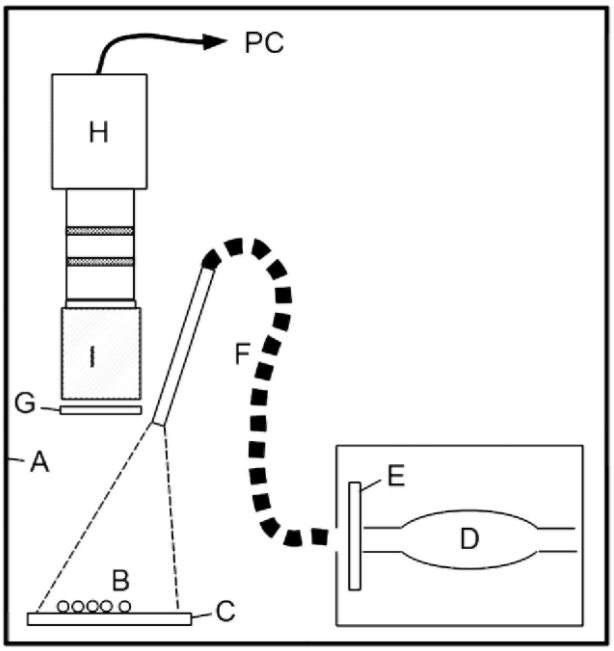
### *2.2. Chlorophyll fluorescence imaging*

A non-destructive imaging device was developed to measure fluorescence of chlorophyll-a of chicory achenes (Ooms & Destain, 2010) (Fig. 5). It was decided to obtain information on chlorophyll-a since it is the most abundant (Papageorgiou, 2004): 70% of all chlorophylls in the light-harvesting complexes of photosystem II, and 100% in the reaction centres (Farineau & Morot-Gaudry, 2006). By application of blue excitation radiation (wavelengths 400-450 nm), red and far-red fluorescence is emitted, with a peak around 680 nm.

The chlorophyll concentration was not estimated from the intensity of CF. Instead, the total CF was used as an indicator of seed quality. To estimate the chlorophyll concentration, it would be necessary to measure the fluorescence at wavelengths 690 nm and 730 nm (Buschmann, 2007) and use narrowband filters. This would be an impediment to the main application, which is the sorting of large amounts of seeds, since root chicory is an industrial crop, due to a lower response of the measurement system and therefore, a lower operating speed.

Since the hypothesis was that the chlorophyll content decreases with advancing maturity, it was necessary that CF is a strictly positive function of chlorophyll concentration. This does not seem to be the case when observing Fig. 2, p. 264 in Buschmann (2007). However, this data indicates that the CF decreases only for chlorophyll concentrations exceeding 7 mg $ml^{-1}$. The chlorophyll concentration in dry chicory seeds is about 1 mg $kg^{-1}$, for a mean dry content of about $7 \times 10^{-4}$ mg $ml^{-1}$ (estimating the dry seeds density at 700 kg $m^{-3}$). Even if the chlorophyll may be concentrated in the pericarp and local concentrations could reach the above limit, this should not be enough to globally affect the results.

***Fig. 5** - Fluorescence imaging system. A: black box, B: seeds sample, C: non-fluorescent support (black - painted metal), D: xenon lamp, E: blue bandpass filter, F: optical fibre, G: lowpass filter, H: camera, I: zoom objective.*




A xenon lamp (Lightningcure L8222 model LC5, Hamamatsu, Hamamatsu, Japan) was used as the excitation source. A blue filter (03FIB002, Melles Griot, Carlsbad, USA) with a central wavelength 410 nm and width at half-maximum of 80 nm was applied to excite the chlorophyll-a. The fluorescence signals were detected using a video CCD camera with a CCD array of 640 x 480 elements (C5405-70, Hamamatsu, Hamamatsu, Japan), equipped with a zoom objective (TV zoom lens 18-108 mm F2.5, unknown maker, Japan). The scale was fixed to 30 pixels $mm^{-1}$. A highpass filter (665 nm, 03FCG107, Melles Griot, Carlsbad, CA, USA) was placed between the seeds and the sensor to select the red and infrared wavelengths corresponding to the fluorescence emission of chlorophyll-a. A frame grabber (Picolo Industrial, Euresys, San Juan Capistrano, CA, USA) and its associated

acquisition software (Easygrab 4.0, Euresys, San Juan Capistrano, CA, USA) digitised the NTSC signal into bitmap digital images. Except for the frame grabber and computer, the system was enclosed within a black box to avoid interference from ambient light. This latter was air-conditioned (Tectro TS27, PVG Int. B.V., Oss, The Netherlands) to maintain a temperature of 20 °C (±1 °C). The lamp was optically isolated and white light did not escape inside the box.

The blue filter transmitted an unexpected, small amount of infrared light between 770 and 900 nm (detected with spectrometer AVS-SD2000, Avantes, Eerbeek, The Netherlands). As this is reflected by the seeds and detected by the camera, the infrared light interfered with the results and caused a positive offset in the fluorescence signal which did affect the results (see Section 3.2). However, this infrared light also allowed the use of non-fluorescent white paper for background correction, and the detection of the edges of non-fluorescent seeds without the need to take another photograph with different lighting or another camera.

To ensure that the CF was effectively detected by the system, the spectrum of the fluorescence of a batch of chicory seeds (Nausica variety, sorted on the basis of density and containing mostly low fluorescent, mature seeds) was determined between 640 and 730 nm wavelength. The lowpass filter at 665 nm was replaced successively by narrow interference filters at 640 ± 5 nm (F43-187, Edmund Optics Ltd., York, UK), 652 ± 5 nm (03FIV022, Melles Griot, Carlsbad, CA, USA), 671 ± 5 nm (F43-191, Edmund Optics Ltd.), 702 ± 5 nm (03FIV024, Melles Griot) and 730 ± 5 nm (F43-195, Edmund Optics Ltd.). Because of the limited response of the system using narrow bandpass filters, only the spectrum of the highly fluorescent zones could be properly estimated (zones corresponding to CF > 125 on the original image taken with the lowpass filter). The spectrum was the one of unbound chlorophyll-a, with the maximum intensity corresponding to the filter at 671 nm.

A preliminary test was conducted to determine if some of the photo systems were still active after the seeds were dried (50 seeds, artificially dried at 30 °C, Nausica variety). Under continuous illumination, the seeds CF was imaged every 0.1 s using a specific code written in C++. No Kautsky curve (Kautsky & Hirsch, 1931) was observed, only a slow decrease was observed which may be due to photo damage. The total decrease of CF intensity was 5% after 1 min. The photo systems were apparently not functional.

### *2.3. Experimental protocol*

To eliminate the effect of the inhomogeneous radiation of the xenon lamp, the measurements were compared to a reference. Two superposed white sheets of paper having a stable reflectance signal, due to the infrared light passing through the blue filter at the light source, were used for background correction. Reflectance images of the papers were taken 15 times each day at regular intervals during the experiment. It was verified that the measured reflectance signal of the paper varied linearly in function of the light intensity.

The fifteen images were filtered (lowpass) to suppress paper texture using a median filter (each pixel was replaced by the median of the surrounding zone of 10 x 10 pixels). The mean of the fifteen filtered images was used for subsequent background correction. The uncertainty (95%) due to the use of paper for background correction was ±3% for individual pixels and ±2.7% for the mean image intensity. The seeds were removed from each *capitulum* (all of them, even if empty), laid on the non-fluorescent support (15-25 seeds at a time, corresponding to one *capitulum*) and irradiated with the blue light. Since photo damage was likely to happen due to strong illumination, two images of each sample were taken: the first after 1 min and the second after 2 min of exposure. A mean difference of 3% was observed between the fluorescence features of both images, meaning that photo damage may have significantly affected the measurements if the exposure time to the excitation light was not controlled. Both images were processed for each *capitulum* (see Section 2.4) and the means of the calculated features were used for interpretation.

### *2.4. Image processing and analysis*

A specific image analysis code was developed with the GNU Octave language (Fig. 6). After applying background correction (the fluorescence values were divided by the reflectance signal of paper and multiplied by 60), the images (A) were segmented (B) and twenty images of individual seeds were created, each of them being rotated (C) along the main axis of the seed. The pappus was automatically detected (D); the mean width of the left half (LW) was multiplied by its mean fluorescence intensity (LF) to obtain a "left pappus score", which was compared to the "right pappus score" obtained from the right half width (RW) and fluorescence intensity (RF). The pappus was located on the left if the left score was greater than the right score and *vice versa*. The accuracy of the detection was greater than 98%. The image was thereafter divided (E) into the "pericarp zone" (Pe, 77% of the seed length) and the "pappus zone" (Pa, 23% of the length). The value of 77% was a compromise based on

the observation of 100 seed images. The mean fluorescence values of the two zones were recorded for data analysis.

### *2.5. Germination tests*

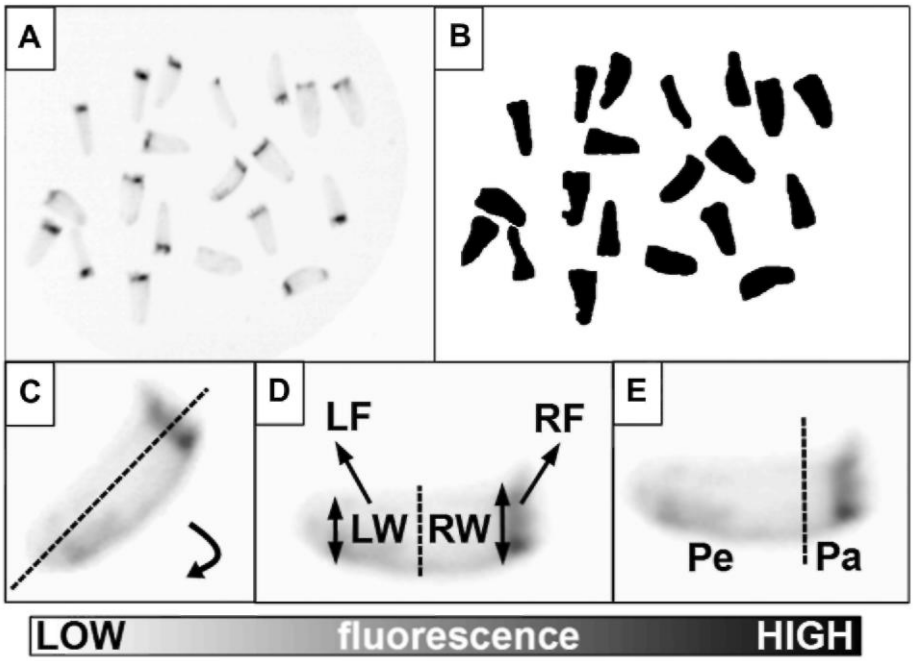
Germination tests can provide information about the seed ability to germinate in the field, by measuring the germination percentage (GP) and the germination rate (GR) in standard conditions.

The GP is the proportion of germinated seeds presenting a healthy, normal embryo after 14 days when placed in standard conditions: controlled temperature and humidity, following the recommendations of the ISTA (2005). The seeds were placed on flat filter papers in closed Petri dishes at 20 °C with enough tap water to ensure unlimited water availability and a saturated atmosphere without suffocating the seeds by flooding. They were observed at regular time intervals (2, 3, 6, 10 and 14 days) to assess the amount germinated and ungerminated seeds. Germinated seeds were put aside at each count.

The GR or germination speed is one of the three key traits related to seed vigour (Finch-Savage, Clay, Lynn, & Morris, 2010). It is a measure of the extent of damage that has accumulated as viability declines (Powell, 2006; Probert & Linington, 2006; TeKrony & Egli, 1991). The GR is defined as "the reciprocal of the time from the start of imbibition (intake of water) for an arbitrarily chosen percentage of seeds to just complete germination" (Halmer, 2006). In our experiments, GR was evaluated simultaneously with GP, with a threshold of 25% which is half of the final GP. A seed was considered germinated when the hypocotyl, root and root hairs were present without anomaly. To assess the GR from the discrete data, spline interpolation was used.

GP and GR were measured for each date of harvest, after performing the CF imaging. All photographed seeds were tested for germination.

***Fig. 6*** *- Image segmentation and analysis. A: after background correction, B: segmentation and individualization, C: rotation, D: pappus detection, E: image subdivision, LF = left fluorescence level, RF = right fluorescence level, LW = left width, RW = right width, Pe = pericarp, Pa = pappus.*



## 3. Results and discussion

### *3.1. Germination tests*

During the germination tests, the seeds from one of the plants had very poor values of GP, less than a quarter of the GP observed on the four other plants. The corresponding data was considered aberrant and removed.

The trend of GP with harvest date (in days after flowering or DAF) is shown in Fig. 7. The large confidence intervals were due to the differences of absolute values of GP observed on the plants used for the experimentation. The correlation between GP and the harvest date was weak for the observed period (18-39 DAF, $r = 0.297$) and the only significant increase in GP was observed between 18 and 21 DAF. Therefore, events leading to the acquisition of the ability to germinate, i.e. achieving histodifferentiation and accumulating enough nutrients to produce a viable seedling, may occur before 21 DAF.

***Fig. 7** - Total germination percentage (overall mean) in function of the harvest date (in DAF), with 95% confidence intervals. Arrow: significant increase.*

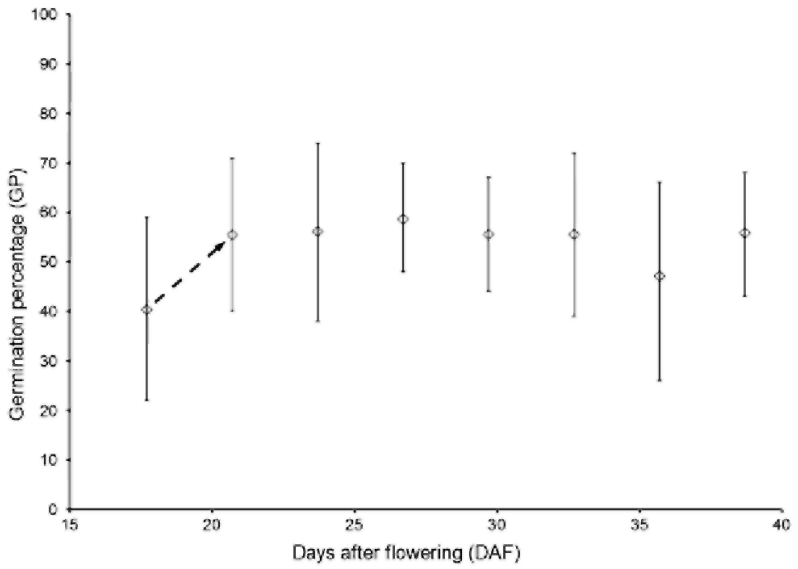



***Fig. 8** - Germination rate (overall mean) in function of the harvest date (in DAF), with 95% confidence intervals. — modelling with a logistic function $GR = 0.221 + 0.449/(1 + \exp((34.3\text{-}DAF)/2.61))d^{-1}$.*

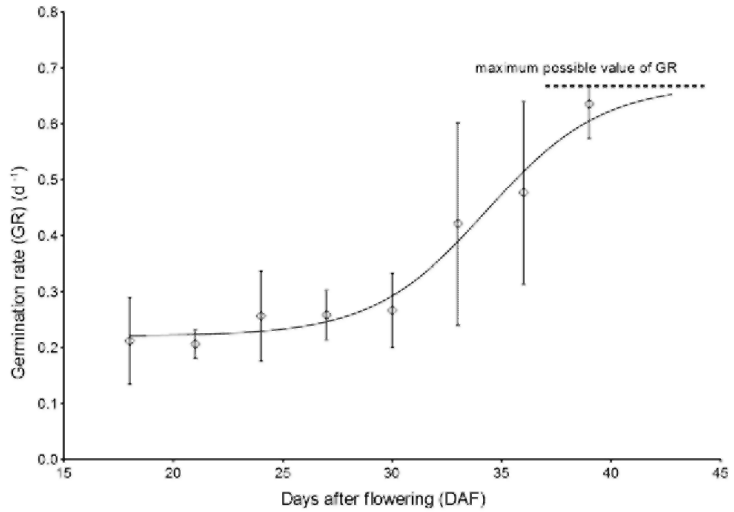
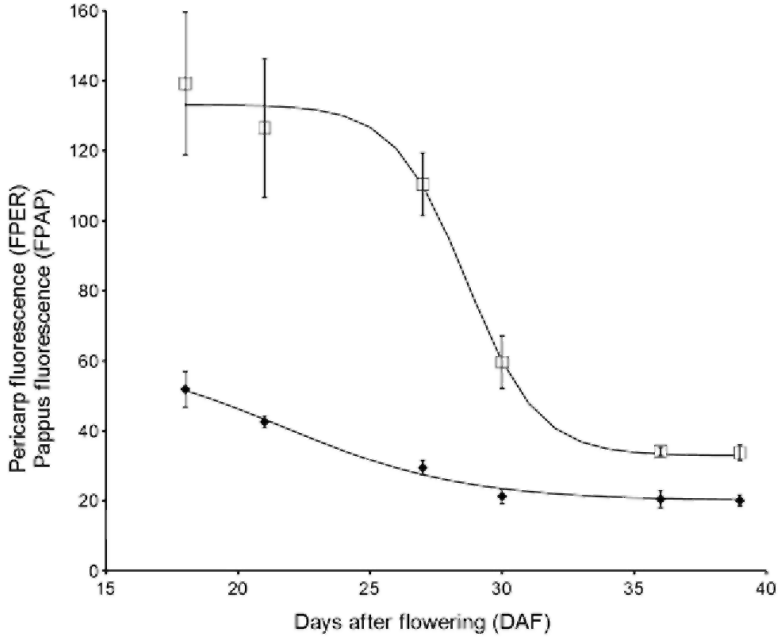


***Fig. 9** - Evolution of chlorophyll fluorescence during seed maturation (means of individual measurements and 95% confidence intervals). □ pappus, ♦ pericarp, — modelling using the logistic functions FPAP = 133-100/ (1 + exp((28.6-DAF)/1.36)) and FPER = 62.4-42.4/ (1 + exp((21.7-DAF)/3.42)), respectively.*




The evolution of the GR in function of the harvest date is shown in Fig. 8. The GR remained nearly constant until 30 DAF, and then increased. At the end of the whole maturation process (at physiological maturity), the GR should attain its maximum value and remain stable for a relatively long period before the onset of senescence. This maximum value corresponds to the GR of healthy, fully mature seeds, which may not be present in the tested samples as the harvest stopped at 39 DAF (i.e. GR was still increasing). The maximum value was estimated by testing several chicory seed lots of varying ages and origins and measuring the time needed for the first (and best) seeds to germinate in standard conditions (36 h). The maximum possible value of the GR was therefore 1/ 1.5 = 0.67 $d^{-1}$. This value was chosen as an asymptote (Fig. 8). The relationship between GR and DAF was modelled using non-linear least square regression (SAS software v. 9.2, SAS Inc, Cary, NC. USA). Among four types of "S" curves: Gomperz, Gaussian modified, logistic and Johnson Shumacher (Debouche, 1979), the logistic model was found to be the most accurate with the following equation:

$$GR = 0.221 + \frac{0.449}{1 + \exp\left(\frac{34.3 - DAF}{2.61}\right)}\ d^{-1} \qquad (1)$$

The correlation coefficient between the harvest time and the GR was globally positive ($r$ = 0.913, 95% confidence interval = [0.584, 0.984]) for the whole experiment. The GR increased mainly between 30 DAF and 39 DAF ($r$ = 0.985, CI = [0.488, 0.9997]). The correlation coefficient was still significantly positive between 18 and 30 DAF ($r$ = 0.896, CI = [0.066, 0.993]).

### *3.2. Chlorophyll fluorescence*

The fluorescence levels of the pericarp and *pappus* are shown in Fig. 9 in function of time expressed in DAF. The fluorescence of the *pappus* (FPAP) was higher than the one of the pericarp (FPER). This could be due to the fact that the *pappus* was more exposed to light in the capitula. The correlation coefficient between the harvest time and FPAP was globally negative ($r$ = -0.967, confidence interval (CI) = [-0.997, -0.721]) for the whole experiment. It was also negative between the harvest time and FPER ($r$ = -0.931, CI = [-0.993, -0.486]). A single exponential or linear model cannot fit to the entire set of data. Using the same method as in Section 3.1, the decrease of FPAP was modelled using a logistic function:

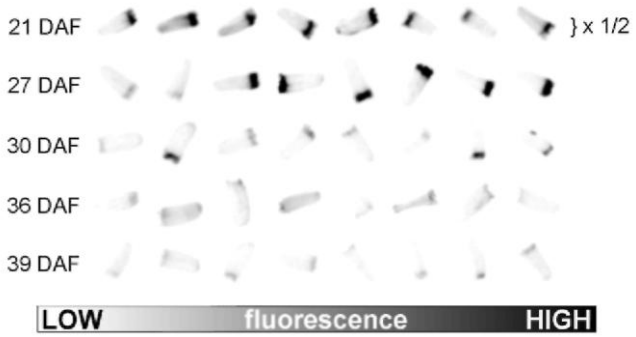
$$FPAP = 133 - \frac{100}{1 + \exp\left(\frac{28.6 - DAF}{1.36}\right)} \qquad (2)$$

The decrease of FPAP was the most marked between 27 and 30 DAF. The decrease of FPER was described by another logistic function:

$$\mathrm{FPER} = 62.4 - \frac{42.4}{1 + \exp\left(\frac{21.7 - \mathrm{DAF}}{3.42}\right)} \quad (3)$$

At 36 and 39 DAF, the ratio FPAP/FPER was the lowest (1.68) and the pappus became less discernable in fluorescence images (Fig. 10).

***Fig. 10*** *- Samples of fluorescence images of seeds for five different harvest dates.*




The lowest value of fluorescence was 20 for FPER and 33 for FPAP. The offset caused by the interfering infrared light reflected by the seeds could be as high, but probably not higher than these figures. The real final values of FPER and FPAP could be equal to or near 0.

### *3.3. Fluorescence of the pericarp and fluorescence of the pappus as predictors of germination performance*

The GR was plotted in function of FPER and FPAP in Fig. 11. A negative correlation was observed between GR and FPAP ($r$ = -0.844, CI = [-0.982, -0.103]) and probably between GR and FPER ($r$ = -0.689, CI = [-0.962, +0.278]). FPER was less correlated to the GR since the GR increased mostly after 30 DAF, when FPER has reached its minimum value. The function GR = f(FPAP) satisfies the parametric Eqs. (1) and (2):

$$\mathrm{GR} = 0.221 + \frac{0.449}{1 + 5.1 \times 10^{5} \left(\frac{\mathrm{FPAP} - 33}{-1.39 \times 10^{9} (\mathrm{FPAP} - 133)}\right)^{0.521}} \quad (4)$$

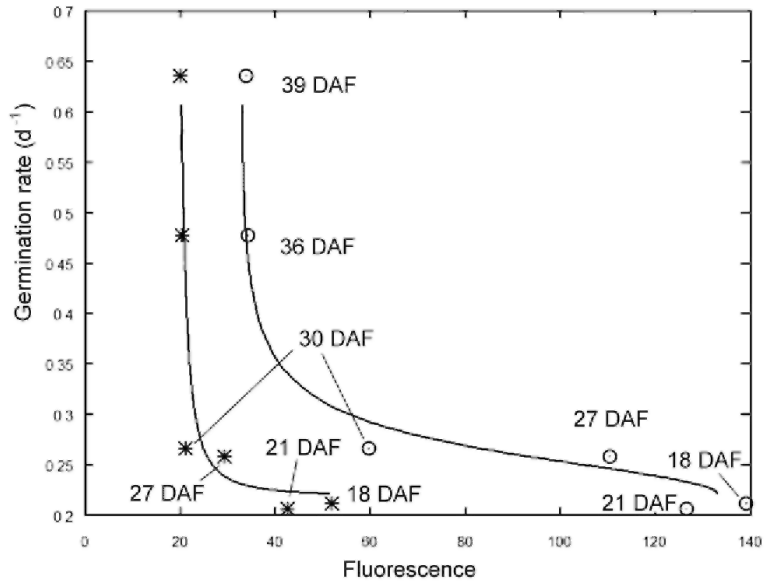
Similarly, from Eqs. (1) and (3):

$$\mathrm{GR} = 0.221 + \frac{0.449}{1 + 5.1 \times 10^{5} \left(\frac{\mathrm{FPER} - 20}{-568\ (\mathrm{FPER} - 62.4)}\right)^{1.31}} \quad (5)$$

FPAP may be used as an indicator of GR when its value is comprised between 35 and 60 (corresponding to seeds between 30 and 36 DAF) when GR and FPAP both vary. Outside this interval, the relationship is unclear. FPER may be used as an indicator of GR when its value is comprised between 25 and 40 (corresponding to seeds aged between 21 and 30 DAF). FPER and FPAP may thus be considered as complementary indicators.

The GP could not be predicted using the gathered data. The observed variation of the GP between 18 and 21 DAF was not long enough to provide information that could be exploited to predict GP using FPAP or FPER.

***Fig. 11** - Germination rate in function of chlorophyll fluorescence, ○ pappus, * pericarp, — modelling GR = 0.221 + 0.449/(1 + 5.1 × $10^{s}$ × ((FPAP-33)/ (-1.39 × $10^{9}$ × (FPAP-133)))$^{0.521}$) $d^{-1}$ and GR = 0.221 + 0.449/(1 + 5.1 × $10^{5}$ × ((FPER-20)/ (-568 × (FPER-62.4)))$^{1.31}$) $d^{-1}$, respectively.*




### 3.4. Discussion

The chicory seeds exhibited a long development and maturation cycle; their ability to germinate in standard conditions was acquired at 21 days after flowering, while full vigour was nearly attained at 39 DAF. It was shown that the CF of chicory achenes decreased and followed a logistic model during maturation, between 21 and 36 days after flowering. CF was related to the germination rate, the fluorescence of the *pappus* being a better indicator that the fluorescence of the pericarp at a later development stage (30-36 DAF), and *vice versa* between 21 and 30 DAF. To establish the relationship between germination percentage and CF, collection of data should begin sooner than 18 DAF. The reproducibility of the observed relationships must be ascertained. Future work will focus on the replication of the experiment with different weather conditions, associated with fresh and dry weight measurements of chicory achenes, in order to locate more precisely in time the main steps of the maturation cycle such as nutrients filling and natural maturation drying. The interfering infrared light could be eliminated from future measurements by adding a filter to block it, but this has two disadvantages: future results could not be compared with the present results and the segmentation of the images would be more difficult.

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