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Autofluorescence of grape berries following *Botrytis cinerea* infection

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Gray mold is caused by *Botrytis cinerea* (anamorph of an ascomycete fungus) infecting over 200 plant species worldwide and causing tremendous harvest losses in vineyards. Even though all grapevine cultivars (*Vitis vinifera* L.) are susceptible to the disease, defense mechanisms are induced to counteract or slow down infection and colonization by the pathogen. One of the key inducible defense molecule is resveratrol, a blue fluorescent stilbenic compound. Considering early fungal detection as a crucial step in disease control strategies, we evaluated the potential of plant fluorescence to achieve early detection of *B. cinerea* in grapevine berries. By comparing control and artificially inoculated berries, images of fluorescence were taken in the laboratory at six combinations of emission/excitation wavelengths (F440/UV, F520/UV, F690/UV, F740/UV, and F690/436-blue F740/436-blue). Data treatments include proper image correction, interest area selection, fluorescence ratios computation and edge detection. In the early stages, before 3 DAI (days post inoculation), the discrimination (of - between ?) wounded and symptomatic berries could not easily be achieved using blue fluorescence solely (440 nm). However, using blue out of red

fluorescence ratio (F_{440}/F_{740}), it was possible to detect infected berries starting as early as 4 DAI. Using image analysis and edge detection over UV-epidermal transmittance measured at 690 nm, it was possible to detect botrytised berries as early as 6 hours after inoculation. The potential of these detection methods for use in the epidemiological field are discussed.

Key words: Bunch rot, detection, epidemiology, fluorescence, remote sensing, phytoalexins, precision viticulture, *Vitis vinifera*.

Introduction

Gray mold of grapevines is caused by *Botrytis cinerea* Pers.:Fr., the anamorph of an ascomycete fungus (*Botryotinia fuckeliana* Whetzel). This cosmopolitan fungus is a necrotrophic pathogen attacking over 200 plant species (Jarvis, 1980). In grapevine, it can infect leaves, in spring, where it grows in a concentric way at the margin of the blade and inflorescences which are highly susceptible, particularly dehiscent floral parts (Pezet *et al.* 2004). Various infection pathways have been described to establish epidemiological relationships between these first attacks, occurring early in the season, and the typical infections of maturing berries (Elmer and Michailides, 2004). Gray mold development on grape berries depends on the genetic structure of the pathogen population (Martinez *et al.*, 2005), but is also driven by some key factors, including climatic conditions, cluster architecture and berry susceptibility (Pezet *et al.* 2004; Elmer and Michailides, 2004). Berry infection result mostly from i) conidia germination and penetration favored by the presence of wounds at the surface of the fruit, in particular insect injuries (Fermaud and LeMenn, 1992; Miklota *et al.* 2003; Holz *et al.* 2004), ii) mycelial spread from one berry to the next which has been associated with severe losses. Depending on climatic conditions at the end of the season, the estimated losses for French vineyards can account for up to 40% of harvest (Mathys, 1982), and the estimated grape losses due to *B. cinerea* represent two billions \$US

in the world per year (Elad *et al.*, 2004). *B. cinerea* can reduce drastically both yield at harvest and wine quality, for all types of white and red wines (Marchal *et al.*, 2001, Ribéreau-Gayon *et al.*, 1980; Ribéreau-Gayon *et al.*, 1998). In particular, botrytised wines are less acceptable because of mushroomy, mouldy and rotten smells even when the infection reaches low-medium levels (approx. 10%). However, the berry infection by *B. cinerea* can also be desirable as ‘noble rot’ when present on some white grape cultivars used to produce sweet, late harvest wines (Ribéreau-Gayon *et al.*, 1980; Ribéreau-Gayon *et al.*, 1998). Control of gray mold in vineyards, as in various other crops, requires mostly the use of specific fungicides, but can also rely on modifications of cultivation practices (Leroux, 2004; Elmer and Michailides, 2004). The detection of *B. cinerea* can be performed by scouting in the vineyard, by using ELISA techniques (Auger *et al.*, 1995) or by relying on forecast models, often based on climatic data (Hruby, 2003; Piéri and Fermaud, 2005; Fermaud *et al.*, 2003). Moreover, as gray mold affects wine quality, grape sorting at harvest and/or just before the vinification process is often necessary. Sorting can be performed using polarographic measurement of oxygen consumption or by colorimetry using syringaldazin (Riberau-Gayon *et al.*, 1998). Moreover, polyclonal and monoclonal antibody-based immunoassays have also been developed to detect *B. cinerea* in grape juice (Pezet *et al.*, 2004; Elad *et al.*, 2004). Although precise, these detection methods are time-consuming and could be improved.

During the process of grape berry infection by *B. cinerea*, various biochemical interactions take place. These interactions have been investigated thoroughly with respect to host resistance to the fungus and involve both constitutive factors and induced ones following stress or infection (Van Baarlen *et al.*, 2004). In grapevine, the penetration of *B. cinerea* into the cuticle induces some defence reactions which include the synthesis by the plant of phytoalexins and pathogenesis-related (PR) proteins (Pezet *et al.*, 2004; Miklota *et al.*, 2003). The major phytoalexins are resveratrol and related stilbenic compounds which are induced in leaves (Langcake and McCarthy, 1979). Resveratrol can be easily observed around *B. cinerea*

infection sites because of its blue fluorescence. It has been detected, 48h after inoculation, at the periphery of the lesion on leaves, but not inside the colonized tissue including dead cells (Derckel *et al*, 1999). In grape berries, resveratrol is also produced, but partly constitutively, and accumulate in the skin only. However, resveratrol levels decrease during fruit ripening (Van Baarlen *et al*, 2004). It is generally assumed that *B. cinerea* must detoxify the stilbenic compounds, particularly resveratrol and the highly fungitoxic pterostilbene, in order to colonize grapevine (Pezet *et al*, 2004; Schouten *et al*. 2002). Laccases are inducible phenol-oxidizing enzymes catalyzing the oxidation of phenolic substrates (blue-copper oxidases) which are considered to play a major role in the degradation of the stilbenic compounds by the fungus. Other biochemical interactions, different from those involving defence reaction mechanisms, have also been reported. Karavaev & Polyakova (2000) showed a decrease in the content of chlorophyll in leaves infected with *B. cinerea* and Berger *et al*. (2004) observed an increased photosynthetic activity in the surroundings of a *B.cinerea* lesion on tomato leaves. Such a decrease in chlorophyll content and changes in photosynthetic activity have also been observed on corn plants (McMurtrey *et al*. 1994). Therefore, it may be assumed that such modifications could also be detectable using plant fluorescence on grape berries. Considering all these host-pathogen interactions, particularly the host defence reactions even if *B.cinerea* does not trigger systemic acquired resistance (Elad *et al.*, 2004), we have hypothesized that it would be possible to rapidly detect *B. cinerea* on grape berries using remotely sensed fluorescence.

In our study, we have evaluated the autofluorescence response of mature grape berries to *B. cinerea* infection in controlled conditions and its potential to achieve early detection. To do so, we collected fluorescence images on infected and control berries. We performed standard plant fluorescence ratios analysis as well as image analysis using edge detection algorithm.

Material and Methods

Grape berries

Mature grape bunches (*Vitis vinifera*) of the white cv. *Italia*, of 500, 550 and 660 g were used in experiments A, B, and C, respectively. Ten, randomly selected grape berries were detached from each bunch by cutting the pedicel from the main stalk using a fire-sterilised utility knife. Each berry was surface-sterilized by immersion in 95 % denaturated ethyl alcohol for 30 sec. They were then soaked and rinsed thoroughly using demineralised water.

Artificial inoculations

The *B. cinerea* strain 234, belonging to the *transposa* genetic type (Group-II), was used because strains of this type are more aggressive on grape berries than *vacuma* Group-II strains (Martinez *et al*, 2005). Provided by *UMR Santé végétale* (INRA-ENITA, Bordeaux, France), a detailed phenotype of this isolate has been described by Martinez *et al* (2003). This isolate was obtained originally in 1998, at harvest, in a Bordeaux vineyard (Pessac-Léognan) from mature white berries (cv. Sémillon blanc). Pure cultures were grown in Petri dishes on 1.5% malt agar solid medium (Biokar Diagnostic, Beauvais, France).

In order to inoculate, mycelial plugs (4 mm in diameter) were taken, using a fire-sterilised punch, from pure cultures on malt agar plates incubated at 20°C (the inoculum was transplanted to a new malt agar Petri dish on 09/18/2006). Before inoculating, the skin of all berries was pierced using a fire-sterilized needle. The needle was sterilized again and used to place one inoculum plug per berry, exactly at the point of skin piercing (applying the disc with mycelium facing the berry skin). Five berries were inoculated on October 10th (Experiment A), five on October 18th (Experiment B) and five on November 2nd (exp. C). As a control, for each experiment, the five remaining berries were inoculated with a virgin malt agar disc applied exactly at the point of skin piercing. The berries were then installed on a metallic netting in a plastic box (humid chamber (20x15cmx5). To ensure a high humidity

atmosphere, a moist sterile gauze was installed underneath the metallic netting. The humid chamber was kept in the laboratory, at room temperature (approx. 21°C) under natural daylight. The incubation period corresponded to the time between the inoculation and the first fluorescence measurements, *i.e.* 3, 2, and 0.25 days for *experiment A, B, and C*, respectively.

Fluorescence measurements

Fluorescence measurements were performed exactly 3 & 6 days after inoculation (DAI) for *experiment A*, 2 & 5 DAI for *experiment B*, and 0.25, 1, 4, & 6 DAI for *experiment C*. Fluorescence measurements were realized using an imaging fluorometer as described in Belzile *et al.*, (2003). The FLIM-100 imaging fluorometer used an interline CCD camera (CoolSnapHQ, RoperScientific, AZ, USA) modified for on-chip accumulation capability. The fluorescence was induced by a xenon flash lamp source (Hamamatsu, NJ, USA) producing intense light pulses at 50Hz repetition rate (4 μ s pulse width) using a pulse generator (model 500B Berkeley Nucleonics Corporation, CA, USA). For plant leaf fluorescence applications, UV (360 \pm 40nm) and Blue (436 \pm 20nm) excitation wavelengths were selected using band pass filter (Chroma Technology Corp., VT, USA). The excitation pulses were synchronized with camera acquisition using proper electronics and control software developed by Telops, Quebec, Canada. Image acquisition for each light pulse was done using 5 μ s integration time. Charges were accumulated on a CCD side layer. The charges were transferred to the frame grabber at the end of image acquisition process. This system enabled gated detection of low light level signal without using an image intensifier. Fluorescence imaging requires between 75 to 2500 acquisitions depending on the sample and on detected wavelength. Four narrow band pass filters (CVI Laser, NM, USA) were placed in front of the camera allowing precise detection of 440, 520, 690 and 740 \pm 10 nm fluorescence. UV-induced fluorescence was measured at the four emission bands whereas blue-induced fluorescence intensities were measured at 690 and 740 nm.

Lastly, for the fluorescence measurements, the grape berries were extracted from the plastic box using a pair of pliers rinsed thoroughly in 95% denaturated ethyl alcohol (beforehand and between each measurement). Grape berries were 50 cm away from the excitation source, thus effective excitation area was approximately 20 cm². All fluorescent measurements were done in the dark.

Image and data analysis

After proper image correction (Belanger *et al.*, accepted), including for example corrections for the number of acquisitions, the transmittance of the filters and the sensitivity of the camera, we conducted image analysis and data treatment as summarized schematically in figure 1. For all fluorescence images, a mask was delineated manually to determine the berry area and exclude non-berry pixels for subsequent data treatment. Fluorescence ratios for each berry pixel were computed. The fluorescence ratios computed were F440/F520, F440/F690, F440/F740, F520/F690, F520/F740, F690/F740, all induced by a UV light source, and UV epidermal transmittance measured at 690 nm (ET690) and at 740 nm (ET740) [Eq.1] induced by UV and blue light sources (Bilger *et al.* 1997).

$$ET_{\lambda} = \frac{F_{\lambda UV}}{F_{\lambda blue}} \quad [1]$$

where λ = the selected wavelength, either 690nm or 740nm
 $F_{\lambda UV}$ is the UV-induced fluorescence detected at $\lambda = 690nm$ or $740nm$
and $F_{\lambda blue}$ is the blue-induced fluorescence detected at $\lambda = 690nm$ or $740nm$

Once the fluorescence ratios were computed and the mask outlined, we computed the spatial average for each grape berry, resulting in one value per berry for each ratio and at each date of measurements. These values (or transformed ones when necessary) were used for the computation of the analysis of variance (ANOVA). Parameters presenting heterogeneous variances were transformed using a logarithmic, square root or Box-Cox transformation

(Peltier, 1998). The completely randomized ANOVA calculations were performed using the glm procedure from the SAS software (SAS Institute, NC, USA).

An edge detection algorithm was also conducted on each ratio image using the canny algorithm in the Image processing toolbox of Matlab (The Mathworks, v 7.0). The canny algorithm sets up two different thresholds in order to detect strong and weak edges, and includes the weak edges in the output only if they are connected to strong edges. For each image, we extracted the number of edge pixels and we computed an ANOVA on this parameter using the SAS software as described above.

Results and discussion

Gray mold development and incidence on grape berries

Because the malt agar inoculum plug showed a strong fluorescence at 440 nm, it was removed from the surface of both inoculated and control berries before the first fluorescence measurements, *i.e.* 3, 2 and 0.25 days after inoculation in the experiment A, B, and C, respectively. Consequently the visible development rate of *B. cinerea* was influenced **by the inoculation period**. For experiment C, the inoculum plug remained only 6 hours on the berries resulting in a slower *B. cinerea* colonization than in experiments A and B. For experiment A, six days after inoculation (6 DAI), all five berries expressed typical symptoms of gray mold. The corresponding digital images of control and symptomatic berries (Figure 2) showed i) on control berries, a circular scar darker and slightly bigger than the needle diameter; ii) on infected berries, a colonization process leading to a brown, rotted lesion surrounding a berry splitting from which exudates were leaking. On control berries, such a scar may include lignified tissue as well as plant phenolic compounds (Gonzalez-Barrio et al., 2005; Elad et al., 2004). For experiment B, at 5 DAI, 4 out of 5 inoculated berries developed symptoms of gray mold. In experiment C, four days after inoculation, typical *B. cinerea* symptoms expressed on all 5 inoculated berries. When compared with

control berries, Botrytised berries in this last experiment were characterized by a deeper berry split and a larger area of lignificated tissue surrounding the split area.

Fluorescence data based on spatial average over the berry area

A preliminary result was obtained by comparing Red-Green-Blue (RGB) images of grape berries under visible light (Fig 3a) and under long wavelength UV radiation (360 nm) (Fig 3b). On figure 3b, a strong blue fluorescence around the contaminated area was noticeable on the inoculated berry (Exp. C, 6 DAI). The fluorescence distribution, associated with a spreading lesion caused by *B. cinerea*, was consistent with observations on infected detached grapevine leaves (Langcake and McCarthy, 1979). The rotted area was surrounded by a band of blue fluorescence due to the presence of resveratrol in an apparently healthy zone of tissue up to 5mm in advance of the visibly rotted area (Langcake and McCarthy, 1979). However, on the control berry, a blue fluorescence was also detected clearly, although restricted at the wounded site (Fig 3b). Therefore, this could mislead a detection based on blue fluorescence solely.

In table 1 are summarized the fluorescence ratios calculated from the fluorescence images based on a spatial average over the berry area. The earlier detection made using fluorescence ratios was achieved at 3 DAI in experiment A. By using F_{520}/F_{690} or F_{520}/F_{740} , the differences between control and infected berries were significant at $P = 0.05$. By considering a significant result in all three experiments, the best detection was provided using F_{440}/F_{740} which increased in botrytised berries when compared to control ones. The earliest detection made using this fluorescence ratio was achieved at 4 DAI in experiment C. Ariana *et al* (2006) also observed an increase in F_{440}/F_{740} for black rot tissue on apples compared to healthy ones. Fluorescence ratios not involving blue-green fluorescence (from 440 nm to 520 nm) did not achieve successful detection of *Botrytis* using a spatial average over the berry area.

Furthermore, it was noticeable that the results calculated with F_{440} as the numerator showed, most often, significant ($P = 0.05$) differences between control and infected berries, *i.e.* in two or three of the experiments (Table 1). By looking at F_{440} fluorescence images (Fig. 4), it was detected on the control berries that the wound fluoresced. This was presumably due to the healing process, involving lignification and accumulation of plant phenolics at the point of wounding, because mechanical wounding in plants can induce rapid activation of genes that are involved in healing and defense against pathogens (Bowles, 1991). Such compounds fluoresce at 440 nm when exposed to UV-light (Machado *et al.*, 2001). Furthermore and although the capacity of berries to synthesize resveratrol decreases during ripening, stilbenic compounds should also be induced by the mechanical wounding in grape berries as also shown in leaves (Blaich and Bachmann, 1980; Bavaresco *et al.*, 1997; Pezet *et al.*, 2004). Furthermore, it was noticeable that the fluorescent area was almost constant in size and shape in control berries, whereas it was evolving in the infected ones. However, the central area within the developing lesion was no longer fluorescing at 440 nm when observed at 3, 4 or 6 DAI. This could be due to the fact that *B. cinerea* produces various extracellular enzymes to colonize berries, particularly laccases that can have digested plant phenolics, particularly the stilbenic compounds, originally present in the inner part of the expanding lesion (Sbaghi *et al.*, 1996; Van Baarlen *et al.*, 2004). The boundary of the colonized lesion showed a higher F_{440} intensity resulting presumably from the induction of defence mechanisms activated by the berry following *B. cinerea* infection and colonization. All these observations were totally in agreement with the results on grapevine leaves infected by *B. cinerea* (Derckel *et al.*, 1999). Once under a Botrytis attack, the plant reallocates some polyphenols, such as resveratrol and associated stilbenic oligomers, at the point of infection (Langcake and McCarthy, 1979; Pezet *et al.*, 2004). Thus, fluorescence observed at 440 nm clearly showed the colonized area by *B. cinerea*, but in the early infection stages (before 3 DAI), it can not be easily discriminated from any wound the berry might suffer (mechanical, insects, for instance).

Fluorescence data based on edge detection

Examples of earliest images of edge detection performed on UV-epidermal transmittance (ET690) are shown in Figure 5. In both experiments A and C, it was possible to detect exclusively the infected area when compared to (with ?) control berries. On control berries, the mechanical wound was not detected and no fluorescence pattern was observed, except for the berry surroundings only.

In Table 2, the proportion of edges detected from the fluorescence images was compared to the total berry area (evaluated by the mask delineation). In experiments A and C, but not in experiment B, infected berries showed a significantly ($P = 0.05$) lower proportion of edges detected than control berries. The results based on ET690 showed significant ($P = 0.05$) differences which were detected at early dates of assessment, *i.e.* 3 and 1 DAI in experiments A and C, respectively. ~~As for inoculated berries, the *Botrytis*-colonized area at the surface of the berry was easily detected by an increase in ET690.~~ In experiment C, the ~~de~~increase in ET690^[U1], associated with the *Botrytis*-colonized area, was detected as early as 6 hours after inoculation ~~and was significant at $P = 0.1$.~~ To account for this increase (decrease ?) in UV-epidermal transmittance (ET690)^[U2] it can be hypothesized that UV-absorbing compounds were catalyzed by laccases during the infection process enabling higher amount of UV-light getting^[U3] to chlorophyll. A similar mechanism was suggested to interpret a decrease in ET690 measured on crop leaf following nitrogen deficiency (Samson et al., 2000). The reduction of UV-induced chlorophyll fluorescence, compared to blue-induced chlorophyll fluorescence, was attributed to an accumulation of UV-absorbing secondary metabolites in the leaf. .

NB : Karavaev & Polyakova (2000) showed a decrease in the content of chlorophyll in leaves infected with *B. cinerea*. *Est ce une info incorporable ici en discussion ?*

Conclusion

In this study we evaluated the autofluorescence response of grape berries to a *B. cinerea* infection. Under UV-light, it was possible to detect the blue fluorescence of accumulated resveratrol and associated stilbenic compounds (Langcake and McCarthy, 1979; Pezet *et al.* 2004; van Baarlen *et al.* 2004). Although a strong signal, this blue fluorescence can be confounded with the fluorescence surrounding any mechanical wound on the berry. Some defence mechanisms, particularly the production by the berry of stilbenic phytoalexins, are not specific and can be activated by biotic as well as abiotic stresses? (e.g. hail, insects ...) (Blaich and Bachmann, 1980; Bavaresco *et al.*, 1997; Pezet *et al.* 2004; van Baarlen *et al.* 2004). This can be misleading in natural conditions, particularly in vineyards where insect injuries are associated with *B. cinerea* development on berries (Fermaud and Le Menn, 1992; Holz *et al.* 2004). Thus, by using measures of UV-induced fluorescence at 440 nm solely, the discrimination between wounded and botrytised berries could not be easily achieved. However, other interesting results based on UV-induced fluorescence measurements, such as the ratio F_{440}/F_{740} , could eventually be used in a handheld device. According to our results and using a spatial average of F_{440}/F_{740} over the berry area, the detection of berries infected by the fungus could be achieved starting as soon as 4 Days After Inoculation (DAI). The designed instrument should acquire UV-induced F_{440} and F_{740} fluorescence in a fast and efficient way. Further studies are already initiated by Cemagref (UMR ITAP), Montpellier, on a prototype built using LED, filters and photodiodes. If it can be implemented directly in the field, the detection of gray mold lesions as soon as 4 days after infection (DAI) can be considered as an early detection. Such a delay in detection would allow potential applications to be developed either i) in the vineyard, during the season, to improve control strategies and/or ii) at the beginning of the wine making process, to sort infected berries from healthy ones. In the field, the disease can be expressed on maturing berries during 1 or 2 months between the veraison stage and harvest, following a possible period of latency in the

herbaceous berries (Pezet *et al*, 2004). The visual detection of infected berries at or just after the veraison stage has to be improved, especially on black cultivars, because the early symptoms are not easily distinguished and a long incubation period is associated with these relatively resistant tissues (Elmer and Michailides, 2004). However, a delay in the detection should also be considered because the initial infectious foci develop often within the grape cluster and have to expand to the outer berries to become visible and easily detectable (Holz *et al.* 2004; Miklota *et al.*, 2003). Furthermore, our results may be useful to implement a more precise detection method at the beginning of the wine making process, when bunches are exposed individually on sorting tables. It can be assumed that fluorescence ratios, which were computed as spatial average over an area, or image analysis based on UV-epidermal transmittance (ET690) would be more rapid and adapted than visual assessments. The latter method could be more powerful than fluorescence ratios allowing berries recently rotted by *B. cinerea* to be detected as early as 1 day after infection.

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Table 1: Means of spatial average for each fluorescence ratio for control and infected berries in the three experiments (A, B and C) taken at (on ?) different dates post inoculation.

Ratio	Experiment A		Experiment B		Experiment C				
	Date of measure	3 DAI	6 DAI	2 DAI	5 DAI	0.25 DAI	1 DAI	4 DAI	6 DAI
F₄₄₀/F₅₂₀	Control	0.5756	0.5380	0.4485	0.4516	0.5249	0.5359	0.5498	0.7274
	Botrytis	0.6161	0.8167 ***	0.4658	0.5323 *	0.5183	0.5191	0.6451 **	0.5559 ***
F₄₄₀/F₆₉₀	Control	0.3663	0.3621	0.1653	0.1803	0.3199	0.3543	0.3473	0.3062
	Botrytis	0.3305	0.3647	0.1825	0.2493 **	0.3032	0.3392	0.6088 **	0.5687 **
F₄₄₀/F₇₄₀	Control	0.2403	0.2456	0.3890	0.4323	0.6475	0.7497	0.7641	0.7512
	Botrytis	0.4752 *	1.8437 ***	0.4258	0.6066 ***	0.6361	0.7217	1.3431 **	1.3862 *
F₅₂₀/F₆₉₀	Control	0.1321	0.1561	0.3796	0.4046	0.8319	0.8347	0.7721	0.7639
	Botrytis	0.2164 **	0.4416 ***	0.3930	0.4273	0.8331	0.8331	0.9291 ***	0.9002 **
F₅₂₀/F₇₄₀	Control	0.8898	1.0051	0.8901	0.9698	1.4959	1.4769	1.4537	1.4413
	Botrytis	1.3712 **	2.5876 ***	0.9068	1.0370	1.5079	1.5074	1.3162	1.1218 **
F₆₉₀/F₇₄₀	Control	2.3114	2.2626	2.3570	2.4083	2.0370	2.1276	2.2144	2.4728
	Botrytis	2.2804	2.2115	2.3201	2.4844	2.1312	2.1588	2.2166	2.4020
ET₆₉₀	Control	0.1757	0.1822	0.1552	0.1616	0.2020	0.1872	0.1932	0.1992
	Botrytis	0.1991	0.1919	0.1648	0.2016 *	0.1946	0.2054	0.2036	0.2378
ET₇₄₀	Control	0.1938	0.1962	0.1739	0.1731	0.2192	0.1968	0.2042	0.1985
	Botrytis	0.2060	0.2096	0.1866	0.2078 *	0.2072	0.2142	0.2160	0.2007

Where DAI=Days after inoculation

*, **, *** : significant at $P \leq 0.1, 0.05, 0.01$, respectively

Table 2: Means of edge proportion (%) detected for each fluorescence ratio for the three treatments, taken at different dates, for *exp.A, B, and C* data.

[U4]Edge proportion	Experiment A		Experiment B		Experiment C				
	Date of measure	3 DAI	6 DAI	2 DAI	5 DAI	0.25 DAI	1 DAI	4 DAI	6 DAI
F₄₄₀/F₅₂₀	Control	15.79	13.96	15.62	15.39	17.57	16.84	14.17	15.87
	Botrytis	14.17 *	16.09 **	15.72	15.27	13.69	13.58	13.21	11.82 **
F₄₄₀/F₆₉₀	Control	12.71	14.01	12.26	11.86	15.89	15.86	11.38	13.92
	Botrytis	11.74	11.78 **	14.71	12.93	12.41	12.55	11.74	10.79 ***
F₄₄₀/F₇₄₀	Control	15.03	15.75	14.56	13.87	18.12	17.69	14.39	14.01
	Botrytis	12.48 **	12.15 ***	15.90	13.46	15.01 *	15.13	14.24	12.46
F₅₂₀/F₆₉₀	Control	15.90	16.46	16.94	16.63	15.81	15.90	15.78	15.63
	Botrytis	15.03 **	13.43 ***	16.74	15.01	15.41	15.88	15.29	14.09 *
F₅₂₀/F₇₄₀	Control	17.76	18.20	17.29	17.68	18.78	18.37	18.03	14.77
	Botrytis	16.34 **	14.49 ***	17.78	16.02	18.15	18.15	17.11	14.80
F₆₉₀/F₇₄₀	Control	21.09	21.02	20.36	20.44	20.45	20.31	20.39	16.62
	Botrytis	19.63	19.39 ***	20.45	20.13	19.80 *	19.91	20.12	18.04
ET₆₉₀	Control	16.83	17.05	16.98	17.09	18.49	18.38	18.19	17.85
	Botrytis	15.25 ***	14.56 ***	16.81	15.42	17.47 *	17.09 **	17.72	16.42
ET₇₄₀	Control	21.14	21.17	20.18	20.41	20.93	20.72	20.71	19.42
	Botrytis	16.23	19.20 ***	20.58	19.07	20.75	20.41	20.26	17.34

Where DAI=Days after inoculation

*, **, *** = significant at $p \leq 0.1, 0.05, 0.01$.

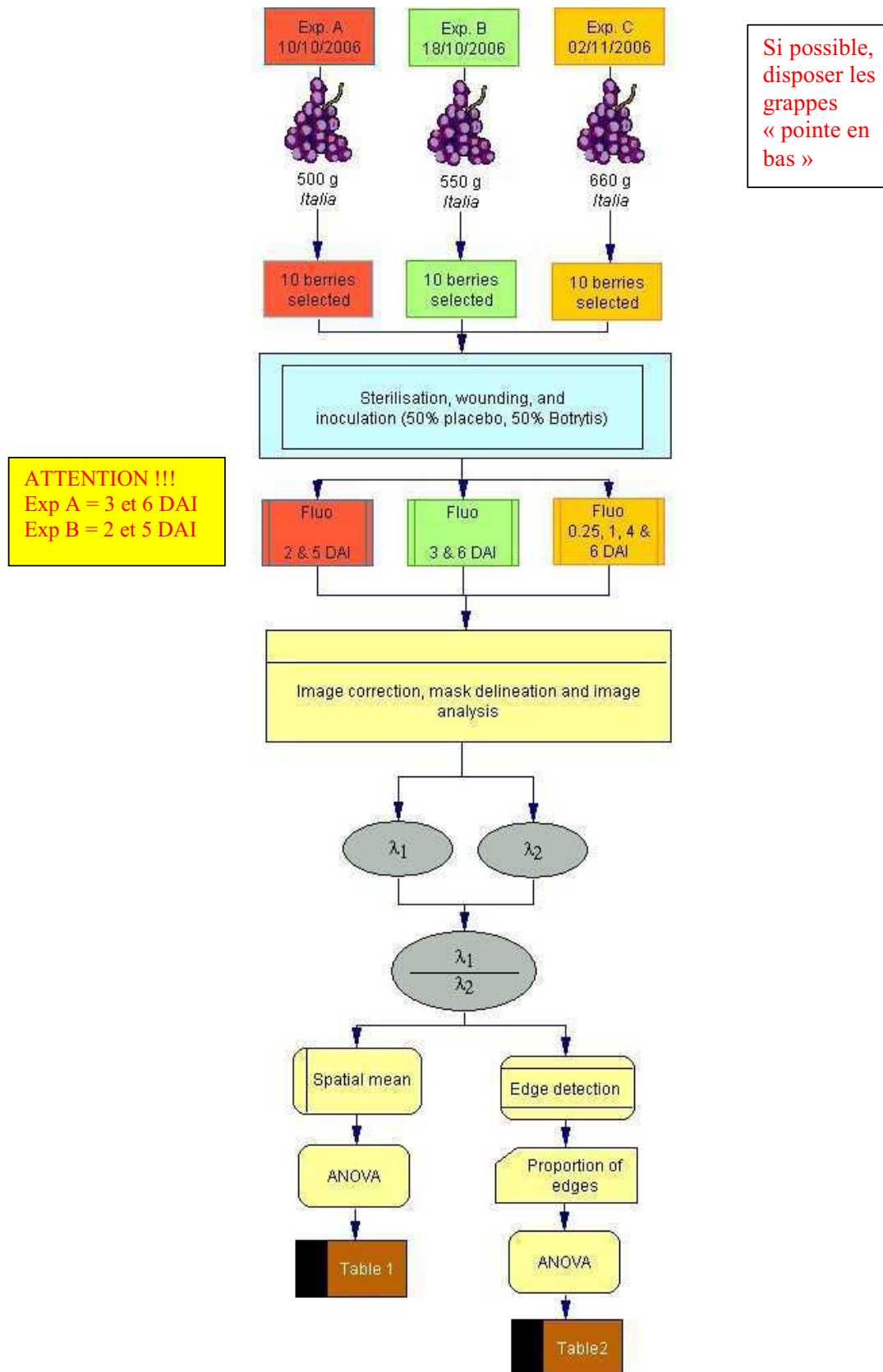


Fig. 1 Schematic representation of methodology and data analysis



Figure 2 : Digital picture of control berries (left) and botrytised berries (right)

Exp. A, 6 DAI

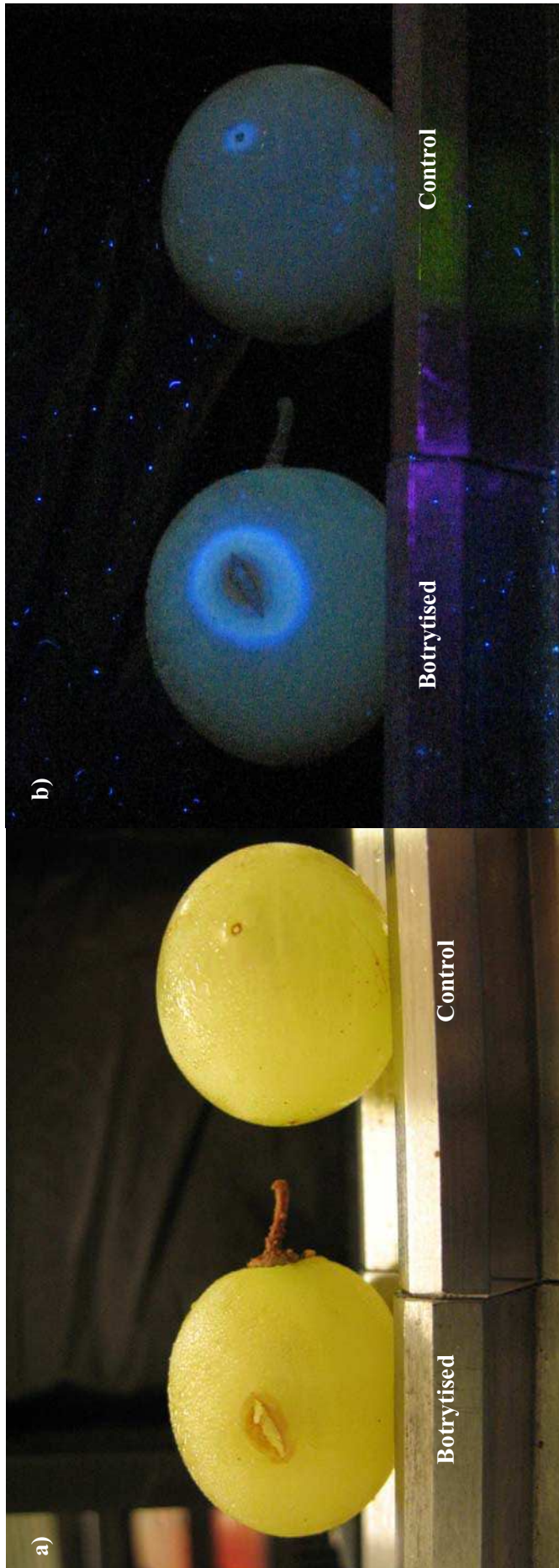


Figure 3: RGB digital images taken under a) visible light and b) UV-light (360 nm)

Exp. C, 6 DAI

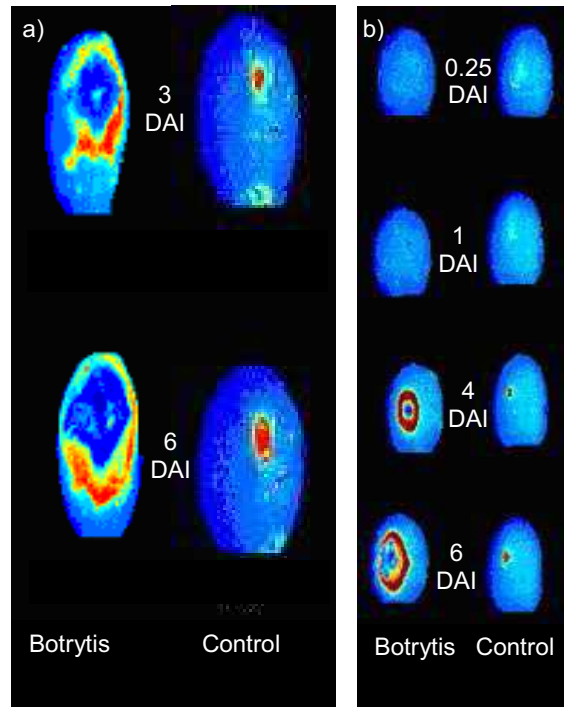


Figure 4 : UV-induced fluorescence images taken at 440 nm on a) experiment A botrytised and control berries, at 3 DAI and 6 DAI and b) experiment C botrytised and control berries, at 0.25, 1, 4 DAI and 6 DAI.

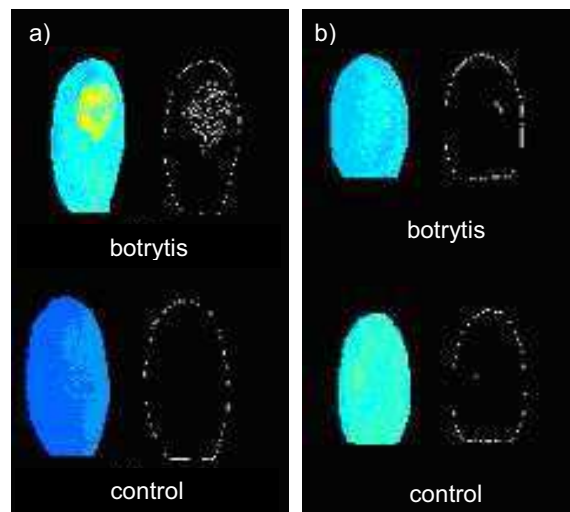


Figure 5 : ET690 fluorescence images and edges detected for each experiment, on the earliest dates of measurements a) Exp A 3 DAI b) Exp C, 0.25 DAI.

[U1] Nous ne comprenons pas comment une diminution de la mesure se traduit par une visualisation accrue du phénomène de fluorescence sur la fig. 5 (Peux tu éclairer notre lanterne ?)

[U2]A nouveau, c'est une diminution des chiffres qui est constatée dans le tableau 2, mais l'explication qui suit tient elle alors toujours ?

[U3]To get to ? N'y aurait il pas un autre verbe biologique plus adapté ? « enabling chlorophyll to be excited by UV radiations ?

[U4]Tab à uniformiser avec tab. 1 (voir mes précédents commentaires)