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Autofluorescence of grape berries following *Botrytis cinerea* infection

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Grey mould is caused by *Botrytis cinerea*, a fungus infecting over 200 plant species worldwide and causing tremendous harvest losses in vineyards. We evaluated the potential of plant fluorescence to achieve early detection of *B. cinerea* in grapevine (*Vitis vinifera* L.) berries. By comparing control and artificially inoculated berries, six images of fluorescence were taken at four wavelengths under 360 nm excitation (F_{440}/F_{360} (fluorescence emitted at 440 nm under a 360 nm excitation), F_{520}/F_{360}, F_{690}/F_{360} and F_{790}/F_{360}) and at two wavelengths under 436 nm excitation (F_{690}/F_{436} and F_{790}/F_{436}). Data treatments include proper image correction, interest area selection, fluorescence ratios computation and edge detection. In the early stages, before 3 DAI (days after inoculation), the discrimination between wounded and symptomatic berries could not easily be achieved using blue fluorescence solely (440 nm). However, using blue out of far-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 botrytized berries as early as 3 DAI.

1. Introduction

Grey mould of grapevines is caused by *Botrytis cinerea* Pers.:Fr., the anamorph of an ascomycete fungus (*Botryotinia fuckeliana* Whetzel). This cosmopolitan fungus is a pathogen attacking over 200 plant species, including the grapevine (Jarvis 1980). The estimated losses for French vineyards can account for up to 40% of the harvest (Mathys 1982), and the estimated grape losses due to *B. cinerea* represent 2 billion $US in the world per year (Elad *et al.* 2004). *B. cinerea* can drastically reduce both yield at harvest and wine quality, for all types of white and red wines (Ribéreau-Gayon *et al.* 1980, 1998, Marchal *et al.* 2001). In particular, botrytized wines are less acceptable because of mushroomy, mouldy and rotten smells even when the infection reaches low–medium levels (approximately 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, 1998).

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In grapevine, various infection pathways have been described to establish mechanistic relationships between the first attacks, occurring early in the season, and the typical subsequent infections of maturing berries (Elmer and Michailides 2004). In spring, *B. cinerea* can infect leaves, where it grows concentrically at the margin of the blade, and flowers which are highly susceptible, particularly dehiscent floral parts (Pezet *et al.* 2004). In summer, grey mould 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 (Elmer and Michailides 2004, Pezet *et al.* 2004). Berry infection results mostly from (i) conidia (airborne spores) germination and penetration, favoured by the presence of wounds at the surface of the fruit, in particular insect injuries (Fermaud and Le Menn 1992, Gabler *et al.* 2003, Holz *et al.* 2004); and (ii) spread by fungal vegetative filaments (mycelium) from one berry to the next, which has been associated with severe losses.

Control of grey mould in vineyards, as in various other crops, mostly requires the use of specific fungicides, but can also rely on modifications of cultivation practices (Elmer and Michailides 2004, Leroux 2004). Detection of *B. cinerea* can be performed by scouting in the vineyard, by using ELISA (Enzyme Linked Immunosorbent assay) techniques (Auger *et al.* 1995) or by relying on forecast models, often based on climatic data (Fermaud *et al.* 2003, Hruby 2003, Piéri and Fermaud 2005). As grey mould 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 to determine the fungal specific enzymatic laccase activity (Ribéreau-Gayon *et al.* 1998). Moreover, polyclonal and monoclonal antibody-based immunoassays have also been developed to detect *B. cinerea* in grape juice (Elad *et al.* 2004, Pezet *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 epidermis induces some defence reactions, which include the synthesis by the plant of phytoalexins and pathogenesis-related (PR) proteins (Gabler *et al.* 2003, Pezet *et al.* 2004). The major phytoalexins are resveratrol and related stilbenic compounds that 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, 48 h after inoculation, at the periphery of the lesion on leaves, but not inside the colonized tissue including dead cells (Derckel *et al.* 1999). Resveratrol is also produced in grape berries, but partly constitutively, and accumulates 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 (Schouten *et al.* 2002, Pezet *et al.* 2004). Laccases are inducible phenol-oxidizing enzymes catalysing 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 and Polyakova (2000) showed a decrease in the content of chlorophyll in leaves infected by *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.

Remotely sensed fluorescence has already been used to detect plants infected with fungi (Bélanger *et al.* 2008), viruses (Pineda *et al.* 2008) and bacteria (Berger *et al.* 2004). These studies mainly focused on plant leaves and concluded notably that the infection modified their photosynthetic efficiency. An increase in F_{440} was also noted in plants infected with fungi. This fluorescence was produced either by fungus autofluorescence (Lüdeker *et al.* 1996) or by the plant's defence mechanism (Niemann *et al.* 1991). Pineda *et al.* (2008) detected an increase in two fluorescence ratios (F_{440}/F_{690} and F_{440}/F_{740}) during pathogenesis. Bélanger *et al*. (2008) detected an increase in the spatial average of F_{440}/F_{520}. In order to detect the spatial heterogeneity between infected and control leaves, they performed an image analysis using an edge detection algorithm.

In our study, we have evaluated the autofluorescence response of mature grape berries to *B. cinerea* infection in controlled conditions and assessed the potential of this to achieve early detection. 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.

2. Material and methods

2.1 *Grape berries*

Mature grape (*Vitis vinifera* L.) bunches 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-sterilized utility knife. Each berry was surface-sterilized by immersion in 95% denatured ethyl alcohol for 30 s. They were then soaked and rinsed thoroughly using demineralized water.

2.2 *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-ISVV, 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).

For inoculation, mycelial plugs (4 mm in diameter) were taken, using a fire-sterilized punch, from pure cultures on malt agar plates incubated at 20°C (the inoculum was transplanted to a new malt agar Petri dish on 18 September 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 10 October (Experiment A), five on 18 October (Experiment B) and five on 24 November 2006 (Experiment 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 acting as a humid chamber ($20 \times 15 \times 5$ cm). 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 (approximately 21°C) under natural daylight. The incubation period corresponded to the time between the inoculation and the first fluorescence measurements, that is, 3, 2 and 0.25 days for Experiments A, B and C, respectively.

2.3 *Fluorescence measurements*

Fluorescence measurements were performed exactly 3 and 6 days after inoculation (DAI) for Experiment A, 2 and 5 DAI for Experiment B and 0.25, 1, 4 and 6 DAI for Experiment C. Fluorescence measurements were carried out using an imaging fluorometer as described by Belzile et al. (2003). The FLIM-100 imaging fluorometer used an interline charged-coupled device (CCD) camera (CoolSnapHQ, RoperScientific, Tucson, AZ, USA) modified for on-chip accumulation capability. The fluorescence was induced by a xenon flash lamp source (Hamamatsu, Middlesex, NJ, USA) producing intense light pulses at 50 Hz repetition rate (4 μs pulse width) using a pulse generator (model 500B, Berkeley Nucleonics Corporation, Orange, CA, USA). For plant leaf fluorescence applications, UV (360 ± 40 nm) and blue (436 ± 20 nm) excitation wavelengths were selected using band pass filters (Chroma Technology Corp., Bellows Falls, 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 μs 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 and 2500 acquisitions depending on the sample and on detected wavelength. Four narrow band pass filters (CVI Laser, Albuquerque, NM, USA) were placed in front of the camera allowing precise detection of 440, 520, 690 and 740 ± 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% denatured ethyl alcohol (beforehand and between each measurement). Grape berries were 50 cm away from the excitation source, thus effective excitation area was approximately 20 cm^2. All fluorescent measurements were done in the dark.

2.4 *Image and data analysis*

After proper image correction (Bélanger *et al.* 2008), 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

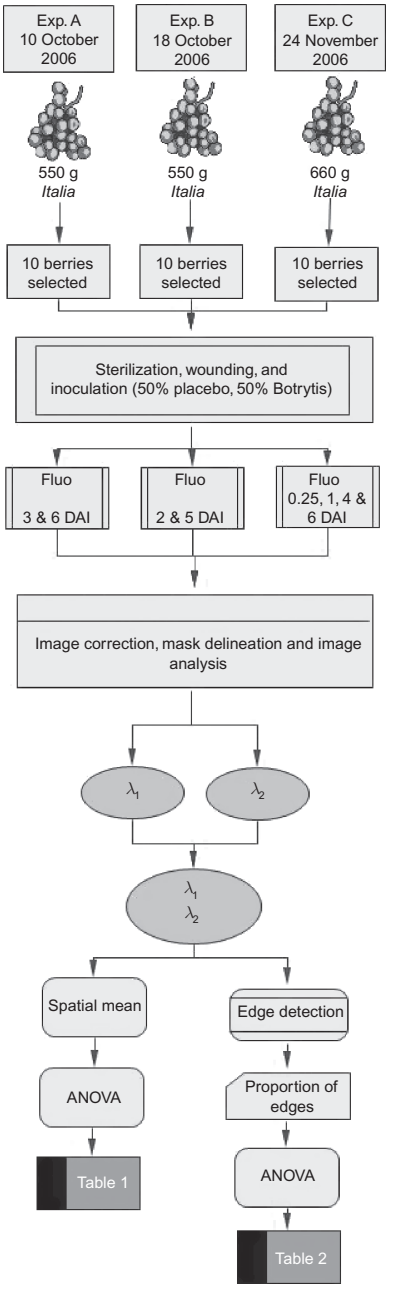

Figure 1. Schematic representation of the methodology and data analysis.

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 F_{440}/F_{520}, F_{440}/F_{690}, F_{440}/F_{740}, F_{520}/F_{690}, F_{520}/F_{740} and F_{690}/F_{740}, all induced by a UV light source, and UV epidermal transmittance measured at 690 nm

(ET_{690}) and at 740 nm (ET_{740}) (equation(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 is the selected wavelength, either 690 or 740 nm, $F_{\lambda UV}$ is the UV-induced fluorescence detected at $\lambda = 690$ or 740 nm and $F_{\lambda blue}$ is the blue-induced fluorescence detected at $\lambda = 690$ or 740 nm.

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 *et al.* 1998). The completely randomized ANOVA calculations were performed using the glm procedure from the SAS software (SAS Institute, Cary, 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, Natick, MA, US). 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.

3. Results and discussion

3.1 *Grey mould 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, that is, 3, 2 and 0.25 days after inoculation in Experiments A, B and C, respectively. Consequently, the visible development rate of *B. cinerea* was influenced by the inoculation period. In Experiment C, the inoculum plug remained only 6 h on the berries, resulting in a slower *B. cinerea* colonization than in Experiments A and B. In Experiment A, 6 days after inoculation (6 DAI), all five berries expressed typical symptoms of grey mould. In Experiment B, at 5 DAI, four out of five inoculated berries developed symptoms of grey mould. In Experiment C, 4 DAI, typical *B. cinerea* symptoms expressed on all five inoculated berries. 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; and (ii) on infected berries, a colonization process leading to a brown, rotted lesion surrounding a berry split from which exudates were leaking. On control berries, such a scar may include lignificated tissue as well as plant phenolic compounds (Elad *et al.* 2004, Gonzalez-Barrio *et al.* 2005). When compared with control berries, botrytized berries in this last experiment were characterized by a deeper berry split and a larger area of lignificated tissue surrounding the split area.

3.2 *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 (figure 3(*a*)) and under long wavelength UV radiation

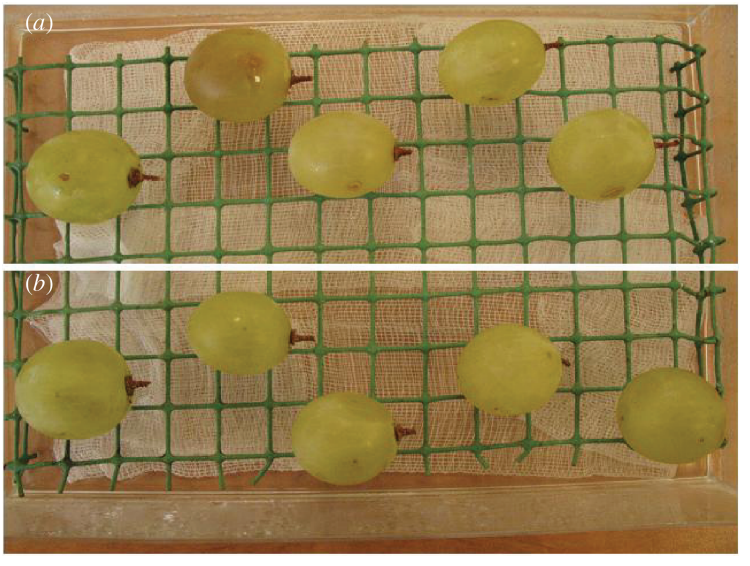

Figure 2. Photographs of (*a*) botrytized berries and (*b*) control berries taken from Experiment C at 4 DAI.

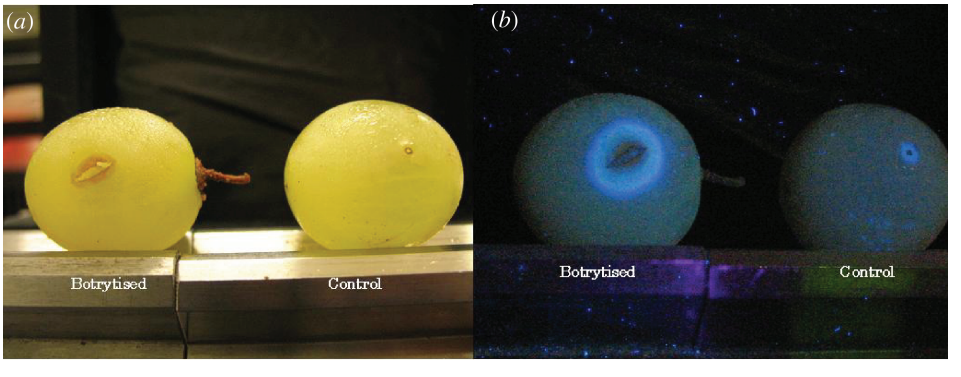

Figure 3. RGB digital images taken under (*a*) visible light and (*b*) UV-light (360 nm) from Experiment C at 6 DAI.

(360 nm) (figure 3(*b*)). In figure 3(*b*), a strong blue fluorescence around the contaminated area was noticeable on the inoculated berry (Experiment 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 5 mm 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 (figure 3(*b*)). Therefore, this could mislead a detection based on blue fluorescence solely.

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

	Experiment A		Experiment B		Experiment C			
	3 DAI	6 DAI	2 DAI	5 DAI	0.25 DAI	1 DAI	4 DAI	6 DAI
F_{440}/F_{520}								
Control	0.5756	0.5380	0.4485	0.4516	0.5249	0.5359	0.5498	0.5559
Infected	0.6161	0.8167***	0.4658	0.5323*	0.5183	0.5191	0.6451**	0.7274***
F_{440}/F_{690}								
Control	0.3663	0.3621	0.1653	0.1803	0.3199	0.3543	0.3473	0.3062
Infected	0.3305	0.3647	0.1825	0.2493**	0.3032	0.3392	0.6088**	0.5687**
F_{440}/F_{740}								
Control	0.2403	0.2456	0.3890	0.4323	0.6475	0.7497	0.7641	0.7512
Infected	0.4752*	1.8437***	0.4258	0.6066***	0.6361	0.7217	1.3431**	1.3862*
F_{520}/F_{690}								
Control	0.1321	0.1561	0.3796	0.4046	0.8319	0.8347	0.7721	0.7639
Infected	0.2164**	0.4416***	0.3930	0.4273	0.8331	0.8331	0.9291***	0.9002**
F_{520}/F_{740}								
Control	0.8898	1.0051	0.8901	0.9698	1.4959	1.4769	1.4537	1.4413
Infected	1.3712**	2.5876***	0.9068	1.0370	1.5079	1.5074	1.3162	1.1218**
F_{690}/F_{740}								
Control	2.3114	2.2626	2.3570	2.4083	2.0370	2.1276	2.2144	2.4728
Infected	2.2804	2.2115	2.3201	2.4844	2.1312	2.1588	2.2166	2.4020
ET_{690}								
Control	0.1757	0.1822	0.1552	0.1616	0.2020	0.1872	0.1932	0.1992
Infected	0.1991	0.1919	0.1648	0.2016*	0.1946	0.2054	0.2036	0.2378
ET_{740}								
Control	0.1938	0.1962	0.1739	0.1731	0.2192	0.1968	0.2042	0.1985
Infected	0.2060	0.2096	0.1866	0.2078*	0.2072	0.2142	0.2160	0.2007

Notes: DAI, days after inoculation;
*, **, ***, significant at $p \leq 0.1$, 0.05, 0.01, respectively.

Table 1 summarizes 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 $p \leq 0.05$. By considering a significant result in all three experiments, the best detection was provided using $F_{440}/F_{740,}$ which increased in botrytized 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) and Pineda *et al.* (2008) both observed an increase in F_{440}/F_{740}, respectively, for black rot tissue on apples compared to healthy ones and for plants infected with viruses. Fluorescence ratios not involving blue-green fluorescence (from 440 to 520 nm) did not achieve successful detection of *B. cinerea* using a spatial average over the berry area.

It was noticeable that the results calculated with F_{440} as the numerator showed, most often, significant ($p \leq 0.05$) differences between control and infected berries, that is, in two or three of the experiments (see table 1). By looking at F_{440} fluorescence images (figure 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

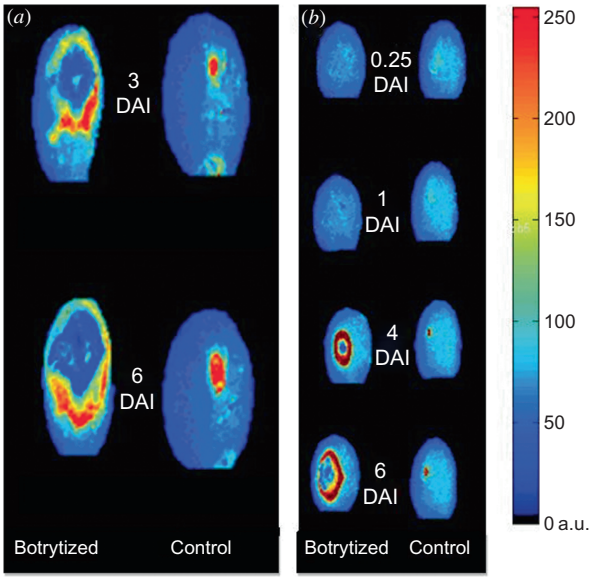

Figure 4. UV-induced fluorescence images taken at 440 nm on (*a*) Experiment A botrytized and control berries, at 3 DAI and 6 DAI and (*b*) Experiment C botrytized and control berries, at 0.25, 1, 4 DAI and 6 DAI.

point of wounding, because mechanical wounding in plants can induce rapid activation of genes that are involved in healing and defence 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 synthetize 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). Moreover, 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, principally 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 *B. cinerea* 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 area colonized by *B. cinerea*, but in the early infection stages (before 3 DAI), it cannot be easily discriminated from any wound the berry might suffer (mechanical or insects, for instance).

3.3 *Fluorescence data based on edge detection*

Examples of the earliest images of edge detection performed on UV-epidermal transmittance (ET_{690}) are shown in figure 5. In both Experiments A and C, it was possible to detect exclusively the infected area when compared 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 is compared to the total berry area (evaluated by the mask delineation). Generally, infected berries showed a significantly ($p \leq 0.05$) higher proportion of edges detected than control berries. The results based on ET_{690} showed significant ($p \leq 0.10$) differences, which were detected at early dates of assessment, that is, 3 and 0.25 DAI in Experiments A and C, respectively.

For inoculated berries, the *B. cinerea* colonized area at the surface of the berry was easily detected by an increase in ET_{690}, resulting in a higher proportion of edge detected. As presented in equation (1), the epidermal transmittance is the ratio of two fluorescences measured at 690 nm, one induced by UV light and the other one induced by blue light but both fluorescences coming from chlorophyll only. The influence of UV-absorbing compound such as resveratrol or other plant phenolics on ET_{690} is not caused by their fluorescence but by their absorption of UV light. Samson *et al.* (2000) determined that nitrogen deficiency induced an accumulation of UV-absorbing secondary metabolites in the leaf. Therefore, if there is an accumulation of UV-absorbing compounds the amount of UV light reaching chlorophyll will be lower, thus reducing the chlorophyll fluorescence induced by UV light, thus decreasing ET_{690}. Considering our botrytized berries, the opposite occurred. It can be hypothesized that the laccase secreted by *B. cinerea* during the infection process catalysed the UV-absorbing compounds of the berries, therefore increasing the amount of UV light reaching the berry chlorophyll and increasing ET_{690}.

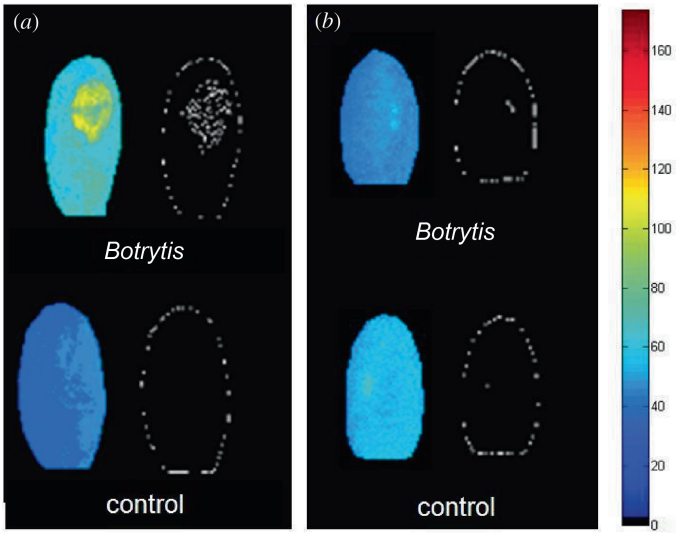

Figure 5. ET_{690} fluorescence images and edges detected for each experiment, on the earliest dates of measurements: (*a*) Experiment A, 3 DAI, (*b*) Experiment C, 0.25 DAI.

Table 2. Means of edge proportion (%) detected for each fluorescence ratio for the three treatments, taken on different dates after inoculation, for Experiments A, B and C data.

	Experiment A		Experiment B		Experiment C			
	3 DAI	6 DAI	2 DAI	5 DAI	0.25 DAI	1 DAI	4 DAI	6 DAI
F_{440}/F_{520}								
Control	8.13	8.00	8.60	10.08	11.30	7.55	9.08	10.50
Infection	13.88**	12.70***	9.24	12.59	8.10	8.17	8.64	10.08
F_{440}/F_{690}								
Control	5.03	5.53	4.81	4.89	6.15	6.48	6.25	6.05
Infection	11.87***	10.94***	4.83	8.78*	8.77	8.26	8.53	8.82
F_{440}/F_{740}								
Control	7.72	8.00	8.55	9.92	15.56	14.13	13.54	14.74
Infection	12.23**	9.52	8.19	11.37	10.69	11.44	6.10**	7.08**
F_{520}/F_{690}								
Control	5.20	6.25	7.54	8.25	6.96	6.83	7.86	8.47
Infection	12.02***	12.49***	7.43	10.25	6.10	7.03	6.58	6.43
F_{520}/F_{740}								
Control	7.77	4.51	7.72	7.48	3.76	2.50	2.53	2.57
Infection	5.79	4.54	8.46	7.14	4.66	3.56	2.79	4.76
F_{690}/F_{740}								
Control	2.00	1.98	2.17	2.15	2.39	2.40	2.39	2.38
Infection	2.08	2.04	2.01	1.99	2.33	2.38	2.41	2.38
ET_{690}								
Control	2.40	2.57	2.54	2.51	2.61	2.51	2.48	2.70
Infection	4.66***	5.81***	2.94	5.43**	3.00*	3.05**	3.61**	4.20*
ET_{740}								
Control	4.77	2.64	2.71	2.90	11.40	10.99	7.62	10.41
Infection	6.64	9.06***	3.86	8.00**	7.73	11.76	12.26	15.85

Notes: DAI, days after inoculation;
*, **, ***, significant at $p \leq 0.1$, 0.05, 0.01, respectively.

When *B. cinerea* infects a grape berry, the first reaction of the berry is to produce resveratrol to refrain the fungal colonization. Therefore, one can see an increase in the fluorescence measured at 440 nm located specifically on the margin of the colonization front. But when *B. cinerea* truly colonizes the berry, it can catalyse the resveratrol and other phenolic compounds, thanks to the laccase *B. cinerea* produces. Because the resveratrol and the other phenolic compounds have been destroyed, there is a decrease in UV light absorption and also in the fluorescence emitted at 440 nm (in the truly colonized area). This is why when we look at figure 3, for instance, we clearly see a blue halo at the margin of the colonization front whereas there is no blue fluorescence emitted from the centre of the colonization area, where *B. cinerea* could excrete its laccase and digest the resveratrol.

4. 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 of stilbenic phytoalexins by the berry, are not specific and can be activated by biotic as well as abiotic stresses (e.g. hail or 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 only 440 nm, discrimination between wounded and botrytized 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 DAI. The designed instrument should acquire UV-induced F_{440} and F_{740} fluorescence in a fast and efficient way. Further studies have already been initiated by Cemagref (UMR ITAP), Montpellier, on a prototype built using light emitting diodes (LEDs), filters and photodiodes. If it can be implemented directly in the field, the detection of grey mould lesions as soon as 4 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 order to discriminate between infected berries and healthy ones, possible threshold values for use by growers should be further investigated and worked out either in the field or at the beginning of the wine making process on sorting tables. From our data and for the ratio F_{440}/F_{740} (table 1), a critical value between, for example, 0.8 and 1.0 could be proposed. However, such a value should be based on more experimental data obtained under various conditions.

Lastly, 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 (Gabler *et al.* 2003, Holz *et al.* 2004). 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 (ET_{690}) 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 possibly as early as 3 days after infection.

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