As a protection mechanism, grapevine produces stilbene phytoalexins. The role of stilbenes in the resistance of grapevine to *P. viticola* is well documented (Jeandet *et al*., 2002; Chong *et al*., 2009; Jeandet *et al*., 2010), but it is still not fully elucidated. Stilbenes show a bright UV-induced violet–blue fluorescence (VBF) (Hillis and Ishikura, 1968) that can be measured *in vivo* on grapevine leaves (Langcake and McCarthy, 1979; Dai *et al*., 1995*a*, *b*). Indeed, under UV illumination, grapevine leaves are known to emit three types of autofluorescence: (i) the red fluorescence of chlorophyll (ChlF); (ii) the blue-green fluorescence (BGF) assigned to hydroxycinnamic acids (Cerovic *et al*., 1999; Pfündel *et al*., 2006); and (iii) a VBF of induced stilbenes in inoculated grapevine leaves (Poutaraud *et al*., 2007; Bellow *et al*., 2012). In a previous study (Bellow *et al*., 2012), confocal spectral microscopy was used for *in vivo* localization at the cellular level of stilbene fluorescence induced by *P. viticola* in grapevine leaves. Compartmentation differed between cultivars (resistant versus susceptible). Moreover, it was shown that due to microenvironment effects, compartmentation should strongly affect the fluorescent yield of stilbenes.

Autofluorescence can therefore be used as a non-destructive indicator of the presence of infection. On the one hand, fluorescence imaging is a particularly useful technique to assess autofluorescence changes due to stilbene accumulation in grapevine leaves at both macroscopic (Poutaraud *et al*., 2007) and microscopic scales (Poutaraud *et al*., 2007; Bellow *et al*., 2012). On the other hand, laboratory spectrofluorimetry allows an accurate and quantitative assessment of the intrinsic spectral signature of stilbenes that has been correlated to the stilbene content of leaves analysed by HPLC (Poutaraud *et al*., 2007). However, there is no report yet on the behaviour of stilbene VBF during the full course of *P. viticola* infections until the late visible symptoms (oily spots).

The aims of this study were 2-fold: (i) to characterize on a daily basis the kinetics of autofluorescence in plant-attached grapevine leaves responding to *P. viticola* infection during at least 2 weeks following their inoculation until characteristic visible symptoms are established; and (ii) to analyse different fluorescence indices as the basis for downy mildew diagnosis in the field. To reach these goals, the monitoring of BGF, VBF, ChlF, and blue-excited green fluorescence (GF) at three spatial scales was performed simultaneously: imaging by fluorescence macroscopy (submillimetric), spectrofluorimetry (millimetric), and proximal sensing (whole-leaf scale) with two sensors, the Multiplex 3 sensor (Ben Ghozlen *et al*., 2010) and a proximal fluorescence sensor prototype with short-wave UV sources.

Materials and methods

Plants of *Vitis vinifera* cv. Cabernet Sauvignon (a genotype susceptible to *P. viticola*) were grown from cuttings in Colmar (France) at 22 ± 3 °C, 13/11 h light/dark in the greenhouse. The study was started when plants attained the stage of ~15 leaves. The plants were settled outdoors next to the greenhouse in a place that was never in the shade in Colmar (latitude 48°05N, longitude 07°20E) in August 2010 for 15 d. This outdoor regimen guaranteed that the plants which were initially grown in the greenhouse protected from biotic or abiotic stresses had a flavonol content equivalent to vineyard leaves at the moment of inoculation (Kolb *et al*., 2001). As the constitutive flavonol have been shown to participate in the resistance of grapevine leaves to *P. viticola* (Agati *et al*., 2008), the outdoor treatment was fundamental for the results to be applicable to vineyard-grown leaves. *Plasmopara viticola* was obtained from naturally infected plants in Colmar. Sporangia were periodically grown in order to prepare inoculum. Sporangia were diluted in distilled water, counted, and then adjusted to a concentration of $\sim 5 \times 10^4$ sporangia ml^{-1}. The fifth fully expanded leaf counted from the apex, still attached to the plant, was inoculated. Three protocols of inoculation were used depending on the experiment. For protocols 1 and 2, the shoot was positioned on a horizontal surface and the leaf laid flat with the abaxial side upwards. For fluorescence macroscopy, protocol 1, five drops of 12 µl of inoculum suspension and five drops of distilled water (control) were applied on the abaxial side of each leaf. For spectrofluorimetry, protocol 2, six drops of 200 µl of inoculum suspension and three drops of distilled water were applied on the abaxial side of each leaf. For proximal sensing, protocol 3, nine fifth leaves of different plants were each totally immersed in a test tube containing 60 ml of inoculum suspension. After 5 h of incubation, leaves were cleaned with distilled water and superficially dried. Plants were then transported (by land transport, in humidified portable mini-greenhouses) from Colmar to Gif-sur-Yvette (France) for fluorescence macroscopy, and to Orsay (France) for spectrofluorimetry and proximal sensing. During the 15 d of experiments, except when being measured, plants were maintained either in their humidified portable mini-greenhouses inside a growth chamber (22 °C, 13/11 h light/dark) or directly in the greenhouse for proximal sensing. The success of infection was attested to by the presence of sporulation on leaves kept in a humid environment, or by oily spots (without sporulation) on other leaves. All measurements were done with leaves attached to the plant.

Fluorescence imaging

Images were acquired using a macroscope (AZ100 multizoom, Nikon, Champigny-sur-Marne, France) equipped with a 130 W metal halide lamp white source (Intensilight, Nikon) and a high-resolution colour camera (Ds-Ri, Nikon). The UV-suppression filter of this source was removed. The images of UV-excited visible autofluorescence were recorded using a custom-made filter block from AHF (Tübingen, Germany) with an excitation bandpass filter 340/26 (FF01 Brightline, Semrock, Rochester, NY, USA), a dichroic filter Q380LP (Chroma Technology Corp., Bellows Falls, VT, USA), and a long-pass 371 nm emission filter (LP02-364RS, Semrock). The images of blue-excited green autofluorescence were recorded using a GFP-B filter set (excitation band pass filter 472/30, dichroic filter 495 nm, and emission bandpass filter 520/35, Nikon). A ×2 objective (NA 0.2, working distanced 45 mm, AZ-Plan Fluor, Nikon) was used, and 24-bit RGB colour images were acquired with a 1284 × 1024 pixel resolution. Imaged leaves, still attached to the plant, were carefully flattened (abaxial side facing the objective) on the glass sample holder (adaxial side lightly moistened for adhesion). The flatness of the imaged area was necessary for a good-quality acquisition. Sporangiophores were washed from the sporulating leaves to avoid their contribution to VBF. Image acquisition was performed using the NIS-Elements software (Nikon). Image analysis, including composition, was performed using the software ImageJ (http://rsbweb.nih.gov/ij/). The presented data are representative results of several experiments.

Spectrofluorimetry

Excitation and emission fluorescence spectra were acquired with a spectrofluorimeter (Cary Eclipse, Varian, Les Ulis, France) using a configuration adapted to attached leaves based on a double-arm optical fibre bundle (C Technologies, Cedar Knolls, NJ, USA) made of 147 randomized fibres. The two arms of the bundle were coupled to the excitation and emission part of the spectrofluorimeter via a fibre-optic coupler accessory provided by Varian (part no. FA-VAR00-AP15). The common part of the fibre bundle was maintained at a fixed distance (5 mm) from the samples by a proprietary clip. Under these conditions, every day from 1 DPI to 15 DPI at about the same time, the spectra of the same marked circular regions (diameter 5.5 mm) of the abaxial side of