each leaf were recorded. Excitation spectra were corrected with a calibrated photodiode (S1337-1010BQ, Hamamatsu, Massy, France), and emission spectra were corrected using a standard lamp with a known spectrum (LI-COR 1800–02, LI-COR, Lincoln, NE, USA) as described in detail previously (Louis *et al*., 2006). In addition, fluorescence was expressed in quinine sulphate equivalent units (QSEU) (Cerovic *et al*., 1999): 1000 QSEU correspond to the fluorescence of 1 µM quinine sulphate dihydrate in 0.105 M perchloric acid for 1 cm light path square cells or, in general, the fluorescence of 1 nmol cm^{-2} of this standard excited at 347.5 nm and emitting at 450 nm under the identical conditions used to acquire the sample fluorescence spectrum.

Proximal sensing

Multiplex® 3 (FORCE-A, Orsay, France) is a hand-held, multiparametric fluorescence sensor based on light-emitting diode excitation and filtered photodiode detection that is designed to work in the field under daylight conditions (for detailed description and specifications, see Ben Ghozlen *et al*., 2010). In the present investigation, the Multiplex 3 (with a 6 cm diameter measuring area) was used for daily measurements of the abaxial and adaxial sides of infected (protocol 3) leaves (fifth from the apex) and control leaves (sixth from the apex).

Several Multiplex 3 indices were followed: (i) SFR_R, a chlorophyll fluorescence emission ratio linked to the leaf chlorophyll content; (ii) R-590, the leaf reflectance at 590 nm, named YF_G in the Multiplex 3, that also reflects changes in leaf chlorophyll content, increasing with chlorophyll content decrease; (iii) FER_RG, a chlorophyll fluorescence red-to-green (635/516 nm) excitation ratio originally designed for fruit anthocyanin content, but which is here inversely correlated to the chlorophyll content of leaves devoid of anthocyanins; (iv) FLAV, a chlorophyll fluorescence red-to-UV (635/375 nm) excitation ratio that is a measure of epidermal flavonols; and (v) YF_UV, the 375 nm excited yellow autofluorescence (590 nm, FWHM 10 nm) of the leaves.

The prototype of a new proximal sensor, the Mx-330 (FORCE-A, Orsay, France), was used to measure daily the *in vivo* VBF of the abaxial and adaxial sides of infected (protocol 3) leaves (fifth from the apex) and control leaves (sixth from the apex), in parallel with the Multiplex 3 measurements (the same region of the leaf). The Mx-330 sensor was based on the Multiplex 3 design (mechanical structure and electronics) but specifically adapted to measure *in vivo* the stilbene VBF on grapevine leaves (335 nm excitation–400 nm emission). The sensor illuminates a 6 cm diameter surface at a 4 cm distance from the source and detectors. The leaves were flattened as much as possible during the measurements.

Statistical analyses

Statistical analyses were performed using the software Statistica 6.1 (StatSoft Inc., Maison-Alfort, France). As normality (Shapiro–Wilk test) or homoscedasticity (Levene test and Brown–Forsythe test) were often violated, the analysis of variance (ANOVA) could not be used. Instead, the significance of the difference between means was assessed by three non-parametric tests: a Mann–Whitney U-test, a Wald–Wolfowitz runs test, and a Kolmogorov–Smirnov test. The three non-parametric tests usually produced the same results. In the event of discordance, the result of the most stringent test was retained and presented.

Results

Daily imaging of autofluorescence of a grapevine leaf infected by P. viticola

For each treatment (inoculated or control), similar results were obtained in five regions. Images presented in Fig. 1D–F are representative of an inoculated region occupied by one inoculum drop of 12 µl followed during 15 d. The first tiny necrotic spots could be seen on transmission images in the middle of infected areoles at 3 DPI (Fig. 1D). A thickening and an extension of the necrotic spots were observed on the following days. At 7 DPI, a yellowing of the abaxial surface was observed due to a local decrease of the chlorophyll content in leaf tissues (Fig. 1D). This chlorosis of infected areoles increased daily during the remaining course of the survey. At 15 DPI, which corresponds to the end of the survey, the chlorosis appeared clearly limited by veins of infected areoles. The UV-induced autofluorescence images revealed a few spots of VBF at 1 DPI (Fig. 1E). At 2 DPI, these early spots of VBF were no longer visible. At 3 DPI, infected areoles displayed an intense VBF. This signal reached a maximum intensity at 5 DPI and then decreased at 7 DPI and remained at a low but noticeable level for the rest of the survey. From 5 to 15 DPI, the VBF appeared clearly limited to the infected areoles. The chlorosis and the stilbene VBF appeared co-localized. Bright GF spots also appeared at 3 DPI (Fig. 1F). A larger and more diffuse GF was present at 7 DPI in infected tissue, but also restricted to infected areoles. Thereafter, GF covered a region (Fig. 1F) seemingly superimposed upon the chlorotic region (Fig. 1D). From 7 DPI, GF progressively increased until the end of the survey. Images of control regions displayed no changes during the whole survey. Transmission images remained green due to the presence of chlorophyll, with the exception of white veins (Fig. 1A), and no variation was observed on fluorescence images (Fig. 1B, C). The UV-induced visible fluorescence of the abaxial side of control regions appeared purple due to the mix between the red ChlF abundant in the mesophyll and the BGF of constitutive phenolic compounds present in epidermal cell walls (Fig. 1B). The GF displayed no variation in the control region (Fig. 1C).

Daily kinetics of autofluorescence measured by spectrofluorimetry at the abaxial side of grapevine leaf infected by P. viticola

Changes in both BGF excitation and emission spectra associated with the *P. viticola* infection have been investigated at the abaxial side of a leaf from 1 to 15 DPI (Fig. 2). The spectra were obtained from samples independent of but comparable with those used for images presented in Fig. 1. The results shown are means of measurements on six infected regions and three control regions. From 1 to 12 DPI on control regions (Fig. 2B) and at 1 and 2 DPI on the inoculated regions (Fig. 2A), fluorescence excitation and emission spectra had the usual shape of control grapevine leaves (Cerovic *et al*., 1999; Poutaraud *et al*., 2007). From 4 DPI onwards, a second peak emerged at 330 nm on fluorescence excitation spectra of inoculated regions due to the accumulation of stilbenes (Fig. 2A). In fluorescence emission spectra excited at 330 nm, this resulted in a hypsochromic shift of the maximum emission from 440 nm to 400 nm (Fig. 2A). These spectral signatures that reflect the accumulation of stilbenes (Poutaraud *et al*., 2007; Bellow *et al*., 2012) were observed from 4 to 15 DPI on the inoculated region and the two last DPI, 14 and 15, on control regions (Fig. 2B). It should be noted here that