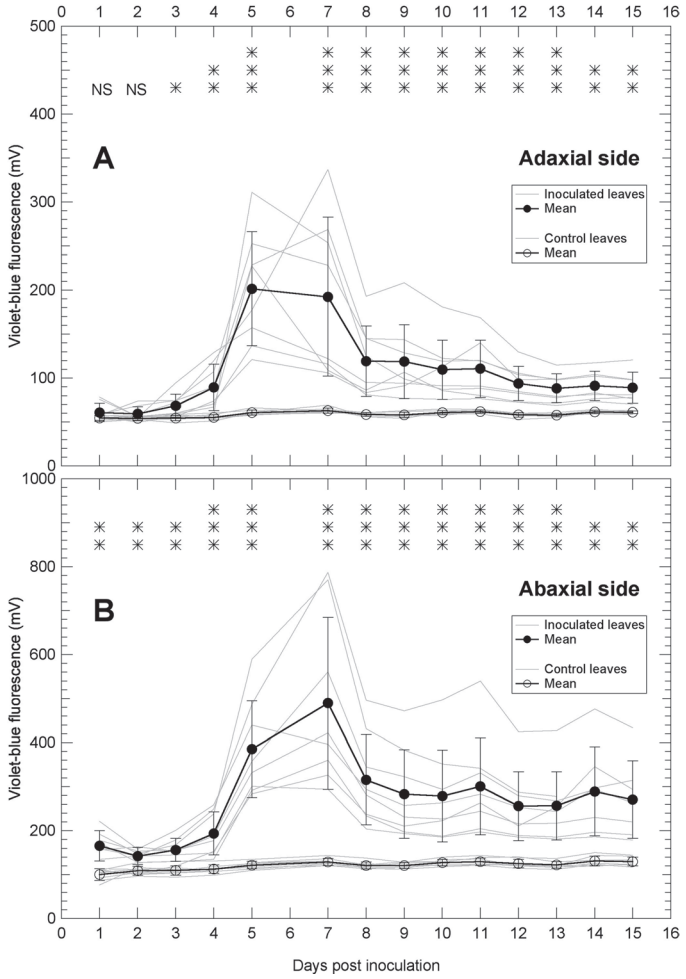

Fig. 4. Changes in violet–blue autofluorescence of grapevine leaves during *P. viticola* infection followed by the Mx-330 sensor. A large surface (6 cm diameter) of both the adaxial (A) and the abaxial (B) side of Cabernet Sauvignon leaves was sensed from the first to the 15th day after inoculation. Note that the *y*-axis scale in B is double that in A. Grey lines are daily kinetics of individual leaves and bold lines are means with standard deviations of eight inoculated leaves and eight control leaves. The significance of the difference between control and inoculated leaves for each day is indicated on the top of the graphs: NS, not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

inoculated leaves, especially at the abaxial leaf side. Although not optimized for the present study, this narrow-band index of yellow fluorescence excited by a 375 nm UV light is related to the GF presented in Figs 1C, F, and 3.

Discussion

Fluorescence measured by spectroscopy or imaging has two advantages: it is very sensitive and can be applied at different scales, from microscopy to remote sensing. Imaging allows assessment of the spatial distribution of signals, while spectroscopy can assess the variation of signals with larger sensitivity and from a distance. Works of Poutaraud *et al.* (2007) and Bellow *et al.* (2012) have shown that *in vivo* VBF is a good indicator of the presence of stilbene phytoalexins in grapevine leaves induced upon infection by *P. viticola.* In addition, changes in variable ChlF were shown to be a good indicator of downy mildew due to its impairment of photosynthesis (Csefalvay *et al.*, 2009).

Spatiotemporal characteristics of the autofluorescence of grapevine leaves during infection by P. viticola

Images acquired in this study showed that the stilbene VBF was strictly limited to the vein-delimited areoles inoculated by *P. viticola*. Outside this region, no extensions of visible symptoms of chlorosis or necrosis were observed. Apparently, the development of *P. viticola* hyphae is generally stopped by veins in leaf tissues (Unger *et al.*, 2007). Therefore, the results confirm that visible symptoms and fluorescence induced by the *P. viticola* infection are both limited to the primary infection site areoles. Sporulation appeared at 4 DPI for the leaves kept in a water-saturated atmosphere in portable mini-greenhouses; this confirmed the success of the infection (not shown). Sporangiophores were washed away daily so they appear neither on transmission (Fig. 1D) nor on fluorescence images (Fig. 1E, F), nor did they influence the fluorescence spectra (Figs 2, 3) (cf. Supplementary Fig. S1 available at *JXB* online). For proximal sensing, plants were kept in the dry atmosphere of a greenhouse and there was no sporulation, hence also no influence of the sporangiophores on the measurements.

The results of this study set the detection threshold of stilbene VBF at 3 DPI at the adaxial leaf side of a susceptible grapevine leaf. On the abaxial side, inoculated leaves had significantly larger VBF than control leaves from day 1 after inoculation. Therefore, the stilbene VBF seems a promising asymptomatic signal to detect infections. These 15 d of the survey revealed the transient nature of the stilbene VBF summarized in Fig. 6, with three characteristic phases: a lag phase, a transient phase characterized by an increase in VBF to a maximum around 6 DPI, followed by a decrease and a stabilization of VBF at a level significantly above the control in the third phase. This last phase was concomitant with the appearance of visible symptoms of chlorosis. The same phases were observed with fluorescence imaging on 0.2 mm^2, measured with the spectrofluorimeter on 20 mm^2, and assessed with the new proximal sensor Mx-330 on 2000 mm^2 on both the abaxial and the adaxial side of the whole infected leaves. Blue-excited GF increased continuously during the third phase. Sporulation appeared at the beginning of the transient phase (4 DPI). The transitory nature of VBF raises the question of the fate of stilbenes during the infection. The dynamics of stilbene VBF suggest that a large proportion of phytoalexins detected in infected grapevine leaves are either degraded or metabolized into molecules with a significantly lower fluorescence yield soon after the transient phase. Laccases are fungal enzymes that have the capability to metabolize stilbene phytoalexins produced by the hosts, as seen in the case of *Botrytis cinerea* infections (Pezet *et al.*, 1991; Sbaghi *et al.*, 1996). Laccase activity is assumed to detoxify *trans*-resveratrol by