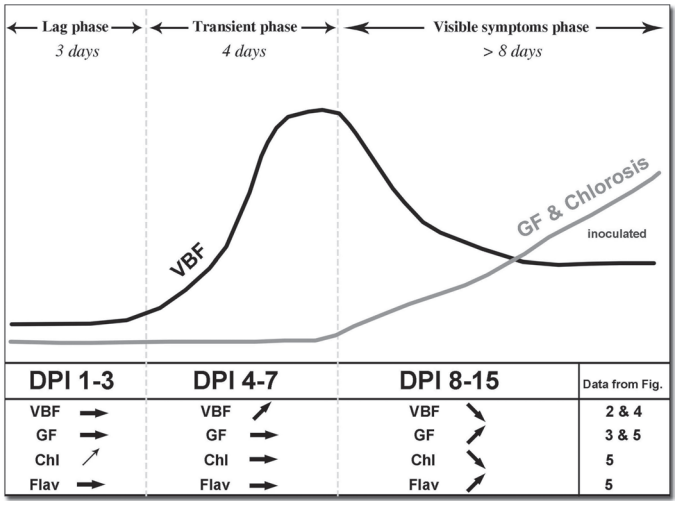

Fig. 6. Schematic summary of optical indicators of downy mildew infection. The initial increase in chlorophyll (Chl) seen during the lag phase is due to the maturation of the leaf. It was identical on control and on inoculated leaves. DPI, days post-infection. The optical indicator increased (↑), decreased (↓), or remained constant (→).

properties and chlorophyll content. Palisade parenchyma had higher chlorophyll content than spongy parenchyma, shown by the SFR difference in Fig. 5A versus B, and the reflectance index (R-590) in Fig. 5C versus D (opposite response to SFR) for both inoculated and control leaves. The chlorophyll content of a leaf will be the sum of the index measured on each of the two sides. The highly diffusive properties of the abaxial leaf side precluded the detection of significant differences between inoculated and control leaves for R-590. The green-to-red fluorescence excitation ratio (FER_RG) has only recently been linked to leaf chlorophyll content when anthocyanins are absent (Z.G. Cerovic, unpublished). It will have higher values for lower chlorophyll contents (opposite response to SFR) due to lower green light absorption. The local loss of chlorophyll, seen by fluorescence indices in the third phase of the infection, will decrease the reabsorption of pre-existing green-fluorescing compounds and lead to an increase in GF (Cerovic *et al.*, 1999). This is supported by the absence of changes in the shape of GF spectra during the infection.

Towards new non-destructive indices to detect downy mildew

The similar results obtained by the three approaches used in this study, albeit a four order difference in sensed area size, prove the universality and robustness of the stilbene VBF and GF as indicators of *P. viticola* infection. In addition to the use of VBF in microscopy to study *P. viticola*–grapevine interactions (Bellow *et al.*, 2012), stilbene fluorescence seems suited for the development of an accurate tool for field detection. The spectra obtained allow the optimal wavelengths (330 nm excitation, 400 nm emission) most specific for stilbene fluorescence in grapevine leaves to be defined. Although the stilbene signal measured at the adaxial side of the leaves was half that measured at the abaxial side, the Mx-330 index allowed a promising discrimination as early as 3 DPI. However, the inoculation performed on entire leaves reached all parts of the leaves. Natural *P. viticola* droplet infections rarely cover the whole leaf surface. So a lower stilbene-dependent signal can be expected in the field. Still, the specificity and advantage of fluorescence over reflectance is its very high sensitivity. Up to a limit (concentration quenching), fluorescence will be the same from a given amount of fluorophore distributed uniformly over an area or aggregated on small spots. Further experiments in the field on spontaneous infections are required, and these are now feasible thanks to the availability of the Mx-330.

VBF appears as an early indicator of downy mildew, while GF is specific to the late stage of the infection, temporally correlated with visible symptoms of chlorosis. Stilbenes are phytoalexins of grapevine, induced by either biotic or abiotic stresses (Jeandet *et al.*, 2002) and not specific to a particular disease. Using stilbene VBF to probe downy mildew in the field might not be specific enough because other factors could lead to the production of stilbenes. Therefore, additional signatures of the infection could be advantageous to discriminate specifically *P. viticola* infections. In the present study, it was found that GF of grapevine leaves was both spatially and temporally correlated with downy mildew late symptoms of chlorosis. Even if this signal does not provide an early diagnosis, it provides evidence of the presence of downy mildew. The comparison of four independent sensing techniques—imaging, specrofluorimetry, UV-excited blue fluorescence sensing (Mx-330), and multiwavelength proximal sensing (Multiplex 3)—has allowed a robust description of the three characteristic phases of autofluorescence of grapevine leaves during the infection by *P. viticola* (Fig. 6). Variable ChlF (Csefalvay *et al.*, 2009) needs imaging and is not available for measurement with mobile platforms. This seems not to be the case with simple autofluorescence signals or ratios such as VBF, VBF/GF, SFR, and FER_RG. Furthermore, the availability of several signals could help to discriminate downy mildew among the biotic and abiotic stresses.

This study was performed on attached leaves in order to maintain them under physiological conditions. However, plants were grown in a controlled environment compared with field-grown plants that are potentially facing multiple stresses. So the next step would be to perform a similar kinetic investigation involving Multiplex 3 and Mx-330 in the field during spontaneous infections. Although demonstrated here only for the *V. vinifera–P. viticola* pathosystem, the approach presented herein can be extended to other pathosystems involving fluorescent phytoalexins found in other species, such as coumarins in sunflower or isoflavonoids in soybean (Grayer and Harborne, 1994).

Supplementary data

Supplementary data are available at *JXB* online.

Figure S1. Images of sporulating leaves before and after removal of sporangiophores.