Non-invasive Raman spectroscopy for monitoring metabolite changes in tomato plants infected by phytoplasma

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The Variable Importance Plot (VIP) displays the most relevant peaks for the discrimination. Very few vibrational frequencies were above the threshold (0.8), such as v_1 = 1005 cm^{-1}, v_2 = 1155 cm^{-1}, v_3 = 1326 cm^{-1}, v_4 = 1525 cm^{-1} and v_5 = 1604 cm^{-1}. The latter has been associated with lignin and polyphenol content. Meanwhile, v_1, v_2 and v_4 are vibrational modes of the same reference spectrum (*i.e.*, carotenoid content), and they can be considered related. Accordingly, only v_4 – the mode with the highest VIP value – was selected. In addition to that, we opted for the use of v_3 (1326 cm^{-1}), typically associated with chlorophyll content. These vibrations have been selected since they also guarantee no overlap between spectral features of different chemical families.
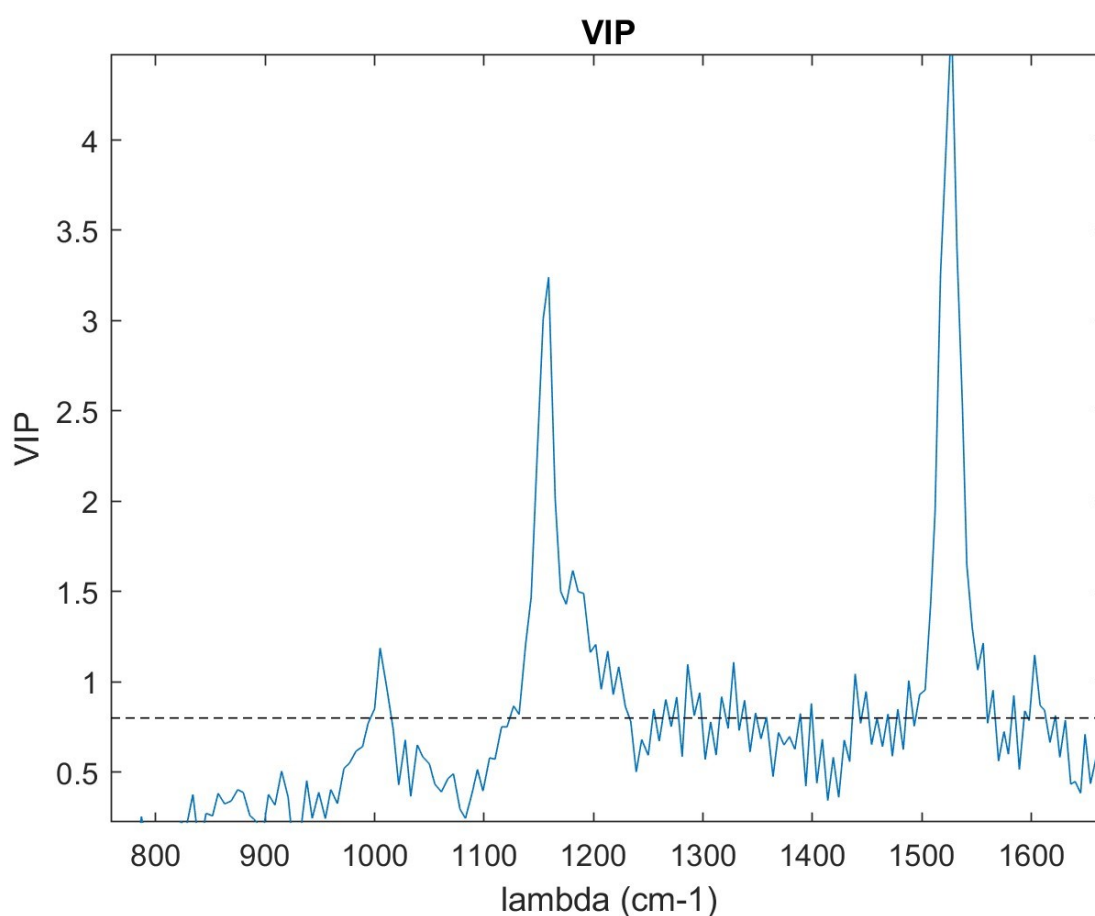

Figure S1 Variable λ Importance in the Projection statistic for the Partial Least Squares (PLS) model applied to the data.
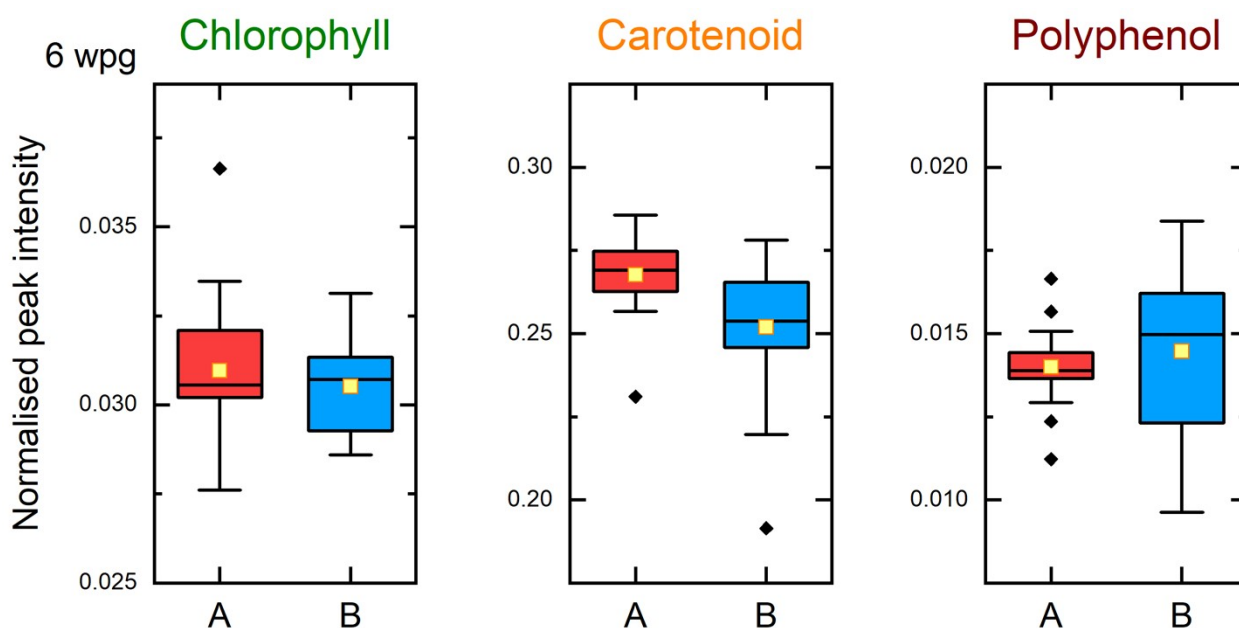
Figure S2 Box-plot of the intensities of v_1 (chlorophyll), v_2 (carotenoid) and v_3 (polyphenol) from group A (infected plants) and group B (control plants). Plants have been grafted for six weeks (6 wpg). Mean values are depicted by a yellow square, while the median is denoted by a straight line. Black diamonds are outliers.

Figure S3 Box-plot of the intensities of v_1 (chlorophyll), v_2 (carotenoid) and v_3 (polyphenol) from group A (infected plants) and group B (control plants). Plants have been grafted for eight weeks (8 wpg). Mean values are depicted by a yellow square, while the median is denoted by a straight line. Black diamonds are outliers.
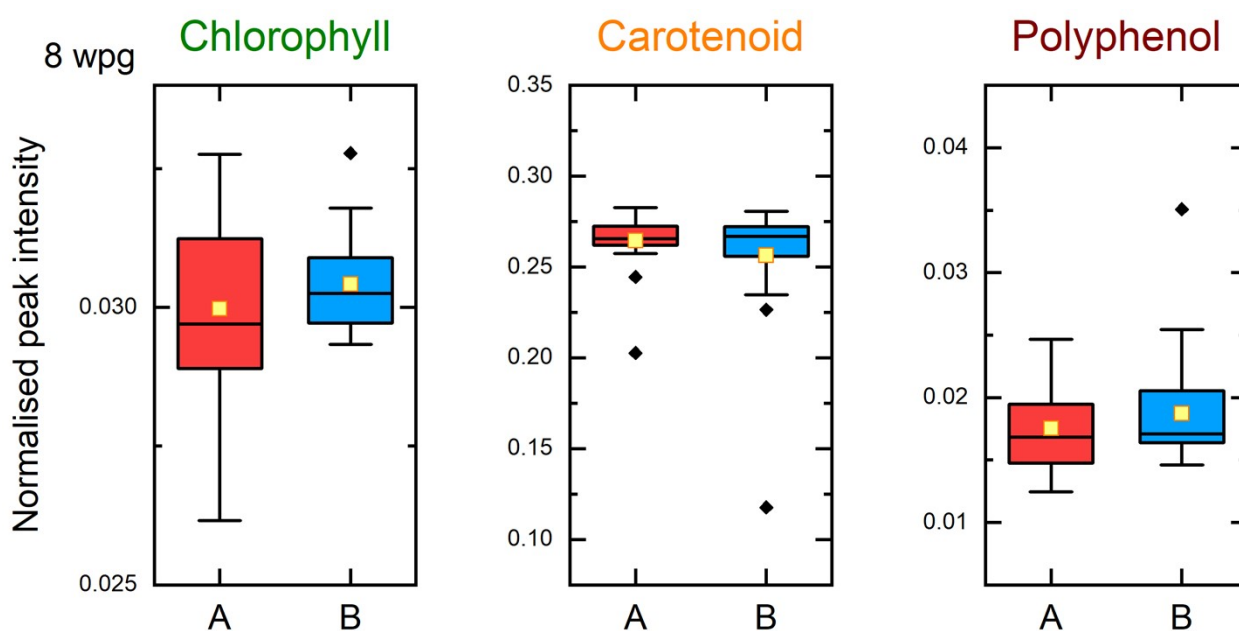
Table S1 One-way ANOVA results used to test the effect of the categorical independent variable "time" on the GU/ng.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Time	2	2.12E8	1.06E8	2.809	0.138
Residuals	6	2.27E8	37774811		

Table S2 Results of ribosomal RNA detection through one-step RT-qPCR: for each group (2 wpg, 4 wpg, 6 wpg, and 8 wpg), one leaflet from three different infected grafted plants was sampled. Each sample was analyzed in duplicate, and the average Ct (cycle threshold) values are presented.

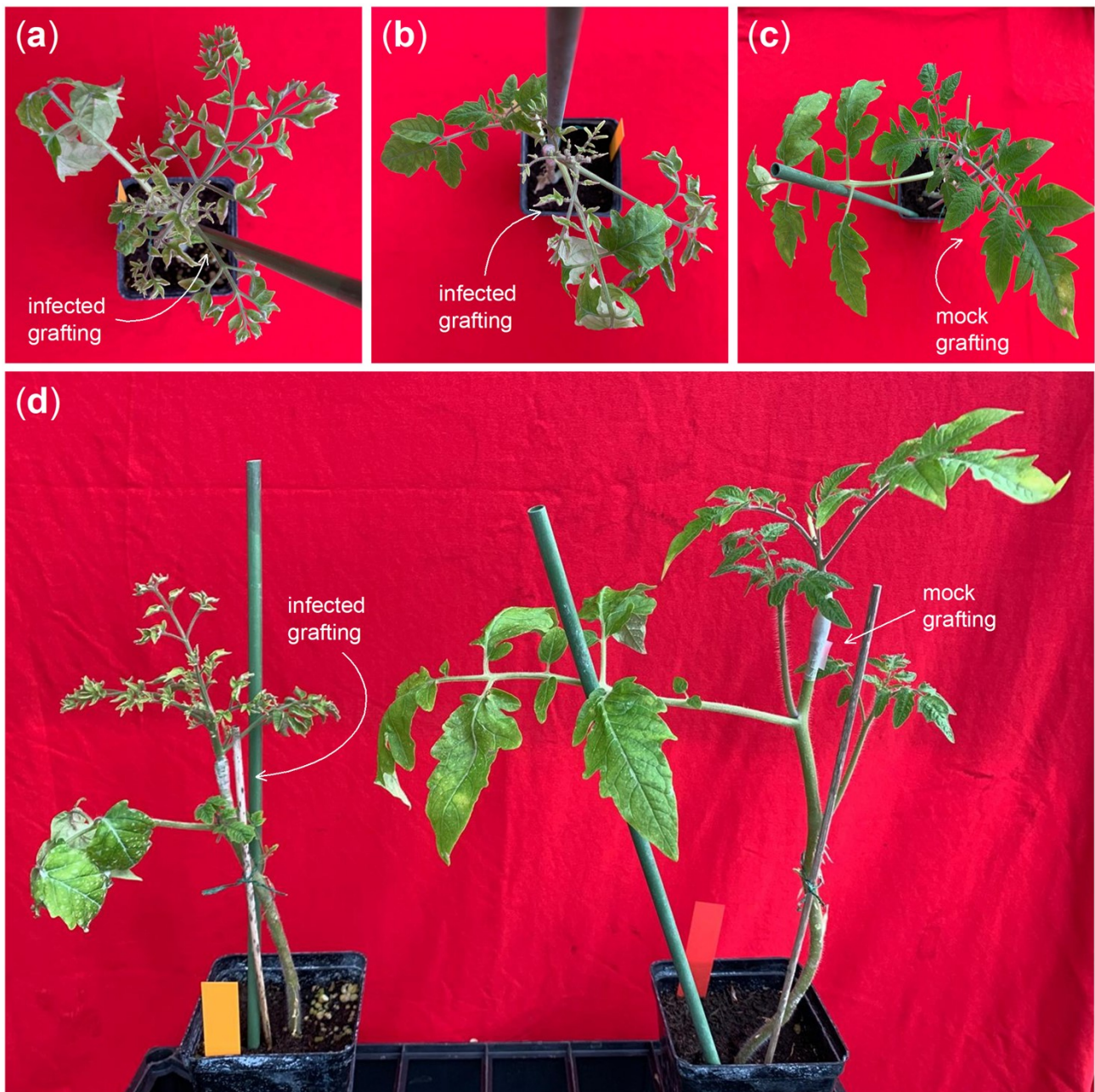| Sample	Ct average
2 wpg	undetected
2 wpg	undetected
2 wpg	undetected
4 wpg	28.0901
4 wpg	29. 6951
4 wpg	29.5763
6 wpg	25.7669
6 wpg	25.4102
6 wpg	26.0058
8 wpg	22.3832
8 wpg	25.3874
8 wpg	22.9493

Figure S4 (**a**) top-view of a grafted tomato plant with infected material; (**b**) top-view of a grafted tomato plant with infected material and asymptomatic new shoots; (**c**) top-view of a mock-grafted tomato plant with healthy material; (**d**) side-view of infected and mock-grafted tomato plants. Symptoms caused by Ca. P. solani in tomato are visible in infected-grafted plants, characterized by reduced growth, upward leaf curling and reddish veins. The development of new shoots following grafting remains asymptomatic until the fourth week, after which symptoms become visible and recognizable.