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Ecophysiological aspects of the interaction between *Cameraria ohridella* and *Guignardia aesculi* on *Aesculus hippocastanum*

Supplementary Material.
Protocol for quantifying leaf damage on crown projection level

Equipment

The need to take photographs from above the plant meant that equipment requirements increased along with the plants height. Digital camera (we suggest those with distortion correction function), lens (we suggest wide angle lens) and spirit levels for achieving parallel plains between camera projection and scaling object are essential. For plants that achieved heights up to 1.5m, we used a tripod stand for camera assembling and a ladder for the operator Dimensions of the scaling object should also be adjusted, hence we used slightly longer than 1m in length slats with marked 1m sections (Figure S1.). When smaller plants are under investigation, photographs could be taken without a ladder and tripod stand. All that this would require is that the camera is levelled using a small spirit level integrated into the camera. Then, a

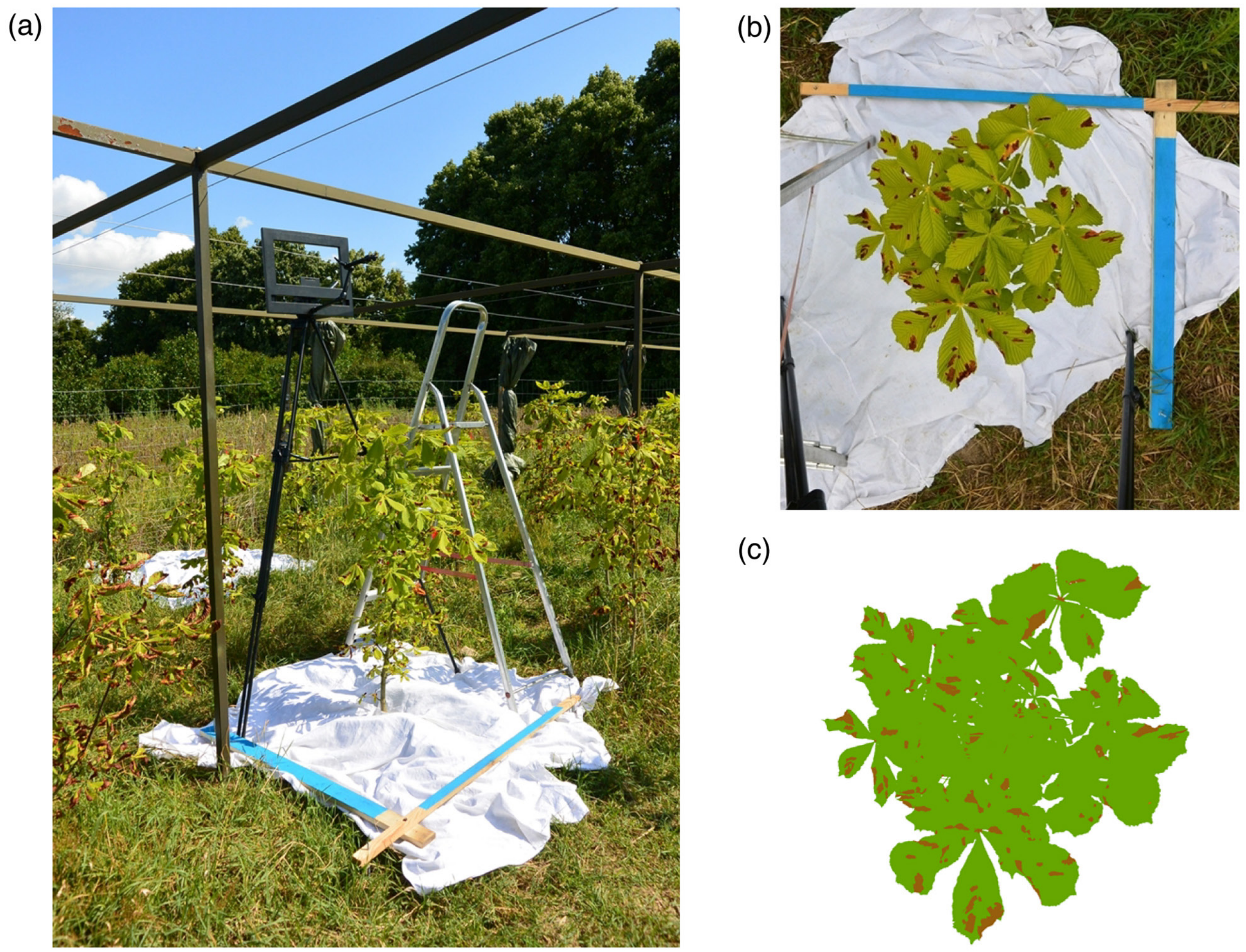

Fig S1. Equipment (a), projection crown area (CPA) viewed from camera (b) and image from WinFolia Pro 2013 after colour analysis (c): white – background; green – healthy area; brown – damaged area photograph can be taken with the camera held directly in the operator's hand. Material for camouflaging the background under the plant should be provided. This is crucial for appropriate distinction between the crown area and ground level plants.

Calibration

To obtain accurate data, calibration will be essential to minimise the distortion effect of the camera lens, even if corrected directly by the camera processor. The calibration procedure is the same as for that of data collection with one key difference – during this procedure we measured a known area of the object. For this purpose, an object of known area (i.e. the black square on white background) must be photographed at a minimum of five different camera heights. The proportion object must be laid on the ground and stabilised. In every position, the object should be photographed at a minimum of five different heights. An example of a scheme for the calibration procedure is shown in table S1.

Table S1. Example of height positions for the calibration procedure. Values adapted for plants with heights between 90 and 150 cm. It is not necessary to set heights of camera and objects precisely, but these values should be measured with an accuracy of up to 1 cm for obtaining appropriate parameters for CF equation

Camera height:	Object height:				
130	0	20	40	60	80
150	0	25	50	75	100
170	0	30	60	90	120
190	0	35	70	105	140
210	0	40	80	120	160

Then, every image must be analysed and the area of the photographed object measured using the scale referring to the lengths of the slats. For this purpose, we used this same software as for further analysis of the crown projection area. When these values are known, the correction factor (CF) is given by:

$$CF = \frac{rAREA}{mAREA}$$

Where rAREA is real area of object and mAREA is measured area. Values from the calibration procedure must be obtained for the correction factor equation described in the next section.

Calculations

Trigonometry was used to compose the equations used for conversion because the geometry of the measured parameters was from a triangle (Figure S2.). Two variables need to be considered, namely,

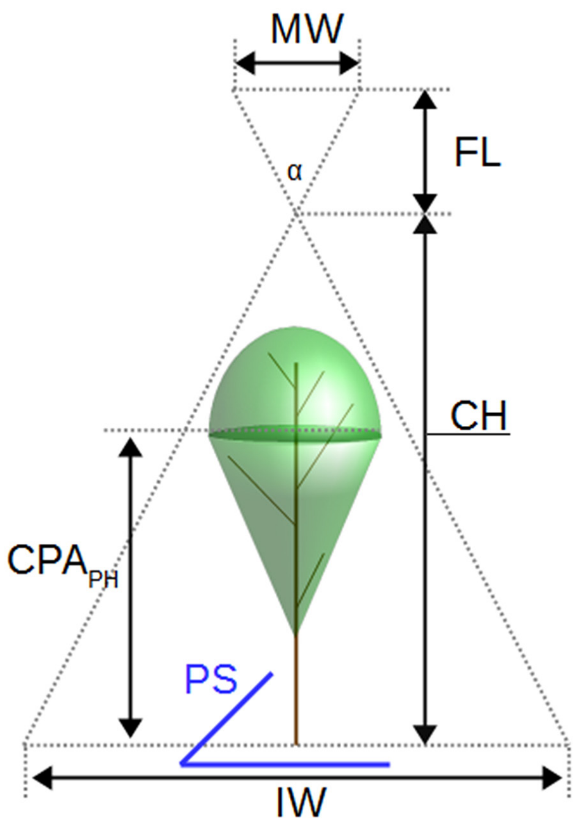

Fig S2. Schematic diagram showing variables used in the equations for converting data from the image into quantitative assessments of foliage damage. IW = image width; MW = camera matrix width; FL = focal length; CH = camera position height; CPAPH = height of crown projection area plane, PS – proportion slats

the height of the camera and the crown projection areas. The camera height (CH) is obtained for every image individually using the technical specifications of the camera lens (matrix width and focal length – also available from EXIF image data) and the image resolution using equation:

$$CH = \frac{PW \times IW}{MW \times FL^{-1}}$$

Where PW is pixel width [meters/pixel]; IW is image width [pixels]; MW – is camera matrix width [mm]; and FL is focal length [mm]. In the calculations, the crown projection area plane height (CPA_{PH}) for each individual tree must be defined. We defined it as the mean height of the branch ends that projected the furthest out from the main stem. Based on the above-mentioned values, a theoretical conversion factor (tCF) is obtained using equation:

$$tCF = \left(1 + \frac{CPA_{PH}}{CH - CPA_{PH}}\right)^{-2}$$

Where CPA_{PH} is the height of the crown projection area plane in meters; and CH is the camera height in meters. This calculation is essential to make measurements comparable, because for every photograph taken, the height of the camera could vary dependent upon the height of the sapling.

The theoretical equation must be translated for the empirical use of data from the calibration. This is obtained by adding three parameters to tCF equation:

$$CF = \left(\alpha + \beta \frac{CPA_{PH}}{CH - CPA_{PH}}\right)^{\frac{-1}{\gamma}}$$

Estimation of parameters α, β, γ could be done using the nonlinear least square method (NLS). The dependent value is from equation 1 and the independent value is the ratio between CPA_{PH} and the difference between CH and CPA_{PH}. Parameters (α, β, γ) could be estimated with nonlinear least-square method (NLS) which is available in R environment ("nls" function in "stats" package) (R Development Core Team, 2008). The following starting values should be used for calculations: $\alpha=1$, $\beta=1$, $\gamma=0.5$, as then equation 4 is equal to equation 3. If a camera without a distortion correction function is used, additional calculations as provided by Zhang (2000) should be considered.

Image analysis

Identifying the extent of foliage damage was dependent on the ability to distinguish the healthy and damaged areas from the background of the image (Fig. 1c in main text). The pixel classification method based on color in WinFolia Pro 2013 (Regent Instruments Inc.) software can be used and it was found to be the most suited for the purpose of the study.

Obtained images must be scaled by measuring the two visible perpendicular slats in pixel scale and converting these to real values in WinFolia (calibration function) – as lengths of slats are known, the pixel width and height are relative to slats lengths. Then every image needs to have the color ranges set up for the layers to be analysed (healthy, injured and background individually). When choosing areas for individual layers, the operator should indicate the whole range of the layer, and shaded areas inclusive. The color settings from previous images could be used for the next image and modified if needed, this speeds up the analysis procedure. WinFolia greatly simplifies this procedure making the whole process very efficient. Time needed for image analysis was not recorded, but took approximately five minutes per image. After analysis, two values should be obtained: healthy and damaged areas. Then equation 4 must be used to change the values to obtain the actual healthy or damaged areas or both. Alternative methods for image analysis are available and several studies have been carried out to determine their accuracy and effectiveness these include: Zhang (2000); Kwack et al. (2005); Shimoji et al. (2006); Barbedo (2014).

Outlines for other use of the method

Apart from determining foliage damage, the proposed method also allows the crown projection area to be calculated at the same time. This information could be used in many other types of investigations, as estimation of foliage area is an important feature for examining growth in plants. The developed method could also be applied to phenological observations. It could be possible to apply the method to larger plants by using an alternative means of suspending the camera, such as the use of drones. The equations derived in this study could then be utilised to analyse the results.

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