Vitis **53** (1), 1–8 (2014)

Initial steps for high-throughput phenotyping in vineyards

K. Herzog[1)], R. Roscher[2)], M. Wieland[3)], A. Kicherer[1)], T. Läbe[2)], W. Förstner[2)], H. Kuhlmann[3)] and R. Töpfer[1)]

[1)] Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany

[2)] Department of Photogrammetry, Institute for Geodesy and Geoinformation, University of Bonn, Bonn, Germany

[3)] Department of Geodesy, Institute for Geodesy and Geoinformation, University of Bonn, Bonn, Germany

Summary

The evaluation of phenotypic characters of grapevines is required directly in vineyards and is strongly limited by time, costs and the subjectivity of person in charge. Sensor-based techniques are prerequisite in order to allow non-invasive phenotyping of individual plant traits, to increase the quantity of object records and to reduce error variation. Thus, a Prototype-Image-Acquisition-System (PIAS) was developed for semi-automated capture of geo-referenced images in an experimental vineyard. Different strategies were tested for image interpretation using MATLAB®. The interpretation of images from the vineyard with real background is more practice-oriented but requires the calculation of depth maps. Different image analysis tools were verified in order to enable contactless and non-invasive detection of bud burst and quantification of shoots at an early developmental stage (BBCH 10) and enable fast and accurate determination of the grapevine berry size at BBCH 89. Depending on the time of image acquisition at BBCH 10 up to 94 % of green shoots were visible in images. The mean berry size (BBCH 89) was recorded non-invasively with a precision of 1 mm.

K e y   w o r d s : image-based phenotyping, grapevine breeding, image analysis, depth maps, BBCH, bud burst, berry size, non-invasive.

Introduction

Accurate phenotyping is a key tool within the scope of plant breeding. A plant phenotype comprises morphological and physiological features and reflects genotype-environment-interaction (Furbank and Tester 2011). In particular, with regard to resistance breeding of grapevine, phenotyping of diseases is possible under lab or greenhouse conditions using leaf discs or potted grapevines (Staudt 1997, Brown *et al.* 1999, Eibach *et al.* 2007, Zhang *et al.* 2009, Schwander *et al.* 2011). Phenotyping of single plant phenology (e.g. time of bud burst), determination of yield parameters (e.g. berry size, number of clusters per shoot), and identification of grape and wine quality are similarly important in grapevine breeding. The investigation of all stated traits requires grapevine plants which have been cultivated beforehand in the field for several years. In breeding programmes up to now phenotyping of grapevines in vineyards is being carried out only by the use of visual inspection – *i.e.* estimating traits by applying the BBCH scale (Biologische **B**undesanstalt, **B**undessortenamt und **CH**emische Industrie) or OIV descriptors (International Organisation of Vine and Wine). The BBCH scale is a common system which is used in order to identify growth stages of grapevines (Lorenz *et al.* 1995) and the OIV descriptors are used in order to evaluate traits, e.g. the berry size (OIV 2001) accordingly. It is thus not only very time consuming and laborious, but also expensive and subjective. Its biggest limitation, however, is the problem of phenotypic data acquisition on several hectares at a time of rapid plant development, thus permitting a broad and detailed evaluation only for limited breeding material. The interdisciplinary competence network CROP.SENSe.net (www.cropsense.uni-bonn.de) was founded to establish practical, non-invasive and high-throughput techniques. The application of automated system technologies in vineyards combined with adaptable sensor-based techniques permits the acquisition of variety-specific, morphological plant features with the objective to open up the phenotyping bottleneck. It is a promising opportunity to increase the quantity of phenotyping samples, to improve quality of recording, and to minimise error variation. Certainly, the implementation of precise sensor-based screening practices to receive grapevine characters combined with increasingly efficient genotyping techniques (e.g. marker-assisted selection – MAS) will greatly enhance the efficiency of grapevine breeding. Currently, the application of robotics or comparable platforms in vineyards equipped with multispectral optical or 3D laser sensors is especially known from precision viticulture (Berenstein *et al.* 2010, Braun *et al.* 2010, Farlie *et al.* 2010, Longo *et al.* 2010, Mazzetto *et al.* 2010, Bates *et al.* 2011, Ramos *et al.* 2012). The published studies aimed at site-specific vineyard monitoring, targeted spraying and vineyard management (Berenstein *et al.* 2010, Braun *et al.* 2010, Mazzetto *et al.* 2010). Sensor-based phenotyping of grapevines often intended for the discovery of water stress or the determination of leaf canopy (Jones *et al.* 2002, Moller *et al.* 2007, Diago *et al.* 2012). Nevertheless, the determination of grapevine phenology or yield estimation using images comprises more detailed detection and the survey of small plant features, e.g. single berries in grapevine clusters (Nuske *et al.* 2011). It requires a comparable application of automated techniques using more detailed machine vision algorithms. The present study aimed at the investigation of initial steps in order to enable high-throughput phenotyping in vineyards by using images and depth maps. Hence, the *Prototype-Image-Acquisition-System* (PIAS) was constructed in order

Correspondence to: Dr. K. Herzog, Julius Kühn-Institute, Federal Research Centre for Cultivated Plants, Institute for Grapevine Breeding Geilweilerhof, 76833 Siebeldingen, Germany. Fax: +49-6345-919050. E-mail: katja.herzog@jki.bund.de.

to capture geo-referenced images with higher throughput in vineyards by trailing the PIAS between the grapevine rows. Two MATLAB® tools were developed and tested for semi-automated image analysis in order to investigate two important phenotypic traits of grapevines: bud burst at BBCH 10 and berry size at BBCH 89. Within this scope an initial experiment was conducted for automated interpretation of (1) one RGB image per grapevine plant with a black artificial background; and (2) one depth map per grapevine which avoids the necessity of using black artificial background.

Material and Methods

Plant material: Tests involved rows of different *Vitis vinifera* cultivars (including 'Riesling', 'Pinot Blanc', 'Silvaner', 'Kerner', 'Pinot Noir', 'Dornfelder' and 'Regent') and 140 individuals of a F1 breeding population (Gf.Ga.47-42 x 'Villard Blanc') at the experimental vineyard of Geilweilerhof located in Siebeldingen, Germany (N 49°21.747, E 8°04.678). The use of different genotypes (altogether 240 grapevines) guarantees a large variation of phenotypes for image interpretation. Inter-row distance was 2.0 m and grapevine-spacing was 1.0 m. To enable precise measurements in images, coloured labels with a total width of 39 mm (Roth® GmbH, Karlsruhe, Germany) were fixed on wires used for scaling as a reference.

Reference evaluations: The BBCH stage 10 (Bud burst – green shoots are visible) and the BBCH stage 89 (end of berry ripening – harvest) were determined according to LORENZ *et al.* (1995). At BBCH 10 reference quantification of shoots was carried out in parallel to image acquisition directly in the vineyard. At BBCH 89 100 clusters per each of four cultivars were sampled after image capture. The sampling of berries, image acquisition and image analysis were carried out as described by KICHERER *et al.* (2013). Afterwards the automated Berry Analysis Tool (BAT) was used to determine precise reference data of the berry size from four varieties ('Riesling', 'Pinot Blanc', 'Pinot Noir', and 'Dornfelder').

Sensors and sensor data: A calibrated single-lens reflex (SLR) camera (Canon® EOS 60D) was used in order to capture *Red-Green-Blue* (RGB) images (3456 x 2304 pixels). A prototype-camera-unit consisting of four calibrated industrial AVT GT-2300 cameras (10 cm difference in camera position between all cameras) was applied for the acquisition of monochrome images (2448 x 2050 pixels). Camera calibration was performed by using a test field calibration according to ABRAHAM & HAU (1997) with an equivalent focal length of 28 mm (SLR camera) respective 8 mm (industrial cameras) in order to determine camera constant, principle point and camera lens distortions. Thus, internal parameters of the camera are known and a post processed correction of aberrations in images is possible to ensure precise measurements. The images were captured in the field under natural illumination conditions with manually controlled exposure. Images were saved for offline processing. All plants of the experimental rows were surveyed using a *Real-Time-Kinematic* (RTK)-GPS system (Trimble® SPS852, Geo Systeme GmbH, Jena, Germany) with 2 cm accuracy. *Universal Transverse Mercator* (UTM) was used as Cartesian coordinate system. The RTK-GPS receiver transmits two different ASCII codes at 5 Hz frequency: *National Marine Electronics Association - Global Positioning System Fix Data* (NMEA-GGA) and *National Marine Electronics Association - Recommended Minimum Sentence C* (NMEA-RMC). Both ASCII codes were used to estimate the orientation angle of the SLR camera and to acquire geo-referenced RGB images.

PIAS – The development of a prototype image acquisition system: Field experiments for image-based phenotyping were conducted in 2012 one day before harvest (BBCH 89) and at the time of bud burst (BBCH 10) in 2013. The images were taken when the BBCH stage 10 was detected by visual inspections at 29th April 2013 (date 1), 2nd May 2013 (date 2) and 6th May 2013 (date 3). In order to enable capturing of standardised images a *Prototype-Image-Acquisition-System* (PIAS) was constructed. PIAS consists of a four wheeled handcart (Fetra GmbH, Hirschberg, Germany) as a carrier vehicle, the calibrated SLR camera and RTK-GPS system for acquisition of geo-referenced RGB images and a laptop (Lenovo® Thinkpad X201, Lenovo GmbH, Stuttgart, Germany) for data recording (Fig. 1A). The SLR camera had variable height mounting above ground level (1.00-1.30 m) to investigate canes, bud burst, and the grape cluster zone and was fixed in the middle of the PIAS with a distance of at least 1 m from the grapevine plants. Image acquisitions were carried out in front of the plants by dragging the PIAS between the grapevine rows. Capturing of images was done

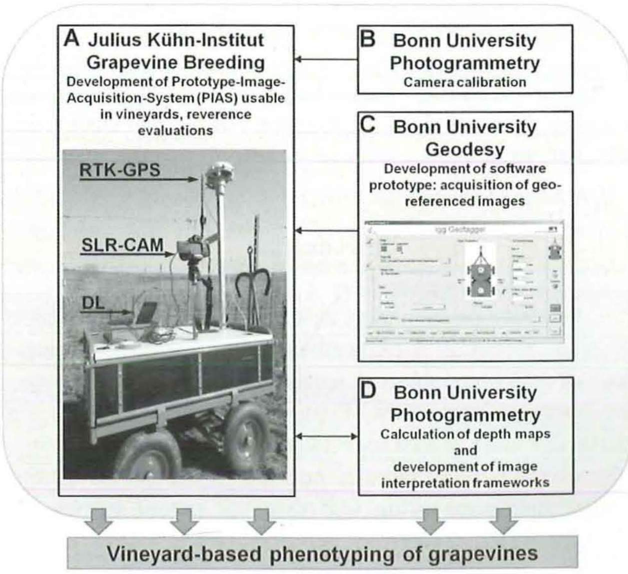

Fig. 1: Interdisciplinary network in order to achieve efficient development of high-throughput, automated and non-invasive techniques for vineyard-based phenotyping. The *Prototype-Image-Acquisition-System* (PIAS) is applied in experimental vineyards (**A**) equipped with RTK-GPS, a calibrated SLR camera (**B**) and a data logger (DL). The prototype software IGG-GEOTAGGER (**C**) on PIAS enables acquisition of geo-referenced RGB images in vineyards. High resolute RTK-GPS is used for plant-image-allocation and image management. The calculation of depth maps from rectified 2D image pairs permits more precise image interpretation which is developed using MATLAB® (**D**).

manually using the prototype software IGG-GEOTAGGER (Fig. 1C) which was developed in LabVIEW (National Instruments® GmbH, Munich, Germany). The IGG-GEOTAGGER software links the SLR camera with the RTK-GPS system. It facilitates the automated recording of actual RTK-GPS coordinates during image acquisition. In order to estimate the orientation angle of the SLR camera, RTK-GPS position data (5 Hz frequency) and an extended Kalman-Filter (EKF) was used. Orientation angle and position data were automatedly memorised into the image EXIF file. Thus, geo-referenced images were received. The geo-reference was used for offline post-processing data management. In a first approach one RGB image was captured per grapevine and in a second approach monochrome images were taken for each plant. A prototype-camera-unit was mounted on the handcart of PIAS but without the application of the RTK-GPS system or IGG-GEOTAGGER. The PIAS was stopped in front of the regarding grapevine plant, adjusted and the prototype-camera-unit triggered manually.

Experiments: Within the present study four experiments were conducted:

Experiment (a): Detection of bud burst at BBCH 10

- (a1) semi-automated (Fig. 2A)
  one RGB image with black artificial background or
  one RGB image with real background;
  Interpretation using semi-automated Matlab tools with user interaction
- (a2) automated (Fig. 2B)
  one RGB image with black artificial background
  Interpretation using automated Matlab tools with user interaction
- (a3) automated (Fig. 2C)
  four monochrome images with real background
  Depth map computation for background identification
  Interpretation using automated Matlab tools with user interaction

Experiment (b): Determination of grapevine berry size at BBCH 89

- (b1) semi-automated (Fig. 6A)
  one RGB image with real background
  Interpretation using semi-automated Matlab tools with user interaction

Image interpretation: The image interpretation was carried out using MATLAB® 7.5 (MathWorks, Ismaning, Germany). Semi-automated MATLAB® tools were developed in order to quantify the grapevine features (Trait Quantification Tool – TQT) and the dimension of such features (Trait Size Tool – TST). Semi-automated means: 1) automated reading of the images (in one folder) in Matlab; 2) automated conversion (pixel to mm) of the diameter from the manually marked berries (circles); and 3) automated tracking of the results (image name and number of shoots or diameter of measured berries) in a txt.-file. Additionally, one prototype automated framework was developed and tested in order to detect bud burst at BBCH 10 and quantify shoots.

Semi-automated tools: The semi-automated quantification of shoots at BBCH 10 was conducted in experiment (a1) by using TQT. Visible buds/shoots in images were marked by mouse clicking. The number of all

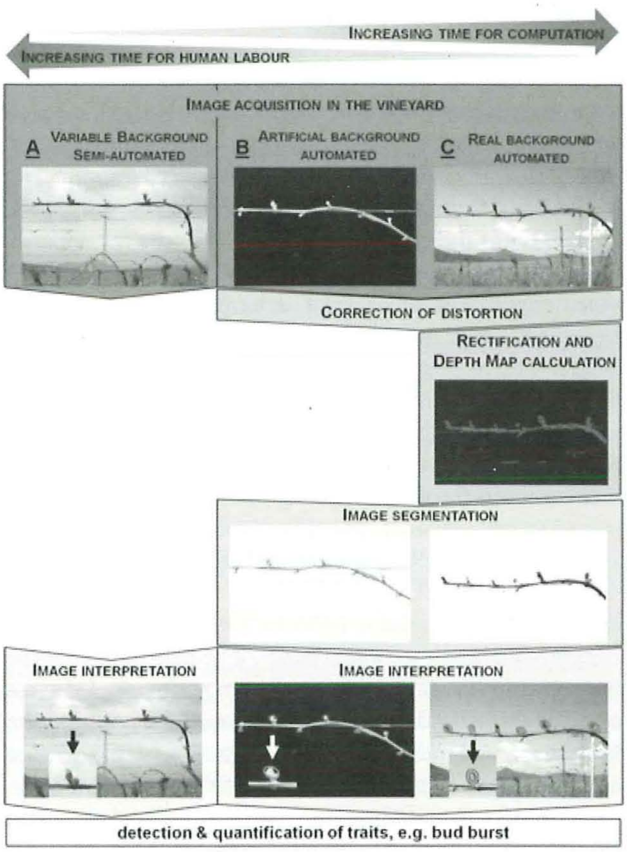

Fig. 2: Image-based phenotyping by using semi-automated and automated image interpretation procedures of the phenotypic feature bud burst at BBCH 10. **A)** The application of semi-automated image analysis in experiment (a1) enables the utilisation of an artificial or real background and the capture of one RGB image. The application of prototype automated framework can be performed using images with an artificial background (**B**) or with real background (**C**). **B**) The artificial background in images of experiment (a2) was used in order to segment the image into 'foreground' and 'background'. Thus, one RGB image per grapevine is required for image classification. **C**) The real background in images of experiment (a3) required the acquisition of four monochrome images per grapevine, following by the calculation of depth maps in order to distinguish between 'foreground' and 'background'. The depth maps were used for image classification. In order to visualise the procedure the depth map was referred to one monochrome image and the ellipses were plotted on the image.

marked shoots per image was automatically stored in a txt.-file (one file for all images in a folder). For semi-automated determination of the berry diameter the TST was used in experiment (b1). The edges of visible berries were marked manually with three evenly distributed points by mouse clicking. These three points define a circle which is automatically fitted through them. The diameter of the circle (*i.e.* the berry) is automatically measured in pixel. The number of pixels was automatically converted to S.I. units (mm) using a reference scale in the images (coloured labels which were fixed on the wires in the vineyard). In order to determine the number of pixel per mm automatically, the labels have to be manually marked in the image and the size of the label needs to be specified in the software.

Automated framework: The development of automated frameworks was divided into three steps: 1) the utilisation of the constructed annotation database in order to identify predefined phenotypic classes in example images; 2) image analysis and machine learning techniques such as active learning of a classification model in order to assign each image object to one or more of the predefined phenotypic classes by utilising the actively acquired training data or low-level features such as circles in images; and 3) the application of the classification model for the test images. In the first step, the phenotypic classes (e.g. 'cane', 'shoot') were defined. The second step determined the application dependent image analysis methods or active learning procedure. Active learning is a technique which provides a higher flexibility than fully supervised methods regarding changing radiometric and geometric features of the classes (Settles 2009). For the automated quantification of shoots after bud burst the classes 'background', 'cane' and 'shoot' proved to be sufficient. For this purpose the images were firstly segmented into 'background' and 'foreground'. The black artificial background in single RGB images of experiment (a2) was used in order to distinguish between the 'background' and the 'foreground'. The four monochrome images with the real background of experiment (a3) were used for the calculation of depth maps. Afterwards, the depth map was utilised for segmentation of the picture into 'background' and 'foreground' within the framework. It was assumed that the class 'cane' is defined as a thin elongated object in images with a defined minor diameter (objects with smaller diameter were disregarded, e.g. the wire). It was also assumed that the class 'shoot' could only be found close to the class 'cane'. Finally, an ellipse was fitted around each 'shoot' segment in order to quantify the number of detected shoots. The depth maps were used in addition to monochrome images with real background.

Annotation database for class definition: Image segmentation is a commonly used tool for the automated interpretation of images. It is defined by the partitioning of the image into assigned regions to predefined classes such as 'background' or 'bud'. Moreover, images are also interpreted using machine vision classification and image analysis methods for the identification of objects (e.g. berry). Each object belongs to one or more predefined classes which were uniquely defined using an annotation database. Classes were predefined by a manual annotation of different morphological features of grapevine (Fig. 3). Depending on the trait of interest the phenotypic classes were appointed with different levels of detail (Fig. 3A). Annotations ranged from low attribute complexity (level 1, segmentation into background and plant) to high attribute complexity (level 5, segmentation into background, wood, shoot, inflorescences, upper side of leaves, lower side of leaves). The database was composed of annotations of relevant growing stages of grapevines according to the BBCH scale (Figure 3B-E). One image per BBCH stage (03, 05, 10, 73, 75, 81, and 89) was used for annotation. The developer for image interpretation frameworks was fed with initial information about the relevant phenotypic classes, their morphological characteristics (e.g. wood, shoot, leaves, inflorescences or grape cluster), their radiometric features and geometric structures. The stated information about object characteristics was required for image classification, e.g. if buds were present near to an elongated object or if one berry was surrounded by other berries.

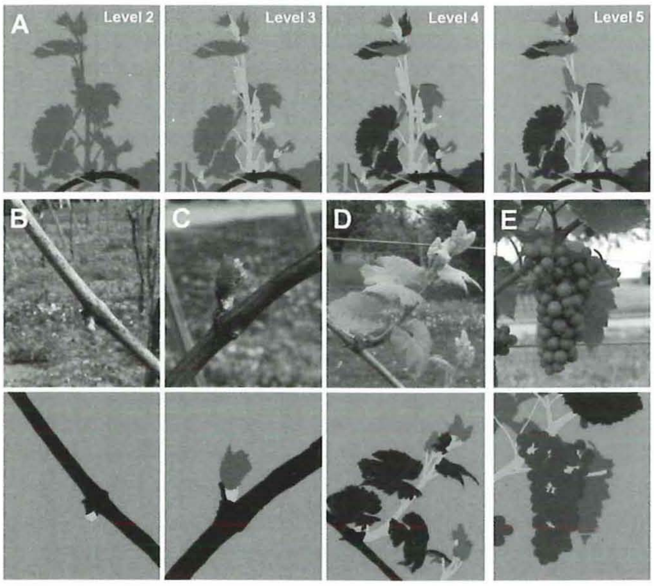

Fig. 3: Annotation of different phenotypic features of grapevines using coloured images. (**A**) Annotations of BBCH stage 51 to predefine classes with increasing complexity starting at level 2 (low complexity) up to level 5 (high complexity). (**A-E**) Images of various developmental stages according to the BBCH scale (top) and associated images from the annotation database (below). Each class is pixel-wise marked using different colours. **B)** BBCH 05 – Wool stage. **C)** BBCH 10 – Bud burst: green shoots clearly visible. **D)** BBCH 51 – Inflorescences visible. **E)** BBCH 81 – Beginning of ripening.

Calculations of depth maps: The detection of the background in images by computer vision procedures is much more practice-oriented compared to the carrying of an artificial background. Thus, monochrome images were captured by using a prototype-camera-unit. The interior and relative orientation parameters of the cameras were determined by camera calibration (Abraham and Hau 1997). Using this data, the image can be rectified in order to correct the non-linear distortion of the camera lenses. The depth maps were derived from 3D point clouds, which were obtained using patch-based multi-view stereo Software (PMVS; Furukawa and Ponce 2010). The software takes four monochrome images as well as the parameters for the internal and relative orientation of the cameras and reconstructs the 3D structure of the scene visible in the images. This approach provides depth maps in a fast way, however with data gaps resulting in pixels with no depth information.

Results and Discussion

High-throughput phenotyping requires the automation of data recording and data analysis. This is carried out both on the laboratory and the greenhouse scale (Granier 2006, Iyer-Pascuzzi et al. 2010, Hartmann et al. 2011). Particularly for perennial plants like grapevine, high-throughput phenotyping in the field is rather new and challenging. In a stepwise process (Fig. 1) an interdisciplinary network was built up which involved the construction of a *Prototype-Image-Acquisition-System* (PIAS) in order to capture standardised images in the vineyard. In addition, several image analysis tools were developed in MATLAB® and were validated by using these images with regard to the detection of bud burst at BBCH 10 and the determination of berry size at BBCH 89.

Semi-automated image analysis: In the first experiment (a1) two different variants of images were used for semi-automated quantification of shoots. 133 single RGB images of grapevines were captured with a black artificial background and 710 single RGB images of the same grapevines were taken with real background at three time points. All images were analysed by applying the semi-automated Trait Quantification Tool (TQT) in order to validate the quality of the quantification results.

Images were captured from 134 grapevines with both real and artificial background at the first beginning of bud burst (BBCH scale 10). With $R^2 = 0.49$, the number of shoots in images with real background was positively correlated with the number of shoots counted directly in the vineyard. Moreover, 76 % of the present shoots were recognised in the images (Fig. 4A). The utilisation of an artificial background in images at the early stage of shoot development improved the contrast, leading to an increased positive correlation of $R^2 = 0.67$ and an improved shoot number detection rate of 89 % (Fig. 4B). Quantification results with comparable quality but without the application of the artificial background were obtained by the analysis of images from date 2, *i.e.* three days after date 1. The counted number of shoots using TQT was positively correlated with the number of shoots counted directly in the vineyard ($R^2 = 0.65$). In addition, 84 % of the shoots were recognised (Fig. 4C). The quantification of shoots in images from date 3, *i.e.* six days after date 1, offered the best and most exact results for this early stage of grapevine development with a positive correlation of $R^2 = 0.81$ and a detection rate of 94 % (Fig. 4D). The reason for the increasing accuracy of the results could be explained by the morphology at this early stage of development. At the beginning of BBCH 10 the shoots are predominantly small, grow close to the cane and not evenly upwards and have similar colours (yellowish, light green, beige or brown) compared to the cane. Thus, the probability increases that buds or small shoots from the backside of the cane are not visible in the images, thus resulting in a lower detection rate. Due to this, it is important to capture images a few days after the first detection of BBCH 10, preferably when the shoots are visibly green and the average size is approximately 2 cm.

In this way, the detection of shoot development at BBCH 10 and the vitality of plants could be determined by analysing images. In grapevine breeding the number of shoots (buds) can be used to characterise individual genotypes from breeding populations, e.g. to receive information about the length of internodes, vital shoots and phenology. The utilisation of PIAS enables the recording of image data from a large number of grapevines in a short period of time. A vehicle speed of one kilometer per hour

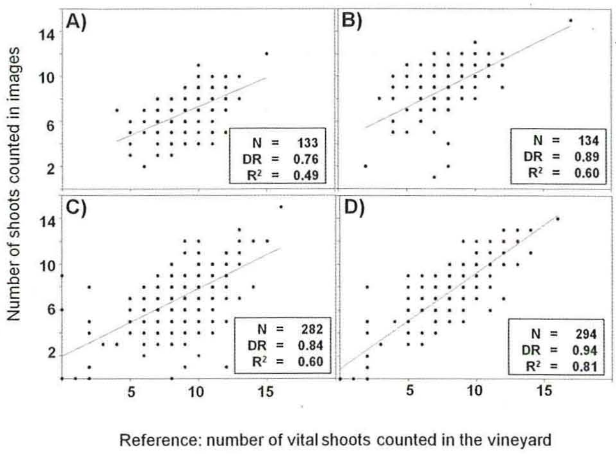

Fig. 4: Quantification of green shoots from grapevines at BBCH 10. Images were analysed by using the semi-automated Trait Quantification Tool. At the first date, images were captured with real (**A**) and artificial background (**B**). At the second (**C**) and third (**D**) date images were captured only with real background. The number of green shoots counted in images is positively correlated in all experiments to the number of vital shoots which were counted directly in the vineyard. **A**) The resulting number of shoots using the semi-automated method and images with real background is positively correlated with $R^2 = 0.49$. The detection rate (DR) was 0.76. **B**) The artificial background in images improves the contrast resulting in an increased positive correlation of $R^2 = 0.67$ and DR = 0.89. **C**) The quantification results of images with real background which were captured three days later (date 2) also showed correlations of $R^2 = 0.65$ and a DR = 0.84. **D**) The images from date 3 (another three days later) offered the best quantification results with a correlation of $R^2 = 0.81$ and a detection rate of 94 %.

permits the acquisition of images from 12 grapevines per minute (planting space 1 m). Subsequent image analysis permits the quantification of shoots for about eight images per minute on a standard computer (depending on image quality). In comparison, the recording of comparable data directly in the vineyard by a person takes one minute for three grapevines. Currently, phenotyping of bud burst is being carried out by visual inspection applying the BBCH scale (LORENZ *et al.* 1995) and the quantification of shoots is not feasible in regular grapevine breeding programmes. In contrast, PIAS enables data acquisition from 4,500 individual grapevines within six hours. It is a high-throughput image acquisition method which allows image-based phenotyping of a clearly increased number of plants.

Automated image interpretation: An automated prototype framework was developed in order to replace the TQT and reduce time for human labour. The prototype framework was tested for the detection and quantification of shoots, with the classes 'background', 'cane' and 'shoot' being used for classification. It was assumed that the class 'cane' was defined as a thin elongated object in images with minor diameter and that the class 'shoot' could only be found close to the class 'cane'. The framework was tested in experiment (a2) by using one RGB image with artificial background and in experiment (a3) by using depth maps (Fig. 2).

Within experiment (a2) the automated framework was tested for 130 single RGB images from 130 different grapevines. An average shoot detection rate of 65 % was observed in comparison with data using TQT. Furthermore, a false-positive rate of 25 % was detected. No correlation was found between the number of shoots in images and the number of shoots counted directly in the vineyard. In experiment (a3) four monochrome images were captured per grapevine and used for the calculation of depth maps. The depth calculation allowed for the generation of three-dimensional (3D) point clouds which provide precise geometric information. Depth maps assign the distance of every pixel in an image to the cameras optical centre which enables the separation between 'foreground' and 'background' in an image. Calculations were done for depth maps from 77 image sets from date 3 and the automated framework used these for the detection of 'shoots'. The detection rate of the class 'shoot' was also 63 % on average. However, the false-positive rate increased to an average of 40 %. The low detection rate combined with a high false-positive rate is thus not acceptable for a phenotyping procedure. Different reasons were determined which probably increase the false-positive detection of traits in images or depth maps. In vineyards, the wires, pheromone capsules, residues of old wooden vine tendrils, binding material or labels proved to be the most often observed sources for false-positively detected 'shoots' because these objects are also often localised close to the cane in the same plane as the real shoots. However, such false-positive 'shoots' could be used as negative training examples due to the fact that it is impossible to remove most of these objects from the vineyard.

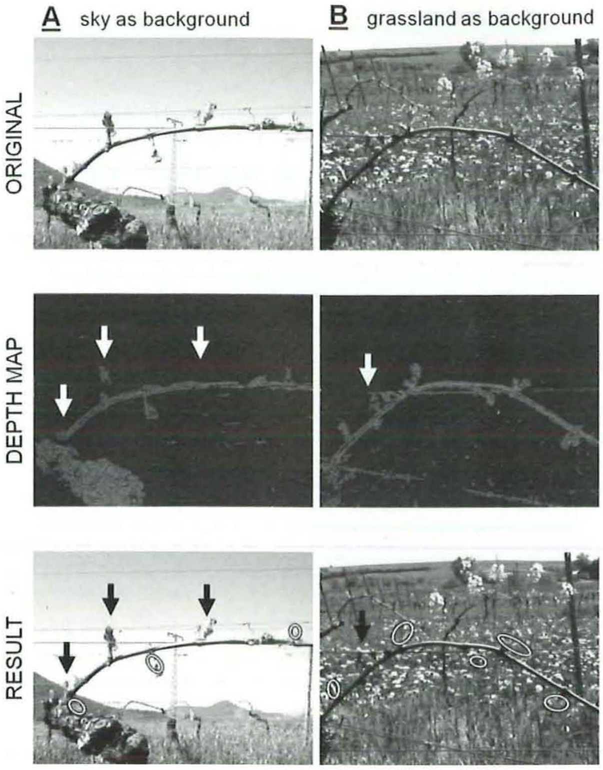

Fig. 5: Sparse depth maps lead to false classification results. The PMVS software detected both types of background with absolutely different properties (sky = far away; grassland = nearby). The used algorithm tracked pixels with vague disparity information as pixels with no disparity information to prevent errors resulting in sparse depth maps. Sparse depth maps led to errors during image classification, i.e. if shoots were not present in depth maps (white arrows), these shoots were consequently not usable for image classification (black arrows).

Additionally, the depth maps were identified as another basic reason for inaccuracy (Fig. 5). The patch-based multi-view stereo (PMVS) software tracked pixels with vague disparity information as pixels with no disparity which resulted in sparse depth maps. Disparity information was computed from points even when the homologous points could clearly be identified in all four monochrome images. This was not the case for homogeneous, non-structured or repeated structures such as plain sky, very dark spots or the wire frame. The sparse depth maps led to false classification results during image interpretation. Consequently, shoots which are not present in depth maps (white arrows in Fig. 5) are not detectable during image interpretation (black arrows in Fig. 5). Hence, adverse illumination (e.g. shadows or overexposure) and artificial objects (e.g. wire frames or trellis posts) increase the possibility for incorrect classification, resulting in low detection rates combined with high false-positive rates. The PMVS software applied for depth map calculation in the present study computes a 3D point cloud and converts the 3D information to a 2,5D depth map. A more sophisticated approach would be the direct computation of a depth, for example using the approach of KLODT et al. (2008).

Furthermore, the various qualities of the images also led to a higher inaccuracy rate. The images were captured in the vineyard under natural light conditions which resulted in shadows (image capture towards the sun) or brightness illumination (black artificial background) of plant traits. Both shadows and brightness on young shoots reduced the contrast of the images and resulted in non-natural colours, e.g. white-coloured instead of green shoots. Colours were thus disregarded from the whole framework. The application of flashes could further improve the quality of images, in turn leading to improved depth map calculation and an increased detection rate because colours could be used as supplementary information (e.g. the green colour of 'shoots' and the brown colour of 'canes'). Interpretation of images with real background was conducted by using depth maps. Improvement of the high-throughput image recording was achieved by the expansion of the calibrated monochrome cameras with an additional RGB camera and the implementation of this camera unit into the IGG-GEOTAGGER software. This also allowed for the consideration of colours within the automated image interpretation.

Semi-automated determination of the grapevine berry size: The semi-automated tool TST was developed in order to enable a contactless and non-invasive determination of the mean grapevine berry size in images. 39 single RGB images were read in MATLAB® and the contour of 50 berries per image marked with three points. TST fitted a circle through these three points (Fig. 6A). The diameter of the fitted circles was automatically measured in mm utilising a predefined scale. Histograms were created to verify the normal distribution of the results (Fig. 6B). In order to confirm the results of TST, reference data were acquired using the fully automated software tool BAT. BAT enables the fast and precise, image-based determination of the diameter from individual berries placed on a specific plate (Kicherer *et al.* 2013). The average berry size from the four investigated cultivars 'Dornfelder', 'Pinot Blanc', 'Pinot Noir', and 'Riesling' (Fig. 6C) which was measured with TST showed an overestimation of 0.9 to 1.8 mm in comparison to the measured sizes applying BAT. Nonetheless, a positive correlation of $R^2 = 0.97$ was found between the observed berry size applying BAT (destructive) and TST (non-destructive). The berry size (TST) of almost all cultivars showed significant differences ($\rho < 0.01$). No significant difference was only found between the berry size of 'Riesling' (average 14.8 mm) and 'Pinot Blanc' (14.6 mm). Thus, differences in berry size of 1 mm could be detected in an easy and non-invasive way by capturing one RGB image directly in the vineyard. The overestimation of the berry size in comparison to the results from BAT could be explained by the quality of images and the applied scale reference. As described previously shadows or brightness on grapevine berries resulted in lower contrast in the images. The fact that the contrast was reduced complicated the clear recognition of the berries′ edge which in turn is associated with increased error variation. In addition, the used reference for scaling in the images was positioned in only one plane. However, plants and grape clusters possess a three-dimensional architecture, meaning that berries can be positioned closer to the camera or further away than the label. Consequently berries being closer to the camera appear greater and berries which are further away appear smaller than they are in reality. The usage of only one scale for the whole image rather than a depth map could be one fundamental reason for the variance of the results from TST and BAT.

For future work the development of an automated framework will be a promising method in order to realise high-throughput image interpretation for non-invasive and contactless determination of the mean berry size. In this way, phenotyping of the berry size could be conducted for a large amount of grapevine varieties or individual plants of e.g. a breeding population.

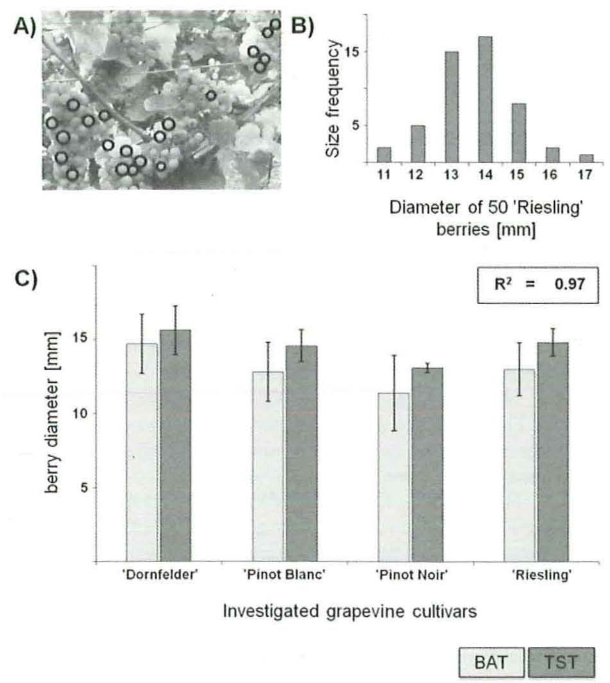

Fig. 6: Semi-automatic determination of the grapevine berry diameter. **A)** Image detail with manually marked circles. **B)** Histogram of the diameter [mm] of 50 'Riesling' berries in one image. The manually marked berries were measured automatically. **C)** Comparison of berry size from different cultivars 'Dornfelder', 'Pinot Blanc', 'Pinot Noir' and 'Riesling'. Light-grey - reference data from BAT; dark-grey – data from TST. A correlation of $R^2 = 0.97$ between BAT (destructive) and TST (non-destructive) was observed. BAT – Berry Analysis-Tool; TST – Trait Size Tool.

Conclusions

Within the present study initial steps were investigated in order to enable high-throughput phenotyping in vineyards by using images and depth maps. First of all, a prototype system (PIAS) was built for higher throughput image capture in an experimental vineyard. In order to phenotype important traits (shoots at BBCH 10 and berry diameter) the captured single RGB images were analysed by applying two semi-automated tools: Trait-Size-Tool (TST) and Trait-Quantification-Tool (TQT). In contrast to visual inspections by persons in the vineyard semi-automated image analysis approaches need less human labour and deliver valid results (detection rate up to 94 %). For the reduction of human labour an automated prototype framework was developed and tested in order to detect shoots after bud burst at BBCH 10 automatically by using 2.5D depth maps. Based on more or less sparse depth maps and lacking colour information a false-positive detection rate of up to 62 % was obtained. Reasons for this were identified. The regarding points should thus be eliminated during image capture. Further improvements were discussed in order to increase the accuracy of the automated framework for future work. The presented approaches and the implementation of the stated improvements facilitate an automated, image-based phenotyping in vineyards with higher throughput, more precise objective data and decreased error rate.

Acknowledgements

This work was supported by the AgroClustEr: CROP.SENSe.net (FKZ 0315534) and PHENOvines (FKZ 0315968A) which are both funded by the German Federal Ministry of Education and Research (BMBF) within the scope of the competitive grants programme Networks of excellence in agricultural and nutrition research. We gratefully acknowledge P. Zeimetz and L. Klingbeil from the group of H. Kuhlmann for supporting the development of Igg-Geotagger. Additionally, we thank T. Wahl (Julius Kühn-Institut, Grapevine Breeding) who provided reference evaluation data. Further we thank I. Fechter, W. Köglmeier and H. Heupel for critically reading the manuscript.

References

ABRAHAM, S.; HAU, T.; 1997: Towards autonomous high-precision calibration of digital cameras. Videometrics **5**, 3174, 82-93.

BATES, T.; GROCHALSKY, B.; NUSKE, S.; 2011: Automating measurements of canopy and fruit to map crop load in commercial vineyards. Research news from Cornell's viticulture and enology. Res. Focus **4**, 1-6

BERENSTEIN, R.; SHAHAR, O. B.; SHAPIRO, A.; EDAN, Y.; 2010: Grape clusters and foliage detection algorithms for autonomous selective vineyard sprayer. Intelligent Service Robotics **3**, 233-243.

BOYKOV, Y.; VEKSLER, O.; ZABIH, R.; 1998: Markov random fields with efficient approximations. Computer vision and pattern recognition, 1998. Proceedings 1998 IEEE Computer Society Conference, 648-655.

BRAUN, T.; KOCH, H.; STRUB, O.; ZOLYNSKI, G.; BERNS, K.; 2010: Improving pesticide spray application in vineyards by automated analysis of the foliage distribution pattern in the leaf wall, 477-486. Commercial Vehicle Technology 2010 - Proceedings of the 1st Commercial Vehicle Technology Symposium (CVT 2010), March 16-18, Kaiserslautern, Germany.

BROWN, M. V.; MOORE, J. N.; FENN, P.; MCNEW, R. W.; 1999: Comparison of leaf disk, greenhouse, and field screening procedures for evaluation of grape seedlings for downy mildew resistance. Hortscience, **34**, 331-333.

CORREA, C.; VALERO, C.; BARREIRO, P.; 2012: Characterization of vineyard's canopy through fuzzy clustering and svm over color images. International Conference of Agricultural Engineering 2012, June 8-12.

DIAGO, M. P.; CORREA, C.; MILLAN, B.; BARREIRO, P.; VALERO, C.; TARDAGUILA, J.; 2012: Grapevine Yield and Leaf Area Estimation Using Supervised Classification Methodology on RGB Images Taken under Field Conditions. Sensors, 12, 16988-17006.

EIBACH, R.; ZYPRIAN, E.; WELTER, L.; TÖPFER, R.; 2007: The use of molecular markers for pyramiding resistance genes in grapevine breeding. Vitis **46**, 120-124.

FAIRLIE, K.; WHITTY, M.; LEACH, M.; NORZAHARI, F.; WHITE, A.; COSSELL, S.; GUIVANT, J.; KATUPITYA, J.; 2011: Spatially Smart Wine – Testing Geospatial Technologies for Sustainable Wine Production. International Federation of Surveyors (FIG) Working Week.

FURBANK, R. T.; TESTER, M.; 2011: Phenomics - technologies to relieve the phenotyping bottleneck. Trends Plant Sci. **16**, 635-644.

FURUKAWA, Y; PONCE, J; 2010: Accurate, dense, and robust multi-view stereopsis. IEEE Transactions on pattern analysis and machine intelligence, vol. **32**, 1362-1376.

GRANIER, C.; AGUIRREZABAL, L.; CHENU, K.; COOKSON, S. J.; DAUZAT, M.; HAMARD, P.; THIOUX, J. J.; ROLLAND, G.; BOUCHIER-COMBAUD, S.; LEBAUDY, A.; MULLER, B.; SIMONNEAU, T.; TARDIEU, F.; 2006: PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in Arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit. New Phytol. **169**, 623-635.

HARTMANN, A.; CZAUDERNA, T.; HOFFMANN, R.; STEIN, N.; SCHREIBER, F.; 2011: HTPheno: An image analysis pipeline for high-throughput plant phenotyping. BMC Bioinformatics, 12.

IYER-PASCUZZI, A. S.; SYMONOVA, O.; MILEYKO, Y.; HAO, Y.; BELCHER, H.; HARER, J.; WEITZ, J. S.; BENFEY, P. N.; 2010: Imaging and analysis platform for automatic phenotyping and trait ranking of plant root systems. Plant Physiol. **152**, 1148-1157.

JONES, H. G.; SERRAJ, R.; LOVEYS, B. R.; XIONG, L. Z.; WHEATON, A.; PRICE, A. H.; 2009: Thermal infrared imaging of crop canopies for the remote diagnosis and quantification of plant responses to water stress in the field. Funct. Plant Biol. **36**, 978-989.

KICHERER, A.; ROSCHER, R.; HERZOG, K.; ŠIMON, S.; FÖRSTNER, W.; TÖPFER, R.; 2013: BAT (Berry Analysis Tool): A high-throughput image interpretation tool to acquire the number, diameter, and volume of grapevine berries. Vitis **52**, 129-135.

KLODT, M.; SCHOENEMANN, T.; KOLEV, K.; SCHIKORA, M.; CREMERS, D.; 2008: An Experimental Comparison of Discrete and Continuous Shape Optimization Methods. Proceeding ECCV 2008 Proceedings of the 10th European Conference on Computer Vision: Part I, 332-345.

LONGO, D.; PENNISI, A.; BONSIGNORE, R.; MUSCATO, G.; SCHILLACI, G.; 2010: Multifunctional Tracked Vehicle Able to Operate in Vineyards Using GPS and Laser Range-finder Technology. International Conference Ragusa SHWA2010 - September 16-18, 2010 Ragusa Ibla Campus, Italy, 487-492.

LORENZ, D. H.; EICHHORN, K. W.; BLEIHOLDER, H.; KLOSE, R.; MEIER, U.; WEBER, E.; 1995: Growth Stages of the Grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L. ssp. *vinifera*)-Codes and descriptions according to the extended BBCH scale. Aust. J. Grape Wine Res. **1**, 100-103.

MAZZETTO, F.; CALCANTE, A.; MENA, A.; VERCESI, A.; 2010: Integration of optical and analogue sensors for monitoring canopy health and vigour in precision viticulture. Precision Agric. **11**, 636-649.

MOLLER, M.; ALCHANTIS, V.; COHEN, Y.; MERON, M.; TSIPRIS, J.; NAOR, A.; OSTROVSKY, V.; SPRINTSIN, M.; COHEN, S.; 2007: Use of thermal and visible imagery for estimating crop water status of irrigated grapevine. J. Exp. Bot. **58**, 827-838.

NUSKE, S.; ACHAR, S.; BATES, T.; NARASIMHAN, S.; SINGH, S.; 2011: Yield Estimation in Vineyards by Visual Grape Detection. 2011 Ieee/Rsj International Conference on Intelligent Robots and Systems.

OIV; 2001: 2nd edition of the OIV descriptor list for grape varieties and *Vitis* species. http://www.oiv.int

RAMOS, A. B.; RIQUELMA, F. M.; GARCIA, F. M.; OROZCO, L.; RONCERO, J. J.; 2012: Precision viticulture using a wireless sensor network. XXVIII International Horticultural Congress on Science and Horticulture for People (Ihc2010). International Symposium on the Effect of Climate Change on Production and Quality of Grapevines and their Products, **931**, 307-313.

SCHWANDER, F.; EIBACH, R.; FECHTER, I.; HAUSMANN, L.; ZYPRIAN, E.; TÖPFER, R.; 2012: Rpv10: a new locus from the Asian *Vitis* gene pool for pyramiding downy mildew resistance loci in grapevine. Theor. Appl. Genet. **124**, 163-176.

SETTLES, B.; 2009: Active learning literature survey. Computer Science Technical Report 1648. University of Wisconsin-Madison, retrieved 2010-09-14.

STAUDT, G.; 1997: Evaluation of resistance to grapevine powdery mildew (*Uncinula necator* [Schw.] Burr., anamorph *Oidium tuckeri* Berk.) in accessions of *Vitis* species. Vitis **36**, 151-154.

STOLL, M.; SCHULTZ, H. R.; BAECKER, G.; BERKELMANN-LOEHNERTZ, B.; 2008: Early pathogen detection under different water status and the assessment of spray application in vineyards through the use of thermal imagery. Precision Agric. **9**, 407-417.

ZANG, J. K.; HAUSMANN, L.; EIBACH, R.; WELTER, L. J.; TÖPFER, R.; ZYPRIAN, E. M.; 2009: A framework map from grapevine V3125 (*Vitis vinifera* 'Schiava grossa' x 'Riesling') x rootstock cultivar 'Börner' (*Vitis riparia* x *Vitis cinerea*) to localize genetic determinants of phylloxera root resistance. Theor. Appl. Genet. **119**, 1039-1051.

Received January 1, 2013