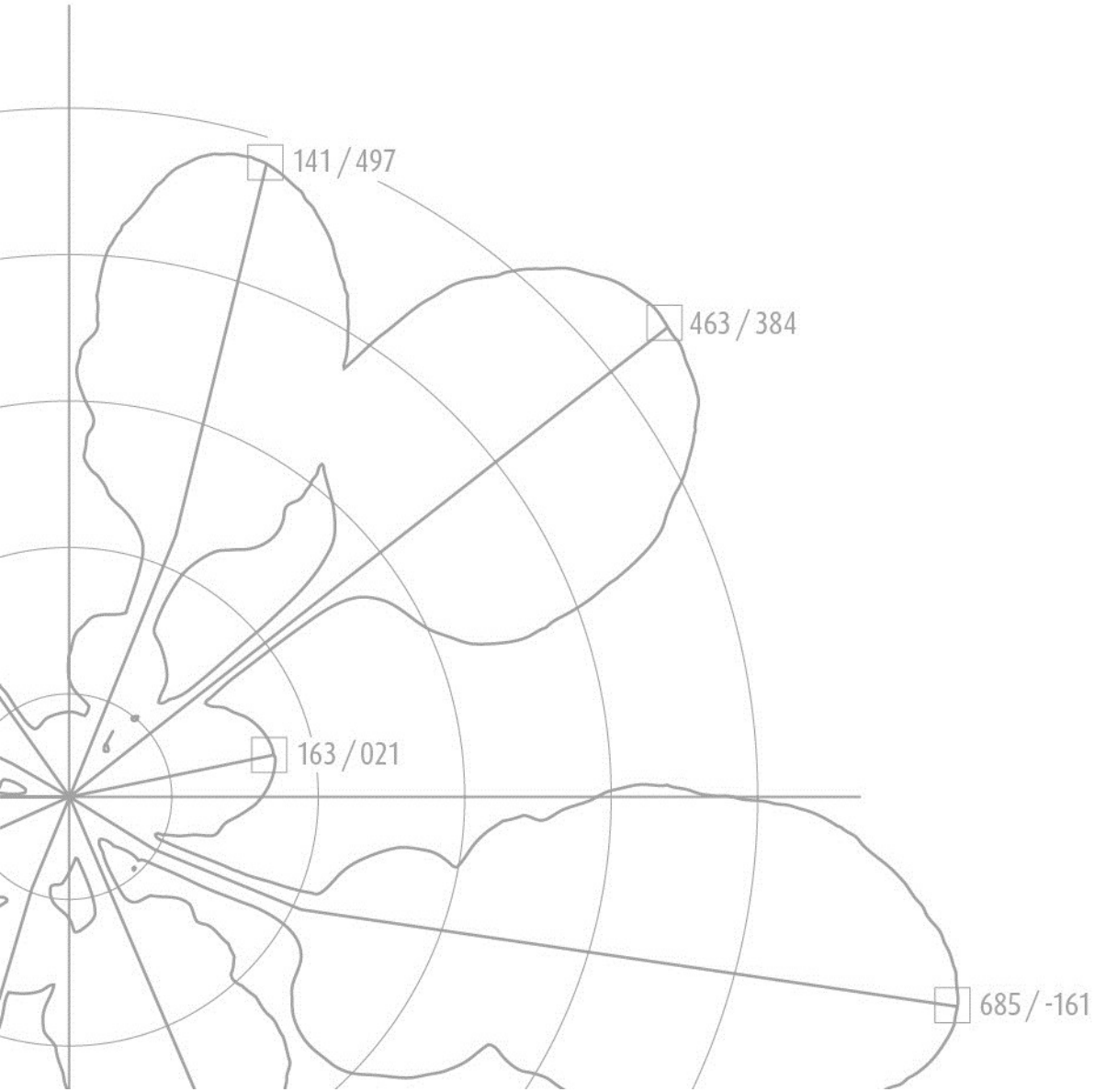
LemnaTec

Leaf assay

Colour Classification

Quantification of disease symptoms on leaf surfaces



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Introduction

Diseases in plant production are a major thread for mankind's supply with food, plant-derived products and ornamental plants. All parts of the plant (roots, shoots, leaves, flowers, fruits) may potentially be subjected to attack by pests or diseases. The mechanism with which a pathogen or pest damages a plant are diverse ranging from eating parts of the plant to infection and destruction on the cellular level. The latter strategy is commonly used by microbes such as fungi or bacteria, and by viruses. Infection usually starts in a small region of the leaf. The organism grows by consuming the plant's resources and eventually kills cells at the infection site and in surrounding tissue. On the other side, plants can respond with various defence mechanisms such as fortifying cell walls, producing secondary metabolites or programmed cell death to kill the pathogen or prevent it from spreading. This interplay between disease spreading and plant defence leads to disease-specific symptoms. For many diseases it is well-known that part of the leaf surface changes its color due to the degradation of light-harvesting pigments and subsequent necrosis. These visible changes can be measured either by manual scoring or by quantitative image analysis. Assessing reliably disease symptoms in a standardized manner and with high-throughput is a major requirement in breeding for plant resistance against pests and diseases, and/or screening for new bio-active compounds. In this paper we present a workflow to quantify colour changes on leaf surfaces, typically denoted as lesions, using LemnaTec imaging technology. As examples, we studied sugar beet and rose leaves. We further demonstrate that the same analysis can be applied to reflection and transmission measurements.

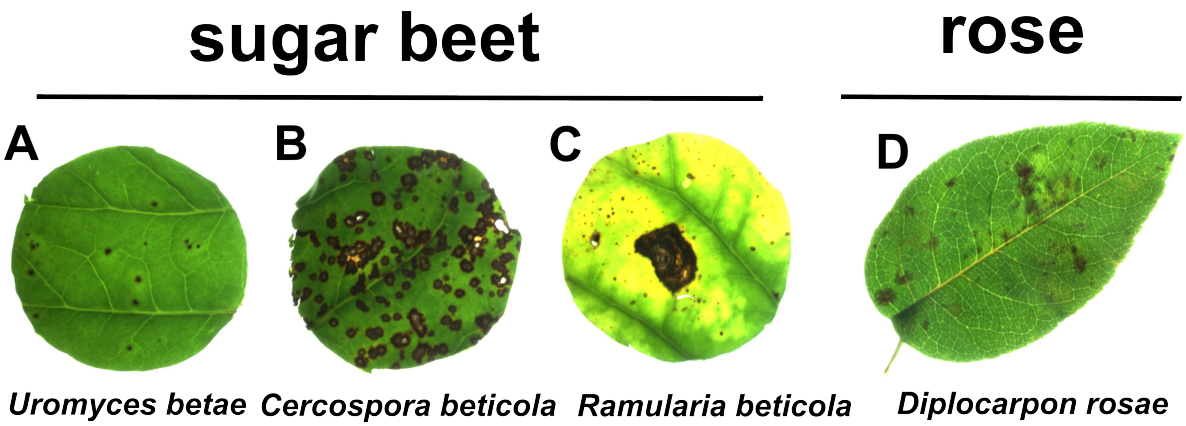

Figure 1: Sugar beet and rose leaves measured with Scanalyer HTS / PL.

Imaging requirements

In principle, 2D imaging can be done in two ways: transmission and reflection. Using transmission measurement has the advantage that light passes through the entire leaf surface thereby collection information on the optical properties of the entire layer it has gone through. However it can only be applied for relatively thin and translucent objects such as leaves. On the other hand reflection measurements give information on the optical properties of the objects surface. Standardized light conditions, ideally diffuse light, are important to ensure image good quality for unsupervised automated analysis. Investigating leaf surfaces mostly light reflection from outermost cell layers is measured. Note that reflection measurements become particularly difficult if leaf epidermis is covered with a glossy layer of wax leading to direct reflection from leaf surfaces and no diffuse light is available. In the following section we present a protocol to analyse digitalised images from leaf samples under

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different lighting conditions. Our goal is to detect and characterise green, yellow and brown regions in leaf tissues.

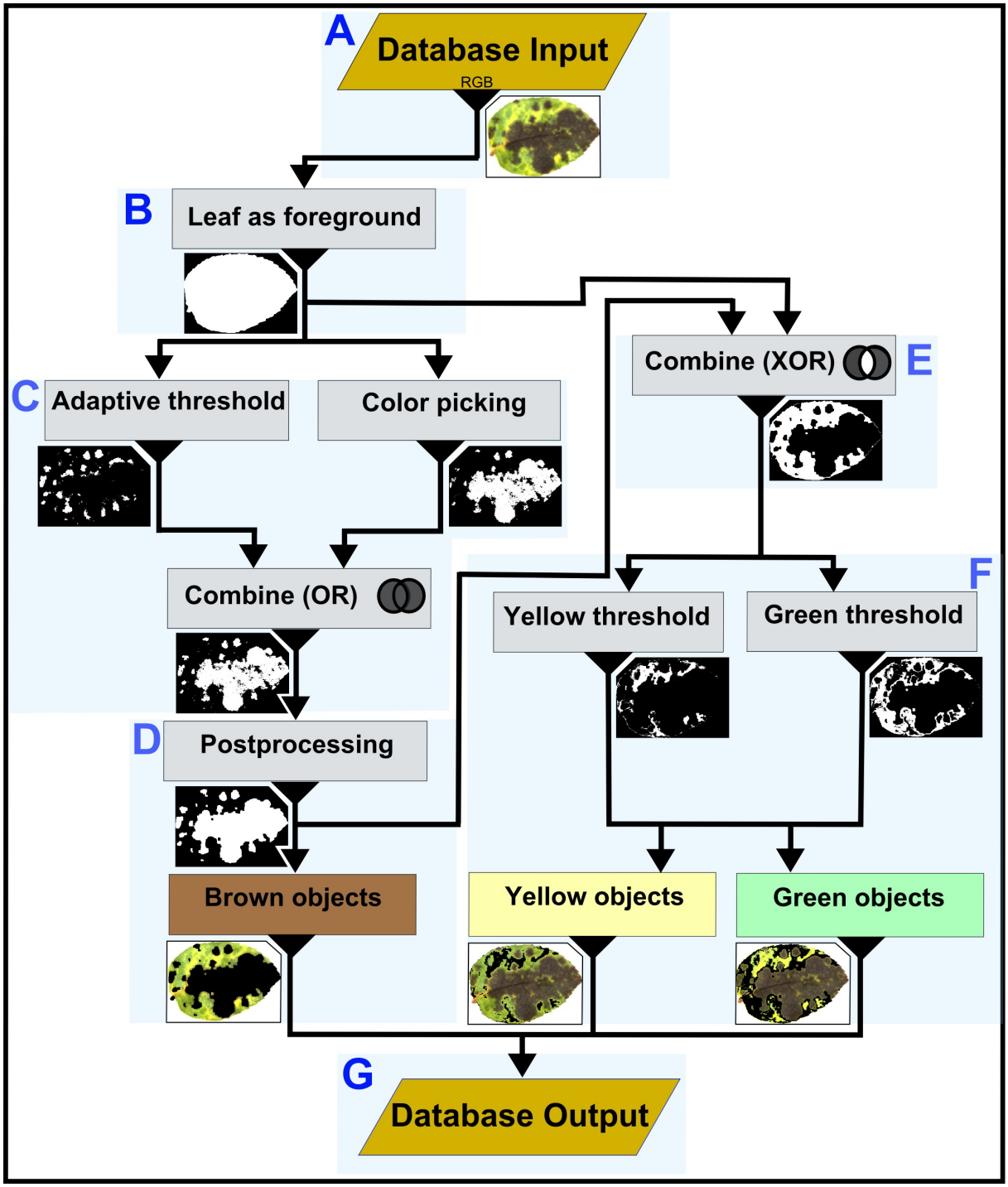

Figure 2: Flowchart to illustrate the image processing approach. Blue boxes highlighted with capital letters represent modules identical to Fig. 3 and are described in the text.

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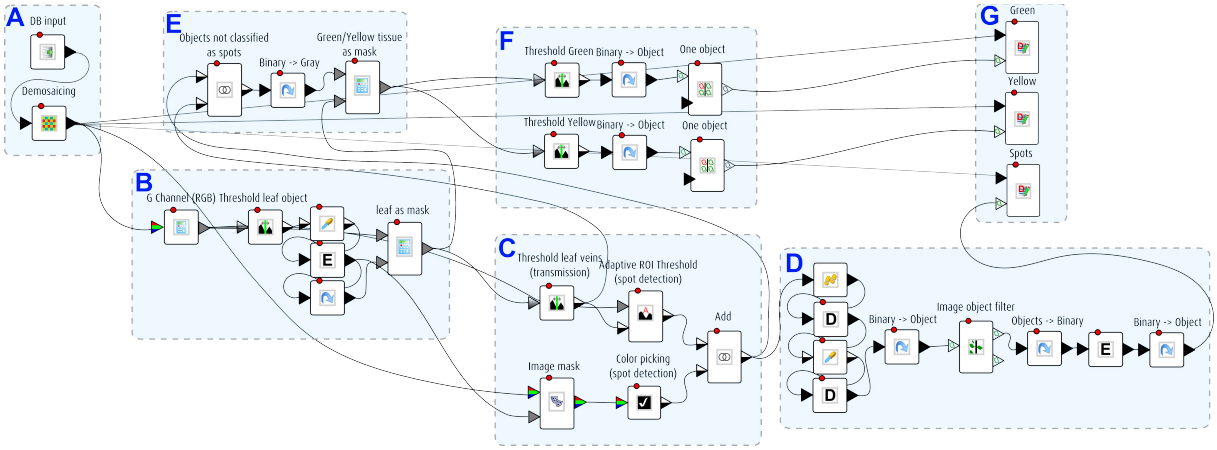

Figure 3: Implementation of the image processing in LemnaGrid software. Icons represent devices and do specific tasks. The dataflow is from left Database Input (A) to right Database writing (G). Blue boxes highlighted wit capital letters represent modules identical to Fig. 2 and are described in the text.

Plant material

We collected sugar beet leaves from a field trial at the Julius Kühn Institut, Institut für Pflanzenschutz in Ackerbau und Grünland, Elsdorf, Germany, with different leaf diseases such as *Uromyces betae*, *Cercospora beticola*, and *Ramularia beticola* (Fig. 1A-C). Leaf discs of 4 cm diameter were cut from these leaves and images were taken in the Scanalyzer HTS with back light illumination for transmission measurements. For reflection measurements we collected ten rose leaves with *Diplocarpon rosae*, a wide-spread rose disease, from a garden. These leaves were imaged in a Scanalyzer PL with diffuse top light for reflection measurements (Fig. 1D).

Approach

LemnaGrid operates in a sequential fashion: reading an input image from the database, applying image processing operations, and writing analysis output back to the database. The workflow is schematically illustrated in Figure 2 and its implementation in LemnaGrid software is given in Figure 3. The principal steps are:

A: Load images from database. Subsequent demosaicing is the process to reconstruct a full colour image from the spatially under-sampled colour channels from the colour filter array (image sensor).

B: The GREEN Channel in the RGB image is used to discriminate the leaf from the white background and to separate leaves into green, yellow and brown tissue. As result one obtains an image mask (binary image).

C: Brown spots are detected using two filters: (i) Adaptive region of interest (ROI) threshold and (ii) Colour-based classification. The adaptive ROI filter computes differences of each pixel value with respect to its local neighbourhood. This method is used to detect small spots. Large spots are detected using the colour-based classification, where a set of manually predefined signature spot colours is used. Both results are combined to a binary image.

D: Post-processing, transformation of interconnected pixels to objects, assignment of colours and shape parameters to each object.

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E: The remaining, not classified green/yellow pixels are filtered.

F: The global threshold for the GREEN pixel value is used to separate between green yellow.

G: Saving data to database.

Note that the analysis parameters were once set for sugar beet and rose leaves. Thereafter all images were analysed using the same set of parameters, which is a prerequisite for automated and unsupervised image processing.

After analysis, data was queried using LemnaGrid Software to extract shape parameters (Tab. 1). Pie charts were plotted using Octave software.

Results

Using the proposed image processing approach with LemnaGrid we were able to detect colour changes in sugar beet images caused by *Uromyces betae* , *Cercospora beticola* and *Ramularia beticola* (Fig. 4). We analyzed 8 leaf discs as control, 6 with *Uromyces betae*, 10 with *Cercospora beticola*, and 3 with *Ramularia beticola*. The mean relative amount of green, yellow and brown tissue per leaf reflected the visual observation well (Fig. 4). Table 1 shows the overview of all standard shape parameters computed by LemnaGrid software. Note that control leaf discs also showed spots, although on average only 0.2% of the investigated leaf tissue. Size and shape of these spots suggest that they could caused either by *Uromyces* or *Cercospora* (Fig. 4, Tab. 1). However it is also probable that they had no pathogenic origin but mechanical damage or random cell death events.

In a second case study we analysed 10 rose leaves using a different imaging set-up (reflection). After readjustment of the image analysis parameters (to detect brown, yellow or green pixels) we were able to classify leaf colours (Fig. 5). The pie chart in Figure 5 summarizes the mean distribution of green/yellow and brown tissue. The analysed shape parameters for *Diplocarpon* leaf spots were different compared to the previously show sugar beet symptoms.

Conclusion

We presented a workflow to quantify disease symptoms and tested this using two different imaging set-ups, leaves of two different plant species and four different diseases. The analysis is robust. Repeating this analysis with a larger dataset would allow establishing a footprint to identify disease symptoms based on the morphological shape properties. Note that the presented workflow would also allow quantify and classify various color changes in leaves caused by various biotic and abiotic stresses.

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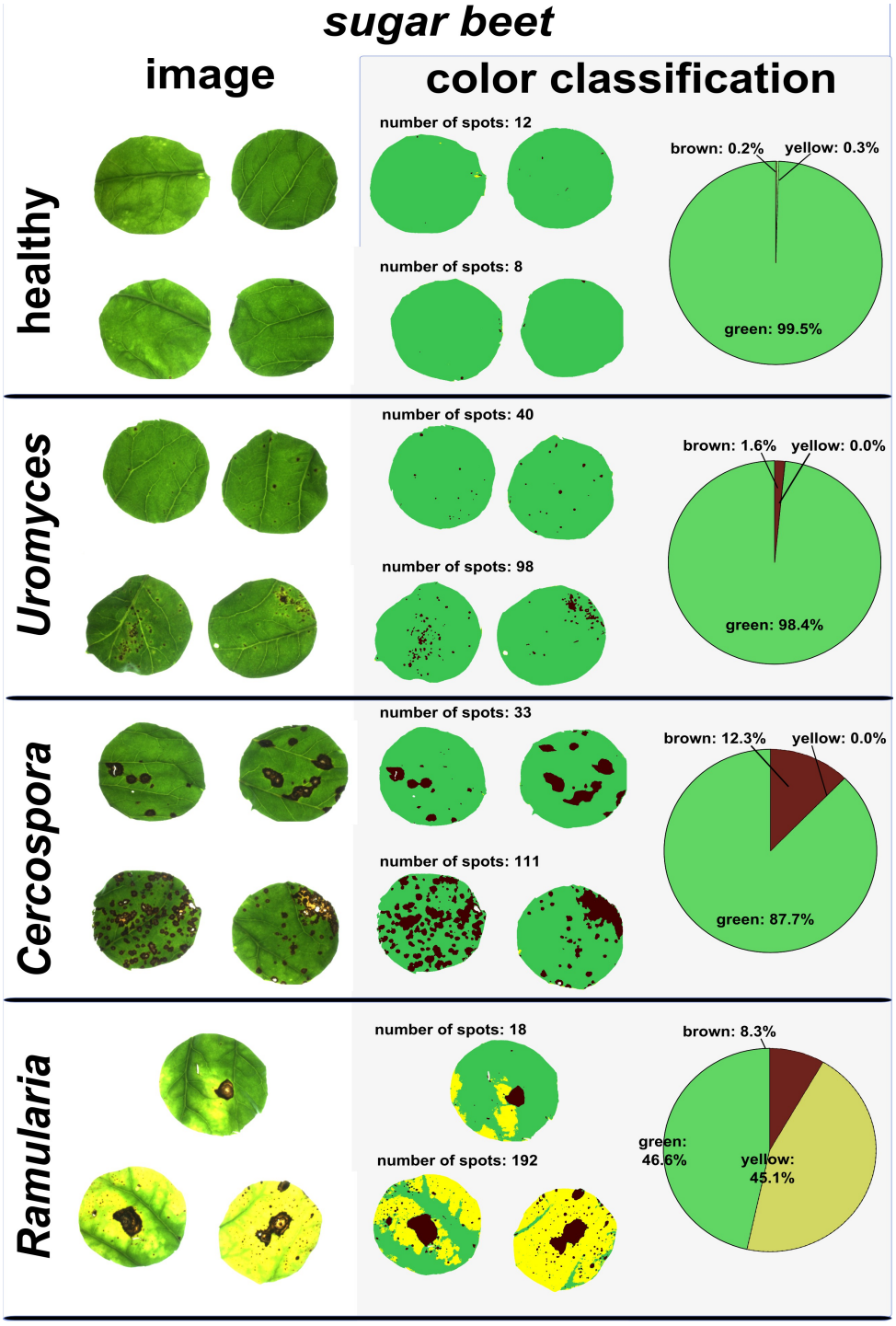

Figure 4: Left: Sugar beet leaf discs with yellow/brown disease symptoms caused by *Uromyces betae*, *Cercospora beticola* or *Ramularia beticola*. Middle: Leaf discs with colour classification into green, yellow and brown areas using the introduced image processing approach. The number of detected spots per image is given. Right: Pie chart summarizing the average colour distribution over all sampled leaf discs. 8 healthy leaf discs, 6 with *Uromyces*, 10 with *Cercospora*, and 3 with *Ramularia* were analysed

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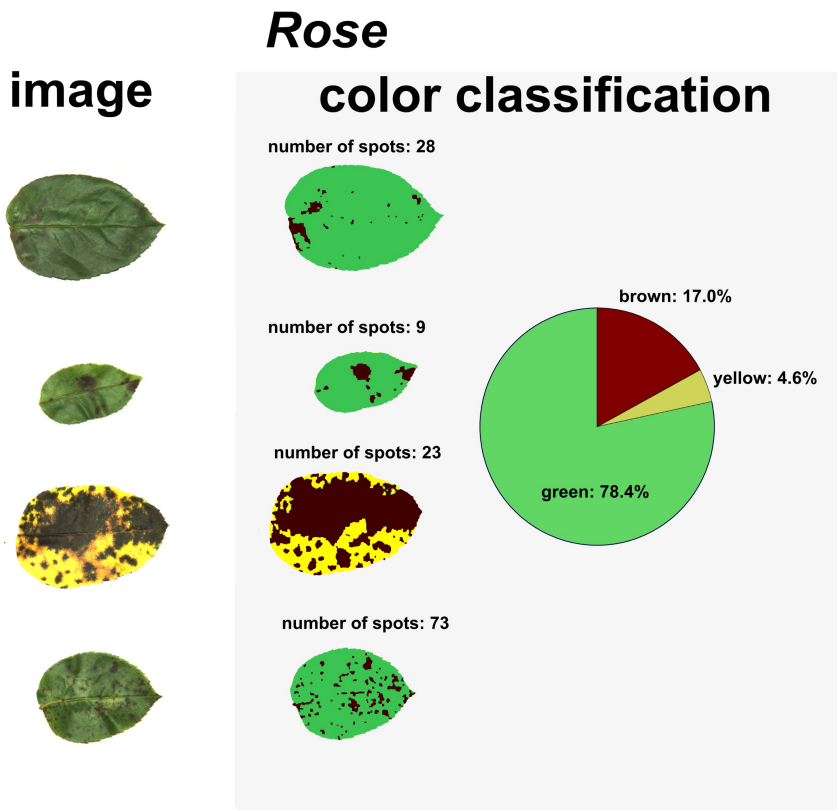

Figure 5: Left: Rose leaf with yellow/brown disease symptoms caused by *Diplocarpon rosae*. Middle: Leaves displayed with colour classification into green, yellow and brown areas using the introduced image processing approach. The number of detected spots per leaf is given. Right: Pie chart summarizing the average colour distribution over all sampled 10 leaves.

Table 1: Summary of mean, minimum and maximum values for shape parameters to characterize disease spots as standard output of the LemnaGrid software.

	Beta vulgaris												*Rose*		
	healthy			*Uromyces*			*Cercospora*			*Ramularia*			*Diplocarpon*		
	min	mean	max	min	mean	max	min	mean	max	min	mean	max	min	mean	max
area (px)	28.8	652.7	8321.0	28.3	412.7	3475.7	30.0	598.6	6901.8	27.5	511.5	4759.0	25.0	787.2	115568
circumference (px)	16.5	63.0	307.8	16.3	60.4	195.6	16.9	68.0	296.3	16.0	66.2	237.0	15.0	76.9	3223.1
compactness (dimensionless)	0.9	1.0	1.0	0.9	1.0	1.0	0.8	1.0	1.0	0.8	1.0	1.0	0.5	1.0	1.0
roundness (dimensionless)	9.4	14.2	26.5	9.4	14.3	27.6	9.5	16.0	33.4	9.3	15.0	28.3	9.0	17.2	89.9
distance center of mass to boundary (px)	1.6	5.6	32.2	1.5	5.3	21.3	0.6	5.1	28.0	1.5	5.4	25.1	-4.3	3.8	60.0
boundary points roundness (dimensionless)	10.7	17.5	31.5	10.6	17.6	32.4	10.8	19.7	40.7	10.5	18.3	33.6	10.2	21.1	153.4
boundary points : area ratio (dimensionless)	0.1	0.4	0.6	0.2	0.4	0.6	0.1	0.4	0.6	0.2	0.4	0.6	0.0	0.4	0.6
2nd moment large principal axis (dimensionless)	0.1	0.1	0.3	0.1	0.1	0.3	0.1	0.1	0.4	0.1	0.1	0.3	0.1	0.1	0.6
2nd moment small principal axis (dimensionless)	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1	0.0	0.1	0.1
2nd moment normalized z-rotation (dimensionless)	42.8	70.2	125.8	36.0	60.2	108.0	39.4	65.2	113.6	34.5	58.5	109.5	0.00	0.19	0.84

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