Digital Processing of Light Microscopy Images in Plant Pathogen Diagnostics

Jiří Sedlář,[1,2] Michaela Sedlářová,[3] Jan Flusser[1]

[1] Academy of Sciences of the Czech Republic, Institute of Information Theory and Automation, Department of Image Processing, Prague, Czech Republic [2] Charles University, Faculty of Mathematics and Physics, Prague, Czech Republic [3] Palacký University, Faculty of Science, Department of Botany, Olomouc, Czech Republic

E-mail: sedlar@utia.cas.cz, michaela.sedlarova@upol.cz, flusser@utia.cas.cz

1 Introduction

The determination of the causal agent of a plant disease is currently based on a combination of host specificity, symptoms, microscopic characters and/or molecular markers [1]. Although the relevance of phenotypic characters has been questioned [3], microscopic investigation remains an indispensable method for determination of fungal diseases (mycoses) in phytopathological practice. However, in comparison to a relatively objective analysis of molecular markers, this method depends on systematic practice and experience of the plant pathologist; the other methods suffer from high costs, availability for a limited number of pathogens, and requirement of a pre-determination.

Downy mildews (order Peronosporaceae, class Peronosporomycota, kingdom Chromista) are highly specific fungi-like parasites which cause severe infections of plants. The diseases are easily spread in wet weather by conidiosporangia [4]. For the purposes of determination, the traditional species concept based on morphometry (size of infection structures) has been discussed with Gäumann's "biological species" and "eco-physio-phenetic" concept [3]. Currently, the relevance of microscopic (light microscopy) vs. ultramicroscopic (scanning electron microscopy, SEM) features and their coherence with molecular data is being re-evaluated by taxonomists [6, 7]. However, this task has not been solved to a satisfactory extent yet so as to serve as a practical field identification system. In this work, methods for (semi)automatic classification from light microscopy images are tested on the model of downy mildews asexual reproduction structures.

2 Methods and Results

For the case study, representatives of five genera (one species per genus) with distinct morphomerical characteristics and frequent occurrence in Central Europe were pre-selected: *Bremia lactucae* (lettuce downy mildew), *Plasmopara halstedii* (sunflower d.m.), *Peronospora destructor* (onion d.m.), *Pseudoperonospora cubensis* (d.m. of cucurbits), and *Phytophthora infestans* (potato late blight). Digital microscopy images of specimens with relatively developed morphological features from the biological point of view were used to develop a semiautomatic classification method based on the properties of conidiosporangiophores and conidiosporangia. The photographs were taken with the Olympus DP70 CCD digital camera attached to the Olympus BX60 light microscope with 200× and 400× magnification, respectively.

In order to suppress degradations introduced during the acquisition process, several preprocessing steps are necessary. In particular, as the specimens are thicker than the attainable depth of field, parts of them appear out of focus. Whereas conidiosporangia are round and their shape can be estimated from a single image, image fusion is necessary in the case of conidiosporangiophores. Images at different focal planes are taken and composed by means of digital multi-focus fusion [8] into one image with the whole specimen in focus. If the microscopic slides are shifted during this process, image registration by means of a rigid-body transformation [9] is used to compensate for the displacements. For every specimen, selected features of conidiosporangia and/or conidiosporangiophores are computed from the preprocessed image. The specimen is then classified according to these features by a classifier.

The conidiosporangia are mainly characterized by their shape (spherical/ovoid/ellipsoid, with/without papilla). They are segmented from image background by means of a convenient segmentation method, such as adaptive thresholding. Mathematical morphology is employed to remove debris and rectify small irregularities on the boundary of segmented objects, as well as to remove artificial holes. The result is a binary image representing the shapes of conidiosporangia. In order to eliminate partly occluded conidiosporangia, the objects that intersect image borders and the objects that represent more that one conidiosporangium are also removed. The remaining objects are then labeled and processed separately.

For each object representing a conidiosporangium, convenient features are computed. Fourier descriptors [5], which classify an object according to the shape of its boundary by means of a set of several Fourier coefficients of its 1D representation, and object area proved best for this purpose. The features

of all conidiosporangia in one image are used to classify the specimen. However, due to high variability within some species (e.g., *Plasmopara halstedii*) and thus, in some cases, high similarity to other species (*Bremia lactucae*), some specimens cannot be well determined by means of conidiosporangia. In such a case, a classification based on conidiosporangiophores is necessary.

The conidiosporangiophores are characterized particularly by their structure. They are segmented from the microscopy images by means of thresholding or edge detection and linking. As the conidiosporangiophores are usually hyaline, morphological closing is used to fill their branches. Their structure is represented by the morphological skeleton, a thin-line continuous curve, which can be computed from the segmented images by means of a parallel thinning algorithm described in [2, Algorithm A1]. The skeleton is then divided into branches, i.e., linear segments corresponding to non-branching parts of the conidiosporangiophore.

Due to common overlapping of branches, local features are used for the classification of conidiosporangiophores, namely the pattern of branching (monopodial/sympodial/dichotomous/sparse) and the curvature of branches. They usually provide satisfactory level of discriminability among the species.

3 Discussion and Conclusions

According to our results, the species of studied crop pathogens were distinguishable by the properties of asexual reproduction structures. The classification based on conidiosporangia is easy and robust to irregularities and outliers but does not provide good discriminability in some cases. This can be usually achieved by classification based on conidiosporangiophores, which is, however, more complex and less robust, particularly due to difficult segmentation.

As the methods kept differences among closely relative genera, many future perspectives may be addressed, e.g. to test the usability for other well-defined taxa, for diagnosis of species within a genus, or to combine it with data from SEM and molecular analysis. Nevertheless, the conception of the defined characteristics of biological taxons is in dynamic progress, which might stand a limitation to suitability of the presented methods.

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