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Editorial

Epidemiology and Control of Fungal Diseases in Crop Plants

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Abstract: Crop fungal diseases are a major threat to crop health and food security worldwide. The epidemiology is the basis for effective and sustainable control of crop fungal diseases. Safe, effective, sustainable, and eco-friendly disease control measures have important economic, ecological, and social significances. This Special Issue, "Epidemiology and Control of Fungal Diseases of Crop Plants", collected one communication and nine original research articles focusing on the identification and detection of the causal agents of alfalfa Fusarium root rot, strawberry black spot, and barley leaf stripe; the semantic segmentation of wheat stripe rust images; the image-based identification of wheat stripe rust and wheat leaf rust; the image-based identification of the severity of wheat Fusarium head blight; the development process of vanilla Fusarium wilt; the regional migration of wheat leaf rust pathogen; the early prediction of potato early blight; the screening of alternative fungicides for the control of alfalfa Fusarium root rot; and the biocontrol potential of endophytic fungi to control of cumin root rot, presenting the progress of research on the epidemiology and control of crop fungal diseases. The studies contained in this Special Issue facilitated the development of epidemiology of the related crop fungal diseases and provided some basis for control of the diseases, which is conducive to the sustainable management of these diseases.

Keywords: crop disease; fungal disease; epidemiology; pathogenic fungus; disease forecast; disease control; disease identification; image processing

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1. Introduction

Food security plays a vital role in ensuring global security. Under the background of global climate changes, crop variety changes, planting system changes, and rapid economic development, the occurrence and epidemics of crop fungal diseases, which can reduce crop yield and quality, threaten the safe production of crops. To study and understand the epidemiology of crop fungal diseases is the basis for sustainable management of the diseases. The interdisciplinary development of sciences, especially the application of molecular biology technology and modern information technology to plant disease epidemiology, has promoted great progress in the studies on detection, monitoring, and epidemic law of crop fungal diseases and pathogens, pathogen population genetics, disease forecasting and early warning, and control of crop fungal diseases [1], and facilitated the development of crop fungal disease epidemiology. Great progress has been made in sustainable and eco-friendly disease control measures and strategies through reducing pesticide applications, and more of these kinds of control measures and strategies have been widely applied.

This Special Issue, "Epidemiology and Control of Fungal Diseases of Crop Plants", collected the original contributions mainly addressing the identification and detection of disease pathogens based on morphology and molecular biology, disease image segmentation, disease identification and disease severity assessment based on image processing technology, disease development based on pathogen-host interactions, population genetic relationship and regional transmission of fungal pathogen, model-based early disease prediction, screening of pesticides for disease control, and biocontrol potential of fungal endophytes. It provides new advances in the epidemiology and control of crop fungal diseases.

2. Special Issue Overview

This Special Issue contains one communication and nine original research articles concerning the epidemiology and control of crop fungal diseases. It involves nine fungal diseases on the crops including wheat, barley, potato, alfalfa, strawberry, vanilla, and cumin.

2.1. Identification and Detection of Pathogenic Fungi

The identification and detection of pathogenic fungi causing crop diseases are crucial for control of the diseases. Usually, the pathogenic fungi are identified and detected based on morphology and molecular biology.

In a study conducted by Wang et al. [2], to identify and clarify the pathogens causing alfalfa Fusarium root rot, a soil-borne and devastating disease on alfalfa, in the Inner Mongolia Autonomous Region, China, from the 305 diseased root samples suspected of being infected with alfalfa Fusarium root rot collected from fives alfalfa growing regions including Hohhot, Ordos, Hulunbuir, Chifeng, and Bayannaoer, 425 pathogen isolates were obtained by using conventional tissue separation method, of which 317 *Fusarium* isolates were achieved and were then divided into 12 groups by using morphological identification method. Subsequently, in combination with the molecular identification method by using the primers ITS1/ITS4, EF1/EF2, and Bt2a/Bt2b based on the internal transcribed spacer (ITS), translation elongation factor 1 alpha (EF-1α), and β-tubulin (TUB) genes, respectively, all the *Fusarium* isolates were identified as 12 *Fusarium* species including *F. acuminatum*, *F. solani*, *F. equiseti*, *F. incarnatum*, *F. oxysporum*, *F. avenaceum*, *F. verticillioides*, *F. proliferatum*, *F. falciforme*, *F. tricinctum*, *F. virguliforme*, and *F. redolens*. The results of pathogenicity assay performed according to Koch's rules showed that all the 12 *Fusarium* species can cause alfalfa Fusarium root rot. The *Fusarium* species differed across the sampled regions, and *F. acuminatum* was the most dominant species among the identified 12 *Fusarium* species. The results of the study also demonstrated that alfalfa Fusarium root rot can be caused by a single *Fusarium* species, a combination of two *Fusarium* species, and a combination of three *Fusarium* species.

In the communication contributed by Sun et al. [3], 108 *Alternaria* isolates were obtained from the strawberry leaf samples with typical symptoms of black spot from Dandong City, Liaoning Province, China, and then 78 isolates were identified as *A. tenuissima* and 30 isolates were identified as *A. alternata* by using morphological identification method and molecular biology identification methods with the primers ITS1/ITS4 based on the ITS gene and the primers H3-1a/H3-1b based on the histone 3 gene. Subsequently, from these identified *Alternaria* isolates, 46 isolates, of which 33 were *A. tenuissima* and 13 were *A. alternata*, were selected for pathogenicity assessments in vitro by using conidia inoculation method, and the results showed that the tested isolates had relatively high pathogenicity to the leaves of a strawberry variety Dandong 99. This study demonstrated that *A. tenuissima* and *A. alternata* are the causal agents of strawberry black spot in Dandong and provided some basis for the control of this disease.

An article contributed by Hu et al. [4] focused on a molecular biology method for the detection of *Pyrenophora graminea*, the causal agent of leaf stripe that is an economically important seed-borne disease on barley (*Hordeum vulgare*). The detection and prevention of the seed-borne pathogen are crucial for the control of this disease. To detect *P. graminea* in barley seeds, the authors developed a loop-mediated isothermal amplification (LAMP) assay. Firstly, the extraction of the DNA of *P. graminea* was performed by using a one-step fungal DNA extraction kit. Based the sequence of the pig 14 gene (GenBank: AJ277800) of the pathogen, five sets of primers were designed, and an optimal set of primers was then selected to carry out the LAMP assay. Subsequently, the LAMP reaction conditions were optimized, and the results showed that the LAMP assay performed at 63 °C for 60 min with the concentrations of $MgSO_4$, dNTPs, and *Bst* DNA polymerase of 10.0 mM, 1.0 mM, and 8 U in a 25 μL reaction volume, respectively, was the optimal. Finally, the sensitivity, specificity, stability, and usability of the LAMP assay were tested. The sensitivity testing results showed that the LAMP assay with a detection sensitivity of 1 ×

10^{-2} ng/μL of *P. graminea* genomic DNA in the 25 μL reaction system was 10 times more sensitive than the conventional polymerase chain reaction (PCR) assay performed with the primers pig 14-F/14-R. The specificity testing of the LAMP assay with the genomic DNA of *P. graminea* and eight other fungi demonstrated that the LAMP assay had high specificity for *P. graminea*. By evaluating with storage treatments and DNA extracts from non-smashed and smashed seeds, it was proved that the LAMP reagent had high stability. The LAMP assay was used to detect *P. graminea* in artificially inoculated barley seeds and naturally diseased barley seeds, and the results showed that the LAMP assay can effectively detect *P. graminea* in barley seeds. The results demonstrated that the developed LAMP assay can be used for the rapid detection of *P. graminea* onsite in barley seed health testing and leaf stripe management.

2.2. Disease Image Segmentation and Image-Based Disease Identification and Severity Assessment

Segmentation operations of plant disease images are important in the image-based recognition and assessment of plant diseases. To distinguish urediospores from disease lesions, Li et al. [5] conducted a study on semantic segmentation of images of wheat stripe rust caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*) by using deep learning. Under the greenhouse and field conditions, 2353 images with different resolutions were acquired by using different mobile devices. Then, a software Labelme 4.5.13 was used to manually label the disease images by annotating three categories including leaf (containing lesions), urediospores, and background. After image grid clipping, 77,152 images in the size of 512 × 512 pixels were obtained. Subsequently, after image filtering, 33,238 images were retained and used to construct an open image dataset of wheat stripe rust called crop disease treatment dataset (CDTS). All the images in the CDTS dataset were divided into training set (19,942 images), validation set (6648 images), and testing set (6648 images). To realize accurate segmentation of the leaf, urediospores, and background, four models including scene parsing network (PSPNet), DeepLabv3, U-Net, and Octave-UNet were trained with the number of training generations of 100, the initial learning rate of 2×10^{-5}, and the batch size of eight. During model training, an Adam optimizer was used to adjust learning rate. Of the four models, the Octave-UNet model was built based on the U-Net model by replacing ordinary convolutions with octave convolutions to realize semantic fusion of high- and low-frequency information. The results showed that the segmentation performance of the Octave-UNet model was the best among the four models. This study was helpful in assessing the percentage of the uredinia to the total area of a diseased wheat leaf infected by *Pst*.

Plant disease image recognition with advantages such as convenience, rapidity, and reducing the dependence on experts has become a very important way to identify and diagnose plant diseases. However, plant disease image recognition is affected by many factors. Wang et al. [6] explored the effects of wheat varieties on image-based identification of two important wheat diseases including stripe rust and leaf rust caused by *Puccinia triticina* (*Pt*) and investigated an image-based method for identifying the two diseases on different wheat varieties. A total of 9087 single-leaf disease images were acquired, including the images of stripe rust and leaf rust from wheat varieties Beijing 0045, Mingxian 169, and Nongda 211 in the field in Shangzhuang Experimental Station of China Agricultural University, Beijing, China, and the controlled-climate chamber in the Laboratory of Macro-Phytopathology, China Agricultural University, Beijing, China, and the images of stripe rust from wheat varieties Longjian 9822, Longjian 9825, and Tianxuan 66 in the field in Gangu Testing Station of the Institute of Plant Protection, Gansu Academy of Agricultural Sciences, Gangu, Gansu, China. The images were preprocessed by using the operations including image scaling, median filtering, and morphological processing. The Otsu threshold segmentation method and the *K*-means clustering algorithm were successively utilized to implement lesion image segmentation. From the lesion images, 140 color, texture, and shape features were extracted. Based on the 1332 multi-variety images acquired in the field in Shangzhuang Experimental Station and the 7280 multi-variety images acquired under laboratory environmental conditions, respectively, the combination methods of the ReliefF, 1-rule (1R), correlation-based feature selection (CFS), and principal components analysis (PCA) feature selection methods and the support vector machine (SVM), back propagation neural network (BPNN), and random forest (RF) modeling methods were used to perform feature selections. The methods ReliefF, 1R, CFS, and PCA were firstly used to conduct feature selections, and then, with the selected features, the optimal disease identification SVM, BPNN, and RF models were built to achieve the optimal feature combinations. Based on the 8612 single-leaf disease images acquired in the field in Shangzhuang Experimental Station and under laboratory environmental conditions, 12 individual-variety image datasets were constructed, and then each dataset was divided into training set and testing set with a ratio of 2:1. The training and testing sets of the 12 datasets were combined into a training set composed of 5741 images and a testing set composed of 2871 images, respectively. Based on the achieved optimal feature combinations and the training sets, the individual-variety and multi-variety disease identification models were built by using the SVM, BPNN, and RF modeling methods. The 475 images acquired in the field in Gangu Testing Station composed an additional testing set to evaluate the performance of each model. The results showed that by using the individual-variety disease identification models, high identification accuracies in the range of 87.18–100.00% were achieved for the training sets and the testing sets on the same individual varieties acquired under the same conditions, but the identification accuracies for most of the other individual-variety disease image testing sets obviously decreased. By using the multi-variety disease identification models built with the merged feature combinations, satisfactory identification accuracies in the range of 82.05–100.00% were achieved on the training set, the corresponding testing set, the individual-variety disease image testing sets, and the additional testing set. The results indicated that the wheat varieties had a great influence on the image identification of the two diseases, but satisfactory identification performances can be achieved by using the models built based on the disease images acquired from different varieties under different conditions. This study provided some basis for accurate image-based identification of plant diseases.

Fusarium head blight (FHB) is a destructive wheat disease. Accurate severity assessment of FHB is crucial for timely control of the disease. Mao et al. [7] contributed an article on qualitative identification of the severity of FHB by using deep learning. The authors constructed an FHB severity grading dataset (FHBSGD) by using 2752 images in five severity levels of FHB acquired under the experimental and complex field conditions. The images were manually labeled by using a software LabelImg, and then were divided into training set, validation set, and testing set with a ratio of 8:1:1. The simple, parameter-free attention module (SimAM), the ghost convolution of the model head (G-head), and the efficient intersection over union (EIoU) loss were applied to improve YOLOX-s, a lightweight version of the YOLOX series, and a lightweight network named GSEYOLOX-s with 8.06 MB parameters was developed. By using this GSEYOLOX-s, a mean average precision (mAP) of 99.23% was achieved. In comparison with five different target detection models including EfficientDet, Mobilenet-YOLOV4, YOLOV7, YOLOX-s, and YOLOX-m, GSEYOLOX-s had the optimal comprehensive performance in severity identification of FHB. Furthermore, a WeChat Mini Program was developed to assess the GSEYOLOX-s model deployed on a cloud server. By using the WeChat Mini Program on mobile terminals, users can upload wheat images taken in the field or in mobile phone albums to obtain real-time information on severity levels of FHB, which is helpful for timely control of FHB.

2.3. Disease Development Based on Pathogen–Host Interactions

Vanilla is a crop with economic importance in food and cosmetic industries. Its growth and productivity are limited by Fusarium wilt associated with *Fusarium* species. Very importantly, the fruits of vanilla crop may be contaminated with mycotoxins, posing a great risk to human health. Disease resistance utilization is an important measure to control vanilla Fusarium wilt. Mosquera-Espinosa et al. [8] investigated *Vanilla* spp.–*Fusarium* spp. interactions to evaluate the pathogenicity of *Fusarium* isolates and the development of Fusarium wilt in vanilla accessions multiplied in vitro. Four vanilla accessions, including two of *Vanilla planifolia*, one of *V. odorata*, and one of *V. rivasii* × *V. trigonocarpa* F1 hybrid, and three *Fusarium* isolates, including two *F. oxysporum* f. sp. *vanillae* isolates and one *F. solani* isolate, were used in the study. Disinfected tissue sections from the plants of vanilla accessions were incubated in Petri dishes with Potato Dextrose Agar acidified at 2.5%, and no fungal endophytes were observed, indicating that the presence of cultivable fungal endophytes was low or absent in the vanilla material. The three *Fusarium* isolates were identified based on morphological characteristics and molecular analyses by using the primers ITS4/ITS5, and the species of the isolates were verified and confirmed. The isolates were inoculated on the plants of vanilla accessions multiplied in vitro. The development of symptoms on plants was observed, the severity assessments were performed, and then the values of AUDPC (area under the disease progress curve) were calculated. The results showed that the isolates caused disease symptoms in all vanilla accessions, but with different development processes. By following Koch's rules, the *Fusarium* isolates were confirmed as the causal agents. According to the bifactorial ANOVA analyses, there were significant differences, when independently considering the design factors, i.e., the *Fusarium* isolates factor and the vanilla accessions factor; there were no significant differences when analyzing the interactions between the *Fusarium* isolates and the vanilla accessions. According to the multiple comparisons of means with the Bonferroni correction test, there were significant differences when independently considering the *Fusarium* isolates factor, but there were no significant differences when independently considering the vanilla accessions factor. The results showed that there were no statistical differences among the values of AUDPC calculated for all vanilla accessions, which were in a range between 0 and 10.0, indicating that the accessions have a slight resistance to the three *Fusarium* isolates. The authors suggested that it is necessary to explore vanilla material with multiple resistance genes for effective control of Fusarium wilt associated with *Fusarium* species.

2.4. Regional Migration of Fungal Pathogen

Wheat leaf rust caused by *Pt* is an important air-borne disease on wheat worldwide. *Pt* urediospores can be spread by airflows to implement the long-distance dispersal and regional migration of wheat leaf rust, thus leading to severe wheat yield losses. Understanding of genotypic diversities and regional migration of *Pt* is of great significance to disease-resistant breeding, disease-resistant variety layout, and control strategy making. Xu et al. [9] carried out a study to reveal the population genetic structure and genetic relationship of *Pt* populations from 15 provinces (Beijing, Hebei, Henan, Shandong, Shanxi, Shaanxi, Anhui, Jiangsu, Hubei, Yunnan, Sichuan, Gansu, Qinghai, Heilongjiang, and Inner Mongolia) of China. Thirteen simple sequence repeat (SSR) primer pairs were used to obtained the loci data, and after removing the loci data with more than 25% missing values, the loci data of 622 isolates were selected from the 709 samples acquired from the 15 provinces of China for analyses on the genotypic diversities and population differentiation of the *Pt* populations. The results demonstrated that 68.8% of the isolates belonged to unique genotypes and there was a high level of genotypic diversity in the 15 populations. The 622 isolates were divided into three predominant populations, including eastern *Pt* populations from Beijing, Hebei, Shanxi, Shaanxi, Anhui, Shandong, Henan, and Heilongjiang, the western *Pt* populations from Gansu, Qinghai, Sichuan, and Inner Mongolia, and the "bridge" *Pt* populations from Jiangsu, Hubei, and Yunnan. The results indicated that there were more frequent exchanges of *Pt* within eastern *Pt* populations than the western *Pt* populations and that the "bridge" *Pt* populations communicated with the other two populations.

2.5. Model-Based Early Disease Prediction

The accurate forecasting of plant diseases can contribute to timing and reducing pesticide applications to control the diseases. Early blight mainly caused by *Alternaria* *solani* on potato (*Solanum tuberosum*) is an important disease threatening the quality and production of potato. To determine the timings of the first fungicide application to control potato early blight, Meno et al. [10] conducted a study to investigate the suitability of existing forecasting models to predict the onsets of the first symptoms of the disease. Monitoring the weather and the phenological stages of potato crops and observations of early blight symptoms were performed throughout five growing seasons in a potato field located in A Limia, Galicia, Northwest Spain. Based on the obtained data, the plant-based forecasting models including the Physiological Days (P-Days) model, the Growing Degree Days (GDDs) model, and the Wang Engel (WE) model, the pathogen-based forecasting models including the Interrupted Wetting Period (IWP) model and the Tomato Forecaster (TOMCAST) model, and the plant-pathogen-based forecasting model (the TOMCAST + Maturity model combining both the TOMCAST model and the P-Days model) were applied to predict the first symptoms of potato early blight. The performance of each model was evaluated by comparing the predicted results with the observed onsets of the first symptoms of potato early blight. The results showed that except the GDDs (1000 cumulative units) model, the plant-based models resulted in the best predicted performances, and that the most accurate forecasting model was the WE (1.4) model with 1.4 risk units. The pathogen-based models were the most conservative, with great risk levels deviations in comparison with the observed onsets of the first symptoms of potato early blight. Among the pathogen-based models, the TOMCAST (25) model with an accumulated disease severity value threshold of 25 was the best. The plant-pathogen-based model, i.e., the TOMCAST (25) + Maturity model, resulted in more accurate predictions than the TOMCAST models. Moreover, the results showed that the existing potato early blight forecasting models need to be adjusted to achieve the best prediction according to the local environmental conditions of a potato crop area.

2.6. Screening of Pesticides and Fungal Endophytes for Disease Control

Pesticides are still used in the control of many crop fungal diseases. In the study conducted by Wang et al. [2], to screen alternative fungicides against alfalfa Fusarium root rot, the toxicities of seven fungicides (including triadimefon, kresoxim-methyl, mancozeb, fine frost · manganese zinc, ene acyl intermediate, metalaxyl-m, and fludioxonil) to *Fusarium acuminatum*, the dominant *Fusarium* species, were determined by using growth rate method. The results showed that all seven fungicides can inhibit the colony growth of *F. acuminatum* and that fludioxonil, kresoxim-methyl, and triadimefon have comparatively strong toxicities to *F. acuminatum*, indicating that these three fungicides can be used as the alternative fungicides for control of alfalfa Fusarium root rot.

Generally, biological control is regarded as an eco-friendly means to control plant diseases. Abo-Elyousr et al. [11] investigated the biocontrol potential of endophytic fungi to control root rot, a destructive disease caused by *Fusarium solan* on cumin (*Cuminum cyminum*) that is a crop with important economic value in food and medicinal industries. From about 25 infected cumin root samples with root rot symptoms from Assiut Governorate, Egypt, seven fungal isolates were obtained and identified as *Fusarium* spp. based on the morphological and microscopic characteristics and the morphological characteristics. The virulence levels of the seven *Fusarium* isolates were determined by evaluating the effects on seed germination of a cumin variety Baladi via pot experiments conducted under greenhouse conditions. The most virulent *Fusarium* isolate was identified as *F. solani* by using molecular identification method with the primers ITS1/ITS4, and was selected for investigation of the biocontrol potential of endophytic fungi. From 10 healthy cucumber root samples collected from Assiut University, Egypt, 12 naturally existing fungal endophytes were isolated and screened for in vitro biocontrol potential against the most virulent *Fusarium* isolate by using a dual-culture assay. The results on the measured mycelial growth reduction showed that all the endophytes had biocontrol potential against this *Fusarium* isolate. Three endophytes with strong mycelial growth reduction were selected for further biocontrol potential evaluation, and were identified as *Trichoderma harzianum*, *T. longibrachiatum*, and *Chaetomium globosum*, respectively, by using molecular identification method with the primers ITS5 and ITS4 with *rpb2* fragments and *tef1* fragments. The results showed that the selected endophytes produced siderophore and indole-3-acetic acid (IAA), and *T. harzianum* had higher production of siderophore and IAA than *T. longibrachiatum* and *C. globosum*. The culture filtrates of the selected endophytes had positive effects on seed germination of the cumin variety Baladi. The results of experiments by using the cumin variety Baladi seedlings in pots in a greenhouse to be firstly inoculated with the suspension of culture filtrate of each selected endophyte and then inoculated with the conidial suspension of the most virulent *Fusarium* isolate after 24 h showed that the application of culture filtrates of the selected fungal endophytes significantly reduced disease severity of root rot on cumin plants. Compared with the control, the activity of the defense-related enzymes including peroxidase (PO), polyphenol oxidase (PPO), and phenylalanine ammonia-lyase (PAL) and the phenolic content production in the cumin leaves treated with culture filtrates of the selected endophytes significantly increased. The results demonstrated the biocontrol potential of the three selected endophytes against *F. solani* causing root rot on cumin.

3. Conclusions and Prospects

This Special Issue demonstrated the latest progress in the epidemiology and control of crop fungal diseases. The studies contained in this Special Issue help us to understand the application of molecular biology technology and modern information technology in crop fungal disease epidemiology. At present, the pathogen identification of crop fungal diseases is mainly implemented by using the methods combining morphological identification method and molecular biological identification method. Molecular biological identification methods have been especially widely used in pathogen identification and the detection of crop fungal diseases, and have played a vital role. Modern information technology will play a greater role in the epidemiology and control of crop fungal diseases, especially in the applications of disease identification and evaluation based on image-processing technology, which will promote the development of smart phytoprotection to ensure crop health and food security [1]. It is pleasing that a study on the regional migration of fungal pathogen based on pathogen population genetics was included in this Special Issue. Studies on crop fungal disease epidemiology in the macro scale need to be strengthened, which is beneficial for the rational layout of crop varieties and macro-management of crop fungal diseases. Safe, effective, sustainable, and eco-friendly control measures such as applications of disease-resistant crop varieties and biocontrol agents available in production practice need to be further explored.

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