Evaluation of deep learning approaches in the identification and classification of fungal spores from microscopy slides

João Antonio T. Guelfi[1], **Lucas Otavio Leme Silva**[1], **Messias Xavier Magalhaes**[1], **Cesar Augusto Dias Batista**[1], **Edivan José Possamai**[2], **Fabricio Martins Lopes**[1]

[1] Departamento Acadêmico de Computação (DACOM)
Universidade Tecnológica Federal do Paraná (UTFPR) - Campus Cornélio Procópio
Avenida Alberto Carazzai, 1640, Cornélio Procópio, PR, CEP 86.300-000, Brasil

[2]Instituto de Desenvolvimento Rural do Paraná, IDR-Paraná
Unidade Regional de Pato Branco
Rua Nereu Ramos, 844, Pato Branco, PR, CEP 85504-012, Brasil

Abstract. *Soybean cultivation is one of the principal crops in agricultural production in Brazil, moving a relevant agribusiness production chain. However, there are problems during the production process, involving care from planting, development to harvesting. In particular, the control of pests such as fungi is of great relevance to production. Monitoring and decision-making for the prevention and application of fungicides in the sector has become a major focus in terms of sustainability, productivity, and healthier production for human and animal consumption. Computational approaches have been applied to pest monitoring, however they focus on detecting the phenotype of diseases, when cultivars already show signs of infection and require fungicide application. However, fungi reproduces through living hosts and uses the wind to migrate its spores to a new host, such as Asian soybean rust. One possibility for early identification, before the manifestation of the pathology, is to detect the presence of spores by spore collectors and microscopy slides. But this leads to manual analysis performed by a specialist, being a time-consuming and tiring process. Deep Learning approaches can assign greater accuracy when counting spores with less time, finding patterns and leading to classification. Therefore, this study presents an evaluation of different deep learning approaches for the automatic recognition of spores that cause soybean diseases: rust, downy mildew, and powdery mildew from microscopy slides.*

Keywords: *Phakopsora pachyrhizi, Microsphaera diffusa, Peronospora manshurica, fungal spore detection, deep learning, pattern recognition.*

1. Introduction

In 2021, Brazil produced around 134.9 million tons, national soybean production grew 10.8% and its production value reached R$341.7 billion in 2021 [IBGE 2023], indicating the importance of Brazil as a producer and the economic impact of soybean production. However, with the opening of new agricultural frontiers and the expansion of soybean cultivation, there has been a rapid spread of the complex of soybean diseases in Brazil. The occurrence of these diseases influences grain quality and productivity, increasing the cost of soybean production. Additionally, they lead to a greater use of fungicides in scheduled applications, even when they could be avoided. These diseases include soybean rust (*Phakopsora pachyrhizi*), powdery mildew (*Microsphaera diffusa*) and downy mildew

(*Peronospora manshurica*). The economic importance of these diseases varies from year to year and from region to region, mainly depending on the cultivar, the environmental conditions and the type of technology adopted [Hirakuri 2020].

Asian soybean rust, considered the major disease of the soybean crop, shows its effects on stems, petioles, pods, and leaves, and can cause yield losses of up to 90% in productivity [Godoy et al. 2016]. At first, small light green or light yellow dots appear and develop into light brown and increase in size, ranging from 2 mm to 5 mm. The leaves with the fungi turn yellow and fall off. Uredia is present at the bottom of the leaf. It has fungi reproductive structures that form the spores called uredospores [Oliveira et al. 2023]. The *Microsphaera diffusa* is one of the oldest soybean diseases, first recorded in 1921. Since then, it has spread throughout the world, including Brazil and leading to severe infection in cultivars, which can cause production losses of 10% to 25%[Igarashi et al. 2020]. The reproduction of this fungus is similar to *Phakopsora pachyrhizi*, which uses the wind for their propagation. The powdery mildew dries out the leaves and falls prematurely because it stops the photosynthesis [Godoy and Canteri 2004]. *Peronospora manshurica* causes the downy mildew, like powdery mildew, downy mildew is a soybean disease that develops on the aerial part of the plant. It is a less aggressive disease, and the damage recorded on crops does not exceed 5% of total production.

Although there are computer vision methodologies for recognizing plants and their problems, the solutions focus on detecting morphological changes, i.e. after the diseases have infested the cultivars at a later stage [Brilhador et al. 2013, Araujo and Peixoto 2019, Pereira et al. 2020, Bevers et al. 2022, Shahoveisi et al. 2023].

Considering that the spread of fungi happens quickly through the wind, to identify its presence early, before the infection affects, it is necessary to carry out monitoring to assist in decision-making for the fungicides applications [Singh et al. 2019]. The spores' early detection makes control more assertive, avoiding unnecessary fungicide applications [de Oliveira et al. 2020].

Figure 1. Spore Collector [de Oliveira et al. 2020].

In response to this challenge, the "rust alert" project, which is being developed by the Paraná Rural Development Institute (IDR-Paraná) in partnership with the Federal University of Paraná (UTFPR) and Embrapa-Soja. This project comprises making traps called spore collectors [de Oliveira et al. 2020] available on the properties of farmers who collaborate with the project, as illustrated in Figure 1. The collectors have glass slides with adhesive inside, which allow air to pass through and particles in the air to stick to the slide, as illustrated in Figure 2. Then, professionals with experience need to handle the slides to perform spore counting [Lei et al. 2018], which requires manually and tediously going through the slides under the microscope to detect the fungi.

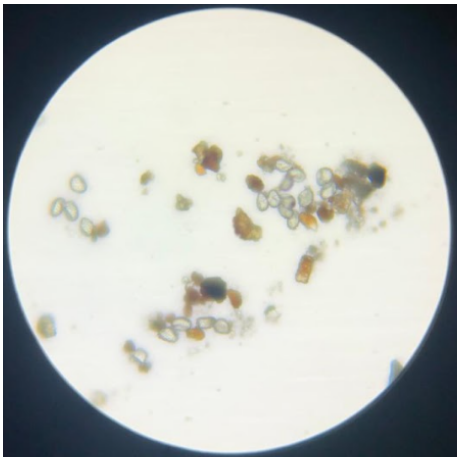

Figure 2. Viewing of the collector slide with the microscope.

The spores automatic identification helps in rapid treatment against the fungus, reducing losses in plantation fields. This identification avoids the use of fungicides as prevention when there are no professionals to identify the fungi. Automating counting reduces human error and the risk of counting the same spore again. Capturing the image of the collector reduces the need for professionals trained in counting, allowing for increased scalability and more analyses.

Therefore, considering the lack of computational approaches in the literature for identifying spores from digital microscopy images and the success of deep learning approaches in recognizing patterns in images, this study presents an evaluation of deep learning approaches for recognizing and classifying *Phakopsora pachyrhizi*, *Microsphaera diffusa* and *Peronospora manshurica* spores.

2. Materials and methods

2.1. Materials

In this study, a database containing 720 images was produced in a balanced way. Thus, comprising 240 spore imagens of each adopted fungi: *Phakopsora pachyrhizi*, *Microsphaera diffusa* and *Peronospora manshurica* spores. The images are spore microscope samples collected by the Paraná Rural Development Institute (IDR-Paraná). Figure 3 illustrates the 3 species. The sub-image "a" is the *Peronospora manshurica* (downy mildew) spore, sub-image "b" is the *Microsphaera diffusa* (powdery mildew) spore and sub-image "c" is the *Phakopsora pachyrhizi* (Asian soybean rust) spore.

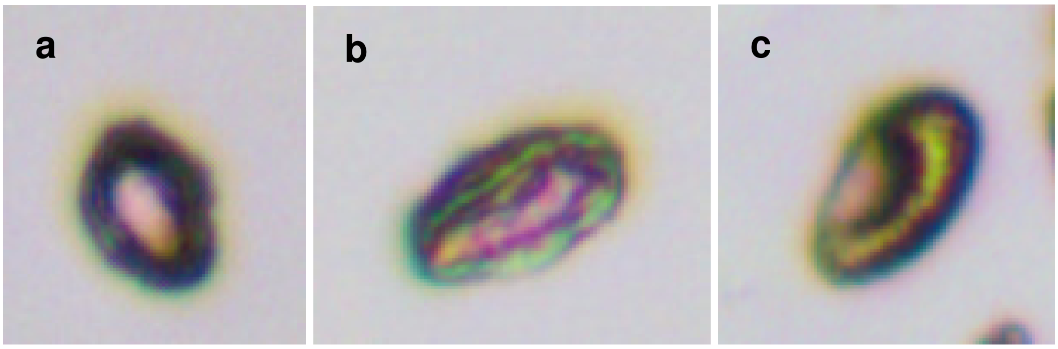

Figure 3. Example of 64x64 images of the fungi adopted in this study.

The experiment consisted of comparing the models for classifying the 3 types of species in the dataset, for this the classification models were trained using the IDR-Paraná dataset, into which it was divided in 72% training, 8% validation and 20% testing. After training the model, the general accuracy obtained by the model was verified using the test data.

2.2. Methods

Considering the success of various deep learning architectures in recognizing patterns and classifying images in different contexts such as Yolo [Im Choi and Tian 2022], Vgg16 [Simonyan and Zisserman 2014], DenseNet [Huang et al. 2017] and ResNet [He et al. 2016]. Then, an important question arises as to which architecture can perform best in classifying spores, the focus of this study. Therefore, the main deep learning architectures available in the literature were adopted and implemented for evaluation in the detection and classification of *Phakopsora pachyrhizi*, *Microsphaera diffusa* and *Peronospora manshurica* spores.

The Yolo approach is a popular object detection algorithm in images and there are different versions of it. The fifth version was made available in 2020 and compared to other object detection methods such as Fast R-CNN it showed superior accuracy [Im Choi and Tian 2022]. Yolo's architecture can be divided into three parts: backbone, neck, and head. The second combines these characteristics, creating three different scales. The last one performs the detection of objects according to the previously generated scale. Yolo has a CSPDarknet53 structure with SPP layer in the backbone and PANet in the neck layer, and has three activation function combinations, being sigmoid, leaky-ReLU and SiLU [Gong et al. 2022].

In addition, YoloV8 version is similar to the YoloV5 version but with superior performance as it does not have an anchor, that is, it identifies the object through its center as it does not have an anchor box [Sohan et al. 2024]. By eliminating the bounding box anchor, the number of predictions within a box is reduced and thus the process is accelerated. Combined with this, some convolutional layers were modified and excluded, making the model more flexible and improving its efficiency. In the backbone of this version, Cross-Stage Partial Networks (CSP), were replaced by C2f (variation of CSP in addition to dividing and combining characteristics as in CSP, C2F adds connections between different stages of the network). Another issue of this version is the data augmentation included, called mosaic data augmentation which is a data augmentation technique in which four different images are joined together and inserted into the model as input helping the model learn the objects in different positions and in partial occlusion.

The VGG16 is known as very deep convolutional network, was proposed in 2014 [Simonyan and Zisserman 2014] and is an advanced version of AlexNet with more layers, with VGG16 it is composed of 16 trainable layers (13 convolutional and 3 fully connected layers), uses 3x3 convolutional filters with padding and stride of 1. After each set of convolutional layers, the pooling layer is applied.

The DenseNet121 [Huang et al. 2017] neural network is a fully connected feed forward neural network. It is characterized by the fact that each layer receives as input the feature map of all previous layers. Furthermore, the model is grouped into dense blocks, interspersed with pooling and convolution layers. The dense connectivity of the model avoids the need for redundant learning. In direct comparison with VGG16, the model has a smaller number of parameters, even though it is deeper.

The ResNet is a deep residual network, proposed in 2015 [He et al. 2016]. The ResNet121 is composed of 121 trainable layers. An important difference to other architectures is that it uses residual blocks with skipped connections, which skip one or more layers. These facilities allow gradients to pass through the network, facilitating training and alleviating the problem of disappearing gradients.

In order to assess different deep learning approaches to the identification and classification of spores, the following were adopted: YoloV5, YoloV8, Vgg16, DenseNet121 and ResNet121. As the number of images available in the dataset were few, the model parameters were kept, and 2 Dense layers were added with an output dimensionality of 512, and finally a Dense layer was added with an output dimensionality of 3 with Softmax activation. The adopted parameters for Vgg16, DenseNet121 and ResNet121 are shown in Table 1.

Table 1. Vgg16, DenseNet121 and ResNet121 adopted parameters.

Parameter	**Parameter Value**
Optimizer	Adam
Learning Rate	0.001
Loss function	Sparse Categorical Cross entropy
callback	Early Stopping
Metric used to Early Stopping	loss validation

Regarding the approaches YoloV8 and YoloV5 the adopted parameters are available in Table 2.

3. Results and Discussions

In order to assess the deep learning approaches, the experiments were performed by adopting the 720 images (see Sec.), divided into 518 training images, 144 test images and 58 validation images divided randomly. Traditional assertiveness metrics were adopted, such as precision, recall and accuracy, which explain the model's ability to identify spores.

Table 2. YoloV5, YoloV8 adopted parameters.

Parameter	Parameter Value
Optimizer	Adam
Deterministic	True
Lrf	0.01
Lr0	0.01
Epochs	100

Table 3 shows the results of the adopted deep learning approaches tested with the same images and 50 epochs for each model. The best results (i.e. classifiers) are highlighted in bold. It can be seen that considering the overall accuracy for the three classes of fungi, the YoloV5 model showed the best result, with an accuracy of 0.79. When considering accuracy, the Vgg16 model performed best with 0.89 for Asian soybean rust spores and again YoloV5 with better accuracies of 0.88 and 0.75 for powdery mildew and downy mildew spores, respectively. On the other hand, when considering recall, the Yolo8 model showed the best results for all spore classes, indicating its suitability for recovering correct results. The DenseNet and ResNet models did not show competitive results when compared to the other deep learning approaches, with a negative highlight for the recall of only 0.56 achieved by the Resnet for downy mildew spores.

In general, it was possible to see that the YoloV5 and YoloV8 approaches showed the best results, indicating the suitability of the fungal spore classification adopted in this study.

Table 3. Assertiveness measurements obtained by deep learning approaches in the classification of fungal spores.

Model	Class	Precision	Recall	Accuracy
YoloV5	Downy Mildew	**0.75**	**0.8**	**0.79**
YoloV5	Powdery Mildew	**0.88**	0.68	
YoloV5	Asian soybean rust	0.86	0.91	
YoloV8	Downy Mildew	0.65	**0.8**	0.76
YoloV8	Powdery Mildew	0.77	**0.95**	
YoloV8	Asian soybean rust	0.75	**0.92**	
DenseNet121	Downy Mildew	0.67	0.75	0.75
DenseNet121	Powdery Mildew	0.84	0.68	
DenseNet121	Asian soybean rust	0.76	0.83	
Vgg16	Downy Mildew	0.63	0.75	0.72
Vgg16	Powdery Mildew	0.71	0.72	
Vgg16	Asian soybean rust	**0.89**	0.7	
ResNet121	Downy Mildew	0.67	0.56	0.73
ResNet121	Powdery Mildew	0.7	0.87	
ResNet121	Asian soybean rust	0.84	0.77	

4. Conclusion

This study presented an evaluation of deep learning models in the classification of fungal spores that cause damage to cultivars, especially soybeans. Three fungi that cause significant damage to soybean cultivars were considered: *Phakopsora pachyrhizi*: soybean rust, *Microsphaera diffusa*: powdery mildew and *Peronospora manshurica*: downy mildew.

An image set was produced with 720 samples containing 240 images of each of the spore types in a balanced way. The following deep learning approaches were evaluated: YoloV5, YoloV8, DeseNet, Vgg16 and ResNet for spore classification. The experiments were performed in order to evaluate the overall accuracy among all fungus classes and the precision and recall for each of the spores and approaches evaluated.

It was possible to see that the Yolo5 approach showed better precision results, while YoloV8 showed better recall results. The Vgg16 approach showed better precision for Asian soybean rust spores. On the other hand, the DenseNet and ResNet architectures showed lower results than the other approaches adopted in this study.

In future work, it is suggested that new deep learning approaches be evaluated, including the new versions of YoloV9 and YoloV10. It is also suggested that the image set be expanded with more samples of each class and that spores causing other diseases in cultivars be considered.

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