Diagrammatic scale for evaluation of *Bremia lactucae* sporulation in lettuce seedlings[1]

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ABSTRACT

In disease assessment, the use of diagrammatic scales provides a standard reference point for comparison. There is still no precise and accurate scale for the evaluation of sporulation intensity of downy mildew in lettuce seedlings. The objective of this study was to propose a diagrammatic scale for the evaluation of downy mildew severity in lettuce seedlings. Thirty lettuce seedlings with different sporulation intensities were used to develop the proposed diagrammatic scale. *Solaris* lettuce cultivar was sown, and fifteen days after sowing, inoculations occurred using distilled water, the pathogen sporangia, and Tween 20. The monitoring of disease development was performed daily.When the first sporulation appeared on the cotyledonaryleaves,the proposed scale was verified to meet the minimum and maximum levels of disease severity. Two evaluators verified the accuracy, precision and performed the estimation of the sporulation and reproducibility using a scale. The images obtained are important in the standardization of a scale to identify resistant, tolerant and susceptible plants to downy mildew. Furthermore, it allows validating a diagrammatic scale of sporulation intensity of *B. lactucae* in lettuce seedlings. Thus, it is the diagrammatic scale developed in the work will guide future research to accelerate the detection of mildew severity in lettuce seedlings.

Keywords: disease severity; downy mildew; *Lactuca sativa*; rating scale.

INTRODUCTION

Lettuce (*Lactuca sativa* L.) is a vegetable widely cultivated and consumed world, being the most consumed leafy vegetable in Brazil (Gnoatto *et al.*, 2018; Gusatti *et al.*, 2019), with a gradual increase in its cultivation area over the years. However, with the expansion of production areas, allied to the intensive cultivation system, has increased the infestation by numerous phytopathogens, especially *Bremia lactucae* Regel.

However, the ideal conditions for growing vegetables, such as mild temperature and high relative humidity, also favor the occurrence of diseases, including lettuce downy mildew, caused by the oomycete *Bremia lactucae* Regel, considered the most common disease devastating culture around the world (Michelmore & Wong, 2008).

The pathogen may persist in crop residues, lettuce grown in home gardens, and wild species of lettuce generating several inoculum sources (Kunjeti *et al.*, 2016). In the abaxial face of the leaves, there is the presence of white sporulation, consisting of sporangiophores and sporangia (Mieslerová *et al.*, 2013). The initial symptoms of the disease are wilting and depigmentation of the older leaves. A brownish green water-soaked lesion then develops in the inner stem, which may progressively cause the entire plant to rot (Colariccio & Chaves, 2017), resulting in yield and

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economic losses for producers.

Initial symptoms are difficult to see and, when observed, almost impossible to control. As such, any strategy aimed at detecting these anomalies in the early stages of development is vitally important, since the disease is aggressive and the growth cycle of lettuce short (Carmo *et al.*, 2021).

Theoretically, the disease progress rate may be reduced by the use of various control measures, such as irrigation shift management, so as not to prolong the duration of leaf wetness and/or the ideal frequency of fungicide application (Castoldi *et al.*, 2014). However, to reduce the use of fungicides in lettuce crops, research has been directed to the use of genes or resistance factors (DM genes or R factors) to obtain resistant cultivars (Castoldi *et al.*, 2014; Jacinto *et al.*, 2019). However, adequate methodologies for assessing disease severity and consequently defining resistant, tolerant or susceptible plants are lacking.

Disease assessment should be easy and quick to perform and produce accurate and reproducible estimates, o diagrammatic scales arevaluable tools for identifying variations in disease resistance between genotypes. Using diagrammatic scales, the accuracy and precision of disease severity estimates are significantly improved, resulting in less experimental errors (De Paula *et al.*, 2016; Lage *et al.*, 2015; Santos *et al.*, 2017). Consequently, there is a greater reliability of heritability estimates for resistance to the disease, increasing potential gains from genetic improvement through selection (Vieira *et al.*, 2014).

The development of methodologies that aimed at an early evaluation of the resistance of materials plants to pathogens is an important step in the selection of superior genotypes in breeding programs.

Despite the existence of diagrammatic scales for the most diverse diseases, there is not yet aprecise and accurate scale to evaluate the intensity of sporulation for lettuce downy mildew. Thus, the present work aimed to develop a diagrammatic scale for the evaluation of the severity of mildew in lettuce seedlings.

MATERIAL AND METHODS

The experiment was conducted at the Seed Analysis Laboratory of the Federal University of Uberlândia (UFU), Monte Carmelo Campus, in Minas Gerais state (MG), Brazil.

To obtain the infected seedlings, 30 seeds of susceptible cultivar Solaris were sown in gerbox type plastic boxes (11 × 11 × 3.5 cm), lined with blotter paper moistened with distilled water, in a proportion equivalent to 2. 5 times the weight of the dried substrate. They were taken to a BOD (biochemical oxygen demand) incubation chamber and kept for 15 days at 13 °C and 12h00 photoperiod.

After this period, the inoculation was performed using a sporangia suspension containing virulence phenotype of *B. lactucae* 63/63/51/00. The inoculation was performed according to the technique of Ilott *et al.* (1987), with the aid of distilled water and Tween 20. The suspension used was a concentration of 5×10^4 sporangia mL^{-1}, being sprayed on the seedlings to the point of dripping. Subsequently, the boxes were replaced in a BOD with a temperature of 13 °C. During the first six hours, they were kept in the darkroom and, after this time, the photoperiod was adjusted to 12h00. Monitoring was performed daily by two evaluators to obtain leaf images with different levels of sporulation intensity.

The diagrammatic scale created was based on the work of Dickinson & Crute (1974), thus obtaining images of leaves with four sporulation intensity levels: 0 (absence of visible sporangiophores on leaves – Figure 1A), 1 (limited sporulation with sporadic sporangiophores – Figure 1B), 2 (less than 50% of the cotyledon leaf covered with sporangiophores – Figure 1C), and 3 (more than 50% of the area of cotyledon leaf covered with sporangiophores – Figure 1D).

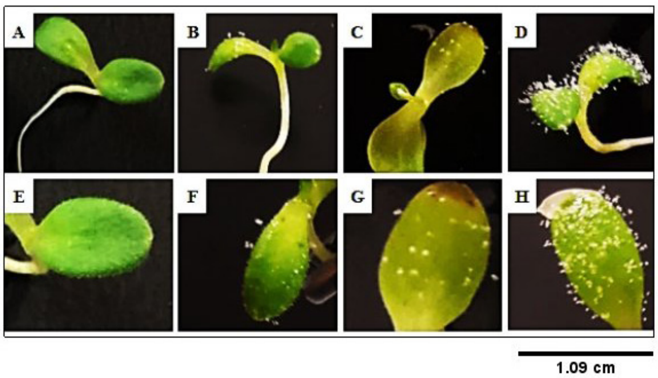

Figure 1: Diagrammatic scale for *Bremia lactucae* sporulation intensity in lettuce seedlings (*Lactuca sativa*). Cotyledonary leaves with no sporulation (A and E). Limited sporulation with sporadic sporangiophores (B and F). Less than 50% of cotyledonary leaves with sporangiophores (C and G). More than 50% of cotyledonary leaves covered with sporangiophores (D and H).

RESULTS AND DISCUSSION

The diagrammatic scale created and based on the work of Dickinson & Crute (1974), allows standardizing scale for the evaluation of sporulation intensity for downy mildew in lettuce seedlings. This being a disease one of the most important and severe diseases of the crop during winter.

Lettuce downy mildew is highly sensitive to climatic factors such as solar radiation and temperature, drastically affecting its survival in the field (Nordskog *et al.,* 2007; Wu *et al.,* 2007). Changes in the producers' management strategy can also interfere in the development of the disease. Applications of specific fungicides for disease control, associated with the use of cultivars resistant to the virulence phenotypes in the producing region, disadvantage the occurrence of this pathogen (Wu *et al.,* 2007). These two strategies should be combined whenever possible to reduce the selection pressure exerted on the pathogen (Barriere *et al.,* 2015).

Tobar-Tosse *et al.* (2017) proposed a latency index to quantify the severity of the disease, but arbitrated notes according to the day when the plant showed the first signs of the pathogen so that those who presented the signs of the pathogen earlier received higher scores. Already in the present work, we tried to arbitrate notes according to the total leaf area of the seedling that was occupied by sporangiophores and to demonstrate them through photographic images (Figure 1).

Such images will be of fundamental relevance in the future to validate a diagrammatic scale of *B. lactucae* sporulation intensity in lettuce seedlings, as well as to standardize a scale to identify resistant, tolerant and mildew susceptible plants. Castoldi *et al.* (2014) identified lettuce strains resistant to *B. lactucae* (100% of non-sporulated seedlings). Still, they did not separate plants with different tolerance levels since there was no diagrammatic scale of disease severity.

CONCLUSIONS

Disease assessment should be easy and quick to perform and produce accurate and reproducible estimates.

In this sense, the diagrammatic scale developed in the work will guide future research to accelerate the detection of mildew severity in lettuce seedlings and subsidize future works in the identification of resistant plants.

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