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DEVELOPMENT OF A DIAGRAMMATIC SCALE FOR THE QUANTIFICATION OF ENTOMOSPORIOSIS (*Diplocarpon mespili*) IN QUINCE

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Dissertação apresentada à Universidade Federalde Lavras, como parte das exigências do Programa de Pós-Graduação em Agronomia/ Fitopatologia, área de concentração em Fitopatologia, para a obtenção do título de Mestre.

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Orientador

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Orientador

LAVRAS – MG

2017

Às mulheres da minha vida, Dona Márcia e Luiza Maria.

Ao homem da minha vida, seu Zé.

Com muito amor,

Dedico.

ABSTRACT

Quince (*Cydonia oblonga*) is appreciated around the world for its uses in the jam and jellie industries. It also has interesting antibiotic and antioxidant properties that may be further exploited. Although its production does not stand out economically, the quince has been considered an "emergent fruit". This is due to its adaptation to acid soils and high temperatures which makes possible its cultivation in regions that are unsuitable for the cultivation of more demanding crops. Quince production is hampered by *Diplocarpon mespili*, a fungus that causes leaf spots, leading to defoliation and low productivity levels. Little is known about the fungus in associtation with quince. In this study, the first chapter presents a theoretical background about entomosporiosis. The second chapter demonstrates the elaboration, validation and comparison of two new diagrammatic scales for quantification of entomosporiosis in quince and the evaluation of the resistance of 29 quince cultivars to the disease. The scales were elaborated in different ways. One of them was structured with six levels, each level (with the exception of level 0) represents a range of disease severity and is illustrated with three images, totaling 16 illustrations. The other was structured as a percentage of diseased area (without levels) and has six illustrations. The elaborated scales were validated with Lin's statistics and linear regression. Although the Lin's statistics did not show a significant difference between the scales, the linear regression indicated a slightly better accuracy of the diagrammatic scale with levels. The resistance of quince cultirvares evaluation showed that Japanese cultivar is the most resistant, while Rea's Mamouth is the most susceptible.

Keywords: *Diplocarpon mespili*. *Cydonia oblonga*. Quince. Resistance.

SUMMARY

1 GENERAL INTRODUCTION

Quince (*Cydonia oblonga* Miller) has originated in temperate climates, but it is now spread around the world (POSTMAN, 2009). Although it does not have a remarkable economic importance, quince is pointed out as an emergent fruit crop. This potential is due to its adaptation to high temperatures and acidic soils, which enables its cultivation in marginal regions that are not suited to more demanding crops (HUMMER et al., 2012). In addition, it is possible to add value to quince by manufacturing jams and jellies and exploit its antibiotic and antioxidant potential (HEDRICK, 1922; PIO et al., 2008; ALVARENGA et al., 2008).

The major obstacle to quince production is a leaf spot caused by *Diplocarpon mespili* (PIO et al., 2005a). The disease is responsible for defoliation and reduction in productivity. *Diplocarpon*, is known to cause disease in many *Rosaceae* species. The species *D. rosae* occurs on roses, *D. earliana* causes leaf spots on strawberry, *D. mali* occurs on apple and *D. mespili* on quince and other related plant species. *D. mespili* is the species with widest host range in the genus *Diplocarpon*. Untill recently the pathogen was known as *Entomosporium mespili*, but taxonomical changes recommended its transfer to the genus *Diplocarpon* (JONHSTON et al., 2014). Little is known about the genetic diversity of *D. mespili* and the other species in the genus.

The ideal control measure for any plant disease is the use of resistant cultivars. However, complete resistance to entomosporiosis does not seem to exist and therefore protective fungicide applications are necessary. Copper fungicides, for example, are sufficient to control disease development in most host species, although variations may occur (RONALD et al., 2001; HOLTSLAG et al., 2004). Although these fungicides are permitted in organic farming, copper might be phytotoxic, contaminates the soil and its microbiota, and may be harmful to humans and animals (NIELSEN et al., 2015; YRUELA, 2005; BED AND MENCH, 2008). Further studies are necessary to investigate alternative methods to control the disease.

Epidemiological studies on entomosporiosis are available for some hosts, but are scarce for quince. Disease quantification is essential in epidemiological studies, however, there are no reliable methods to perform these studies on quince. Usually the quantification is performed subjectively, through visual analysis. Diagrammatic scales are essential tools to deliver more accurate estimates of disease severity (NUTTER JR. et al., 1991; SPÓSITO et al., 2003; PASSADOR et al., 2013).

In this study, a review on the current status of entomosporiosis on quince is presented in the first chapter. In the second chapter, two diagrammatic scales to evaluate to evaluate the

severity of entomosporiosis in quince were elaborated, validated, compared and one of them was used to determine the disease severity in 29 quince cultivars.

2 THEORETHICAL BACKGROUND

2.1 Quince and the entomosporiosis

Quince, *Cydonia oblonga* Miller, is the only species in this genus which to *Rosaceae*. This shrub is native to west Asia and is appreciated all over the world. Nevertheless, there are only 43,000 ha cropped to quince around the globe. Turkey is the largest producer with approximately 25% of world's production, followed by China, Uzbekistan and Marocco (HUMMER et al., 2012; TOPCU; KAJKAS; DOGAN; AKCAY; ERCISLI, 2015). Quince production is not a valued economical activity, but it is interesting due to its taste, and its antibiotic and antioxidant properties. The fruit may be consumed *in natura* or used in the industry to manufacture jams, jellies, marmalades and conserves (DALL'ORTO et al., 1987; SILVA et al., 2006). Quince may also be used as a pear rootstock (PIO et al., 2005b, BETTIOL NETO et al., 2011).

The main phytosanitary problem of quince is entomosporiosis, caused by the fungus *Diplocarpon mespili*. This disease affects several *Rosaceae* species. It occurs in all regions where quince is cultivated (POSTMAN, 2012; PARK et al., 2011).

2.2 Taxonomy of *Diplocarpon mespili*

Diplocarpon mespili is a phytopathogenic fungus that belongs to *Leotiomycetes*, *Ascomycota*. The first reports on this fungus date from the end of the 19th century. The fungus was named according to its forms: *Diplocarpon maculatum*, the sexual form, and *Entomosporium mespili*, the asexual form (LEVEILLÉ, 1856; ATKINSON, 1909). The name *E. mespili* was used until very recently in 2014 (GONÇALVES et al., 2013; GONÇALVES et al. 2014) and is still used to refer to this pathogen, but this status is currently changing. Within the genus *Diplocarpon*, besides *Entomosporium*, there were also species that possessed their asexual forms classified in the genera *Marssonina*, *Entomopeziza* and *Bostrichonema*. This classification was based only on morphological characteristics, such as size and shape of the spores and fruiting structures. There is a similarity between the ascospores produced by these genera, but their conidia are different (JOHNSTON et al., 2014).

The one fungus one scientific name rule was established in the beginning of 2013 (TAYLOR, 2011; MCNEILL et al., 2012). There were some indications that *Diplocarpon*, *Marssonina*, *Entomosporium* and *Bostrichonema* are congeneric on the basis of conidia ontology and the available sequences of the ITS (internal transcribed spacers) of the ribosomal DNA (JOHNSTON et al., 2014). They defend that this name is more frequently used and better-

known to harbor *Rosaceae* and therefore *Diplocarpon* must be used instead of the other names. In this study we adopt the name *Diplocarpon mespili* to refer to the pathogen throughout the text.

2.3 Morphological and epidemiological aspects

The asexual form of *D. mespili* is more frequently reported, perhaps because it is easily recognizable due to its morphological characteristics. The cruciform organization of the conidia is a peculiarity. Such ornamentation has the appearance of an insect, which resulted in its old name: "*Entomosporium*" (SUTTON, 1980). Each conidium is formed by an apical and a basal cell, and between them there are two to four smaller lateral cells. With the exception of the basal cell, all the others have a thin appendix (Figure 2i). Each conidiogenous cell originates three to four conidia. The apical cell is the first to be formed and this happens in a holoblastic way. On the other hand, basal and lateral cells appear in an annelidic enteroblastic way. Each cell of the conidium has a nucleus and is capable of germinating independently, favouring fungal infection (MIMS; SEWALL; RICHARDSON, 2000a).

Conidia germinate and form appressoria, penetration hyphae. Subsequently, the elongated haustoria with a thin neck and a rounded body are formed and the fungus obtains its nutrients from plant cells. Hyphae are formed in the mesophyll of infected leaves and subsequent colonization between the leaf epidermis and the cuticle result in subcuticular acervuli (MIMS et al., 2000b). Therefore, *D. mespili* is a hemibiotrophic parasite, but details of its life cycle on quince are lacking. Studies done on detached pear leaves showed that the pathogen exibited the first necrotic lesions 7 days after the inoculation, but the acervuli were not seen (NAOUI, 2013). On rose leaves, *D. rosae*, a related species, produces acervuli 7 days after the inoculation and the first conidia are released 3 days later (BLECHERT and DEBENER, 2005). These time frames appears to be different for *D. mespili* on quince and pear, but further research needs to be done on this crop.

The sexual fruiting bodies of *D. mespili* are very discrete apothecia that are not easily perceived when the leaf is dry (ATKINSON, 1909). This may explain the small number of reports based on this reproductive phase of the pathogen. They have been observed on pear and quince leaves that remained on the ground during the months that followed the summer. These apothecia contain long and thin asci accompanied by paraphyses. Each ascus contains eight hyaline and bicellular ascospores (PIEHL AND HILDEBRAND, 1936; STOWELL AND BACKUS, 1967).

Observations in temperate regions of the world showed that the asexual acervuli are observed during the spring and summer, whereas the sexual apothecia are found during the autumn and winter months (ATKINSON, 1909; PIEHL AND HILDEBRAND, 1936; STOWELL AND BACKUS, 1966).

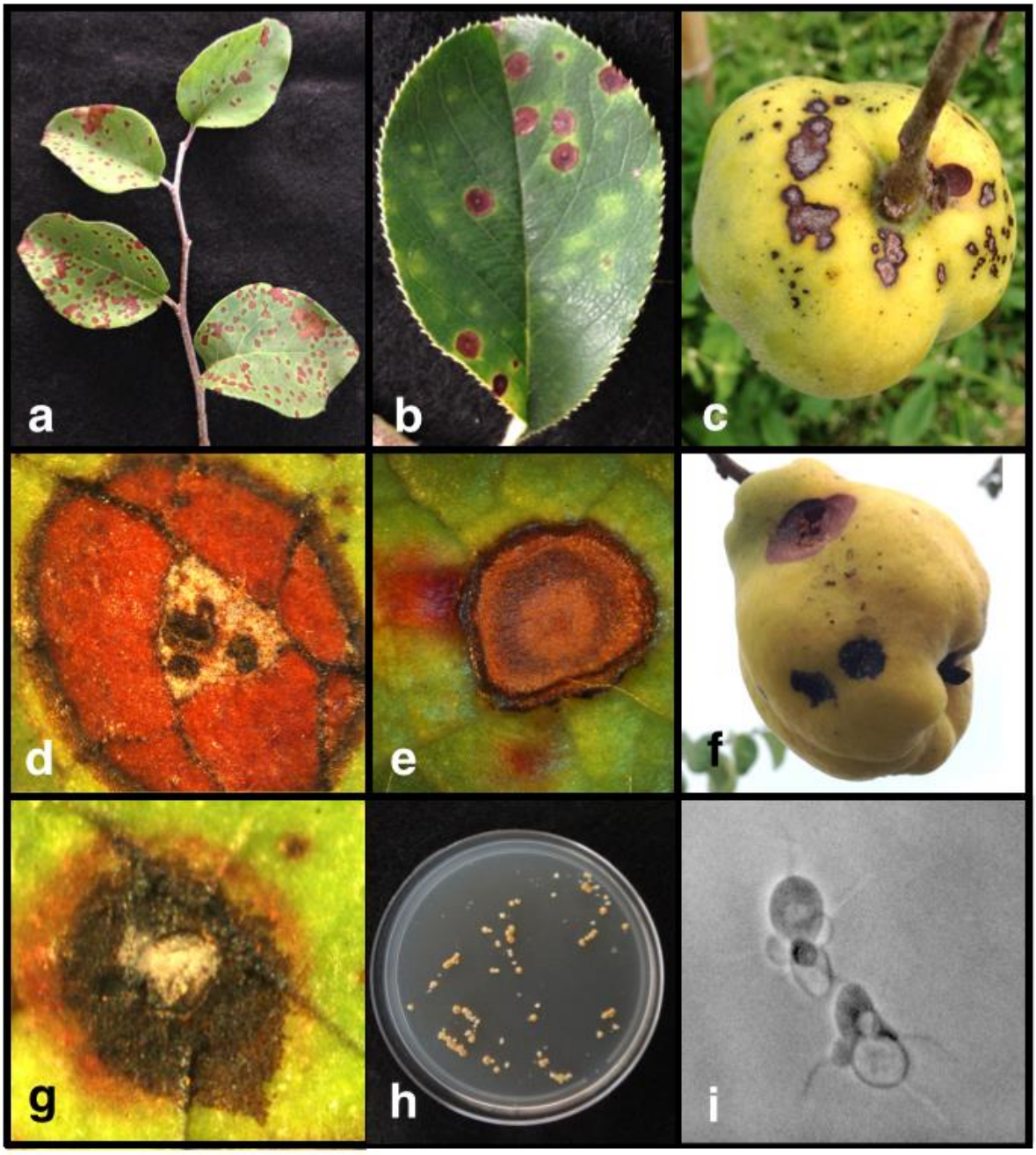

Figure 2. Symptoms and morphology of *Diplocarpon mespili*. *Diplocarpon mespili*-induced leaf spot symptom in leaves of Rea's Mamouth (a) and Japanese (b) quince cultivars. *D. mespili* symptoms on quince fruit (c). Closed acervuli formed by the fungus on a leaf surface (d). Detail on the round red spot leaf symptom on the 'Japanese' cultivar (e). Symptom of a secondary infection caused by saprophytes on a quince fruit (f). Acervulus with a white conidial mass (g). Colonies of *D. mespili* on PDA medium two weeks after transference (h). Insect-like shaped conidia of *D. mespili* (i).

2.4 Host plants and symptoms

Diplocarpon mespili infects several *Rosaceae* species. It has been reported on *Cydonia oblonga* (quince), *Pyrus* spp. (pear), *Amelanchier* spp. (juneberry), *Chaenomeles* spp. (flowering quince), *Malus* spp. (apple), *Eriobotrya japonica* (medlar), *Prunus persica* (peach), *Sorbus sitchensis* (mountain ash), *Photinia* spp. and *Raphiolepsis* spp. (SUTTON, 1980; PARK, 2011).

The symptoms (Figures 2a-g) caused by the fungus are similar in all hosts. On the leaf surfaces, round spots with a coloration that varies from brown, passing through purple, until red are formed (Figures 2abe). At the center of these spots small black crusts that are the acervuli of the fungus are produced (Figures 2dg). On fruits, depressed lesions with colours similar to what was described above for leaves are observed (Figures 2c). These lesions may cause cracks and fruit cleavage, which may favour secondary infections by saprophytes (Figures 2f). On branches symptoms are hardly observed, but they may occur in the format of small cankers (LAMBE AND RIDINGS, 1979; PIO, 2005a; HORIE AND KOBAYACHI, 1979).

2.5 Genetic diversity of *Diplocarpon mespili*

Studies on the genetic diversity are essential to understand the evolutionary processes affecting the relationship between the pathogen and its hosts. However, few studies have focused on the diversity of *D. mespili*. A study with AFLP and RAPD markers revealed a high level of polymorphism among 47 isolates of *D. mespili* from different regions of Canada, but this genetic variability was not linked to their geographical origin (NAOUI, 2013). *Diplocarpon mespili* reproduces sexually and for this reason supposedly generates high diversity among isolates. This high variability may contribute to its capacity to adapt to different cultivated species. However, there are no studies on the genetic diversity of this fungus in Brazil.

2.6 Control of *Diplocarpon mespili*

Diplocarpon mespili may decimate plantations when phytosanitary measures are not carried out. The treatments must be done preventively, in order to delay or hinder the establishment of the fungus in its host. An appropriate spacing between plants is a condition to be considered. This allows air circulation and does not promote a favourable microclimate to the fungus. In addition, it is recommended to remove infected leaves remaining on the ground, as these serve as sources of inoculum for new shoots (LAMBE AND RIDINGS, 1979).

Fungicides application is also necessary. The most used fungicides are the ones with protective action, especially cupric, triazoles and chlorothalonil. The application must follow

the specificities of each pathosystem, since these products present different results depending on the host. Differences range from the occurrence of phytotoxicity to variation in the ability to contain the disease progression. Propiconazole, for example, is effective in containing the development of the fungus in *Amelanchier alnifolia* and *Photinia* spp., but causes phytotoxicity in the latter. Chlorothalonil is capable of inhibiting the pathogen in both hosts without causing phytotoxicity, yet is less efficient in *Photinia* spp. The same happens with some cupric fungicides. While copper oxychloride is ineffective for disease control on *Photinia* spp., it works satisfactorily on quince (COBB; HAGAN; GILLIAM; MULLEN, 1985; LANGE; BAINS; HOWARD, 1998) .

There is no mention of biological control use for any pathosystem involving *D. mespili* in the literature. Despite this, there are four registered and marketed *Bacillus subtilis*-based products that act against the pathogen (RUTGERS, 2012). In the specific case of *Amelanchier alnifolia*, another method was developed to reduce the use of fungicides: a dynamic model of disease prediction. The model is able to provide pressure/severity estimation of entomosporiosis in the host. For this, phenological development of the plant, temperature and leaf wetting time and host susceptibility are considered among other variables (HOLTSLAG et al., 2004).

Using cultivars that present greater resistance to *D. mespili* is also a way of minimizing the damages caused by pathogen. There are no cultivars totally resistant, but responses to fungal infection are different among cultivars of host species. A study analyzed the behavior of different saskatoon (*Amelanchier alnifolia*) cultivars and found differences, which may also explain the different degrees of resistance among them is the fact that these cultivars are hybrids between *A. alnifolia* and *A. stolonifera* (RONALD et al., 2001).

There are few reports highlighting the resistance of other host species. Gonçalves et al. (2014) studied the effect of three quince rootstocks ‘Adams’, ‘EMA’ and ‘EMC’ on the susceptibility of two canopy cultivars of pear ‘Rocha’ and ‘Abate Fetel’ to *D. mespili*. They concluded that the two pear cultivars tested are susceptible to the fungus, but there is a difference in the degree of susceptibility between canopy / rootstock combinations. Both canopy cultivars combined with 'Adams' and 'EMA' quince rootstocks were more susceptible when compared to the ‘EMC’ rootstock.

Specific studies about resistance of quince cultivars to *D. mespili* are scarce. Nevertheless, it is known that Japonese cultivar presents a higher level of resistance to the pathogen when compared to the others. However, this cultivar does not belong in the *Cydonia*

oblonga Miller species like all the others, but in *Chaenomeles speciose* Koehne. It is used as a rootstock of quince and other related plant species because of its ability to contain fungal development. Nevertheless, future studies are needed to understand the differences and mechanisms of resistance in quince cultivars.

2.7 Diagrammatic scales

Disease quantification is essential for epidemiological studies and control strategies (COOKE, 2006). It can be performed in the following ways: 1) disease intensity, where the amount of disease present in a population is quantified; 2) disease prevalence, where the proportion of places where the disease is detected is quantified; 3) disease incidence, where the proportion of symptomatic organs (root, stem, leaf, flower or fruit) of the plant within the sample is quantified, and 4) disease severity, where the diseased area of the organ of the plants being considered is quantified (NUTTER et al., 1991).

The quantification procedure is usually performed subjectively through visual analysis (BADE et al., 2011), which makes it essential to use tools that standardize the process (SPÓSITO et al., 2004; PASSADOR et al., 2013). Diagrammatic scales are widely used for this purpose and are usually elaborated to quantify disease severity (DO VALE et al., 2001). They are able to improve disease quantification process and are broadly used in the Phytopathology. This justifies the elaboration of different scales in the recent years (VIEIRA et al., 2014; BELAN et al., 2014; LAGE et al., 2015; DEBONA et al., 2015; RAMOS et al., 2015; NICOLI et al.; 2015; LIBRELON et al., 2015; OLIVEIRA et al., 2015; BRAIDO et al.; 2015; DE PAULA et al.; 2016).

Diagrammatic scales can classify the amount of disease qualitatively or quantitatively (HORSFALL and BARRATT, 1945; CHESTER, 1950). Those that quantify the disease qualitatively, classify it according to the description of the symptoms as "slight", "moderate" or "severe" (descriptive scales), or grading disease severity into arbitrary classes that represent increasing severity symptoms (ordinal scales) (NUTTER and ESKER, 2006). As these types of measurement are very subjective, their use is considered limited (BOCK et al., 2010). In any case, ordinal scales are widely used for the quantification of viral diseases, which do not present symptoms that are easily quantitatively measured (MADDEN et al., 2007). Anyway, the first diagrammatic scale to be recorded was elaborated with intervals by Cobb to assess quantitatively the wheat rust. It has five illustrations representing five different degrees of severity ranging from 1% to 50% (Figure 3) (COBB, 1892; LARGE, 1966).

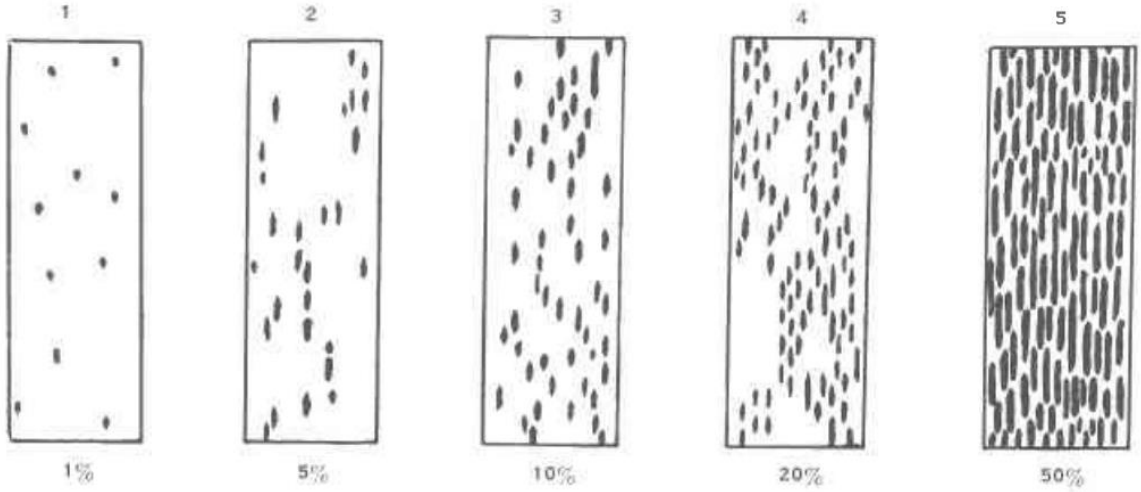

Figure 3. Diagrammatic scale to estimate the severity of rust on wheat proposed by Cobb (1892).

The Cobb scale was the first interval scale developed (COBB, 1892). That is, with its use the amount of disease is classified within a severity range (DEVELLIS, 1991). Interval scales can be structured with or without levels (BOCK et al., 2010). In those without levels, percentage values are assigned to quantify the disease (Figure 4) (DUAN et al., 2015). While with the interval scales with levels the evaluations are made from notes that are later transformed into a disease index. Each note represents a level of the scale, which corresponds to a range of severity (Figure 5) (NUÑEZ et al., 2017). Although most of the scales published to date are interval scales without levels (DEL PONTE et al., 2017), in the last two years the number of interval scales with levels has increased (NUÑEZ et al., 2017). Anyway, there is no information in the literature about which of the interval scales, with or without levels, has better effectiveness.

The elaboration of the diagrammatic scales has been well studied in the last 20 years (BOCK et al., 2010) and the factors that can favor or disadvantage their effectiveness are already known. The individual ability of each evaluator (HORSFALL and COWLING, 1978), the number of lesions per injured area (AMANAT, 1976; NITA et al, 2003; GODOY et al., 2006), structure and size of the plant to be quantified (FORBES and JEGER, 1987; DANIELSEN and MUNK, 2004), time spent to quantify the disease (NUTTER et al., 1993; PARKER et al., 1995), complexity of the disease symptom (BOCK et al., 2008), structure of scale elaboration (descriptive scale or interval scale, scale ilustration) (BRAIDO et al., 2015) and the statistical method used in the validation process to test the effectiveness of the scale

(BOCK et al., 2009; DEL PONTE et al., 2017) are considered source of error that may compromise scale effectiveness.

The diagrammatic scale must be validated to be proposed as a standard method (LAZAROTO et al., 2012). For this, statistical methods are used to verify the accuracy, precision and repeatability of the assessments made with them. The accuracy represents how close the estimated values are from actual (observed) values. Precision measures how many times the same value has been estimated and can be represented by repeatability (intra-evaluator reliability) or by reproducibility (inter-evaluator reliability) (NUTTER et al., 1991, 2001; MADDEN et al., 2007). Some of the statistics that can be used in the validation process are analysis of variance, correlation coefficient, regression analysis and Lin's concordance correlation coefficient. Most of the proposed scales have been validated using the linear regression method, but it is suggested that Lin's statistics are superior for assessing precision and accuracy of measurements (LIN, 1989; DEL PONTE et al., 2017). Nevertheless, Nuñez et al. (2017) validated a scale for quantification of black rot severity (*Xanthomonas campestris pv. campestris*) in the kale with linear regression and Lin's statistics. In any case, one of the obstacles on disease quantification is the lack of a specifically adjusted statistical model for the case. In other words, more studies are needed to improve the diagrammatic scales validation.

Plant disease quantification process began about 100 years ago and has changed since then (BOCK et al., 2010). While efforts are being made to improve the diagrammatic scales development, there are still many issues to be explored (DEL PONTE et a., 2017).

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4 CHAPTER 2

COMPARING DIFFERENT DIAGRAMMATIC SCALES FOR QUANTIFICATION OF ENTOMOSPORIOSIS SEVERITY IN QUINCE

ABSTRACT

Entomosporiosis, caused by *Diplocarpon mespili*, has a worldwide distribution and occurs in most quince producing regions. Disease quantification is essential for epidemiological studies and control strategies. Diagrammatic scales have been widely used for this purpose, but certain aspects of their elaboration still need to be understood. Therefore, this prompted us to elaborate, validate and compare two different diagrammatic scales to assess entomosporiosis severity in quince. A sample of 112 leaves naturally infected were collected and scanned to determine the real disease severity. The scales were developed considering the lowest and the highest limits of disease severity observed in the field. The first scale was elaborated with six levels and each level represents a range of disease severity: 0 (0.00%); 1 (0.1 - 4%); 2 (4.1 - 8%); 3 (8.1 - 16%); 4 (16.1 - 24%); 5 (<24%). The second one was elaborated with percentages of diseased areas, without levels. To validate the scales 50 images of diseased and healthy leaves were presented to 10 inexperienced evaluators. Evaluations were repeated three times in the following order: 1) an initial assessment without the scale; 2) a second assessment with the scale performed seven days later; 3) a final assessment seven days later with the scale to assess the repeatability. According to the Lin's statistics and the linear regression, the scales proved to improve the accuracy and the reproducibility of disease quantification. Nevertheless, some differences on the evaluators' performances were noted according to the scale and the statistical analyses used. The Lin's statistics showed no difference between the elaborated scales. On the other hand, the linear regression analysis showed that the evaluations made with the scale with levels presented an accuracy slightly higher than those made with the scale without levels. In contrast, the repeatability of the evaluations made with the scale without levels were better. The scale without levels was used to evaluate entomosporiosis severity on 29 quince cultivars and showed that the cultivar Japanese was the most resistant whereas Rea's Mamouth were the most susceptible to entomosporiosis.

Keywords: *Diplocarpon mespili*. *Cydonia Oblonga*. Lin's statistics. Linear regression. Resistance.

4.1 INTRODUCTION

Quince (*Cydonia oblonga* Miller) is a *Rosaceae* shrub that produces fruits appreciated worldwide for its uses in the industry of jams and jellies (Hedrick 1922; Pio et al. 2008; Alvarenga et al. 2008). Although grown on almost every continent, Turkey is the world's largest producer. When compared to the production of other crops, quince does not have as much economic importance, but is considered an emerging crop due to its adaptation to high temperatures and acidic soils (Postman 2009; Hummer et al. 2012).

The major problem for quince cultivation is the fungus *Diplocarpon mespili*, causal agent of the disease known as entomosporiosis (Pio et al. 2005). The disease occurs wherever quince is cultivated. However, higher incidences are noticed in Europe, Australia, Canada, United States, Paraguay and Brazil (Park et al. 2011). Disease symptoms are observed mainly on quince leaves, but also occur on branches and fruits. On leaf surfaces, small lesions with colours varying from brown to red occur. These small lesions coalesce over time and turn into necrotic spots. Depressed lesions that may lead to cracks are observed on fruits (Lambe and Ridings 1979; Pio 2005; Horie and Kobayachi 1979).

Disease quantification is essential for epidemiological studies and control strategies (Spósito et al. 2004). The severity is one of the measures used for disease quantification and is expressed as percentage of diseased tissue (Jackson et al. 2006; Boyle, Hamelin and Seguin, 2005). Determining diseased area is too time consuming to be performed with a large number of samples. This makes diagrammatic scales useful for greater accuracy in disease evaluation (Passador et al. 2013).

Although widely used for disease quantification, there is no perfect methodology for the diagrammatic scales elaboration (Large et al. 1966, Del Ponte et al. 2017). Anyway, in the last 30 years their elaboration has been studied in order to improve their effectiveness (Bock et al. 2010). Scales can be in the following ways: 1) Nominal, where the disease severity is subjectively classified as "slight", "moderate", or "severe; 2) Ordinal rating, that classify disease severity within classes that represent increased severity; 3) Interval (with or without levels), when the scale has levels and each level represents an interval of severity, with or without illustrations, and 4) Ratio, where the minimum and maximum limits are consistently defined from 0 to 100%, with or without illustrations (DeVellis, 1991).

Most of the elaborated and validated diagrammatic scales are in interval without levels type with illustrations (Del Ponte et al. 2017), but in the last two years the number of scales

with intervals has increased (Nuñez et al. 2017). It is believed that the disease quantification process is made more quickly when scales with intervals are used (Bock et al. 2010). However, the effectiveness between disease severity assessments made with a scale elaborated in intervals with levels and a scale in intervals without levels was not compared.

Therefore, the lack of a scale to quantify entomosporiosis in quince and the questions on the scales elaboration that still need to be elucidated prompted us to elaborate, validate and compare two diagrammatic scales that were differently elaborated. This study aimed to compare the accuracy of the evaluations made with a scale elaborated in intervals with levels and a scale in intervals without levels. In addition, after being validated the interval scale was used to evaluate the resistance of 29 quince cultivars to entomosporiosis.

4.2 MATERIAL AND METHODS

4.2.1 Leaf sampling and image analyses

To elaborate the scale, 112 naturally infected leaves with different levels of severity were collected from different quince cultivars. They were obtained from an orchard at Federal University of Lavras, Minas Gerais, Bazil. For the confirmation of *D. mespili* as the etiological agent, fungal structures were observed under a microscope. The leaves were photographed with a digital camera and the photos were downloaded in the program MEASURE PICTURE v. 1.0 (Kassler 2016). The program was used to determine the real leaf severity, considering necrotic areas as diseased tissue for quantification. In sequence, the distribution of disease severity frequency was done according to the lowest and highest frequencies observed in the sampled leaves. Using the intervals with higher concentration of leaves with the same percentage of necrotic area, two scales were defined. The first one was elaborated in levels whereas the second was elaborated without levels. The illustrations of the scales were made using real images of diseased leaves.

4.2.2 Validation and comparison of the diagrammatic scales

Collecting data

Diagrammatic scales must be validated before they are used as a standard method for assessments of disease severity. To do this, 50 real images of diseased quince leaves were randomly placed in individual slides (LibreOffice Impress 4.4). Then, ten evaluators without experience with entomosporiosis performed the assessment of disease severity according to each developed scale. For the assessments using the scale with levels, the evaluators assigned grades varying from 0 to 5 to quantify the disease severity. Each grade representing a range of disease severity. When using the scale without levels, the analyses were done directly. That is,

the evaluators assigned values in percentage (0 - 100%) to estimate the entomosporiosis severity. The procedure was performed three times every week with the same evaluators. The first one was performed without the diagrammatic scales and the other two were done with the scales with and without levels.

Data analyses

Evaluator's performances without and with the use of the diagrammatic scales were analysed considering precision, accuracy and intra- and inter-evaluator reliability. The validation and comparison of the developed diagrammatic scales were performed by two different statistical methods: Lin's statistics and linear regression. Lin's statistics calculates Lin's correlation coefficient (LCC), that combines accuracy and precision by the agreement between the real and estimated severity values to the line of agreement (45° = perfect concordance). The accuracy is obtained through bias correction factor (C_b), that is calculated from location shift (where 0 = perfect match between *x* and *y*) and scale shift (where 1 = perfect match between *x* and *y*), variables derived from the means and standard deviations of *x* and *y*, respectively. Pearson's correlation coefficient was calculated to certify the precision of the evaluations. The confidence intervals (CIs) and 95% CIs were calculated on the difference between the groups by the t.test (P = 0.05). Intra-evaluator reliability (repeatability of two estimates done by the same evaluator) was also measured using Lin's statistics. The inter-evaluator reliability (reproducibility of the visual estimates) was calculated with the intraclass correlation coefficient (ICC) (Shrout and Fleiss 1979) and the confidence interval of the ICC estimates were used to measure the influence on the interevaluator realiability for each assessment.

Additional analyses were performed by linear regression. Real severity was considered as the independent variable and estimated severity as the dependent variable. In this case, the accuracy is measured by the coefficients β0 (intercept) and β1 (slope). The values are considered perfectly accurate when β0 = 0 and β1 = 1. The accuracy of the estimates of each evaluator was determined by the *t*-test applied to the slope coefficient (β_1) to verify whether it was significantly different from one, and to the intercept coefficient (β_0) to verify whether it was significantly different from zero. The precision of the estimates was determined by the coefficient of determination (R^2), that indicates in percentage how much the linear model can explain the observed values. The linear model is considered perfect when $R^2 = 1$. Besides the coefficient of determination, the variation of the absolute error (estimated severity minus actual severity) of the evaluations was also considered for precision determination.

All statistics analyses were performed with the R software (R Core Team 2013). The epi.cc function of the epiR package (Stevenson et al. 2012) was used to obtain Lin's CC statistics. The ICC was calculated using icc function of the irrR package (Gamer et al. 2015).

4.2.3 Assessment of the resistance in quince cultivars

During December, 2015 and January, March, and April of 2016, ten leaves of 29 different quince cultivars were randomly collected to assess disease severity. The samples were obtained from an orchard at Federal University of Lavras that harbors a collection of quince cultivars. Each leaf was photographed with a digital camera and had the final disease severity determined with the diagrammatic scale with levels.

4.3 RESULTS

4.3.1 Development of the diagrammatic scales

Entomosporiosis severity on quince leaves ranged from 0.48% to 46.2%. Almost 90% of the sampled leaves presented less than 24% of disease severity and approximately 70% of them presented up to 16% of disease severity. The interval between 0 and 8% of severity showed the highest frequency (43.70%) of diseased leaves among the 112 leaves used in this study. The range and the frequency of severity (Figure 1) were considered to elaborate two diagrammatic scales to quantify entomosporiosis on quince. The first scale was elaborated with 16 real illustrations of quince leaves in six levels that represent the following intervals of disease severity: 0 (0.0%), 1 (0.1 – 4%), 2 (4.1 - 8%), 3 (8.1 – 16%), 4 (16.1 - 24%) and 5 (>24%) (Figure 2). The second scale was elaborated with six real illustrations representing percentages of diseased area, without levels (Figure 3).

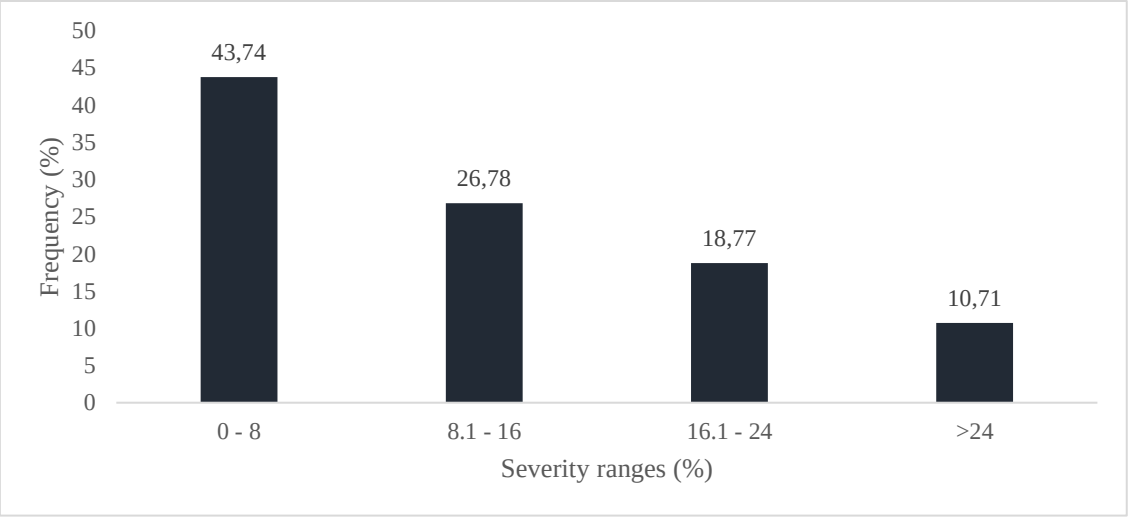

Figure 1: Frequency of entomosporiosis severity in a sample of 112 quince leaves.

4.3.2 Validation of the diagrammatic scales

Lin's statistics

Lin's statistics was the first method used to validate and compare the diagrammatic scales. It calculates the agreement between real and estimate severities for each evaluator (Lin, 1989). The Lin's concordance coefficient (LCC - ρ_c), that combines the measures of accuracy and precision, ranged from 0.46 when not using diagrammatic scales to 0.86 and 0.88 when using the diagrammatic scale with and without levels, respectively (Table 1). The bias correction factor (C_b), that corresponds to the accuracy, did not presented significant difference with and without using the scales. When not using the diagrammatic scales its value was 0.97, whereas it was 0.96 using the diagrammatic scale with levels, and 0.97 when using the diagrammatic scale without levels (Table 1; Table2).

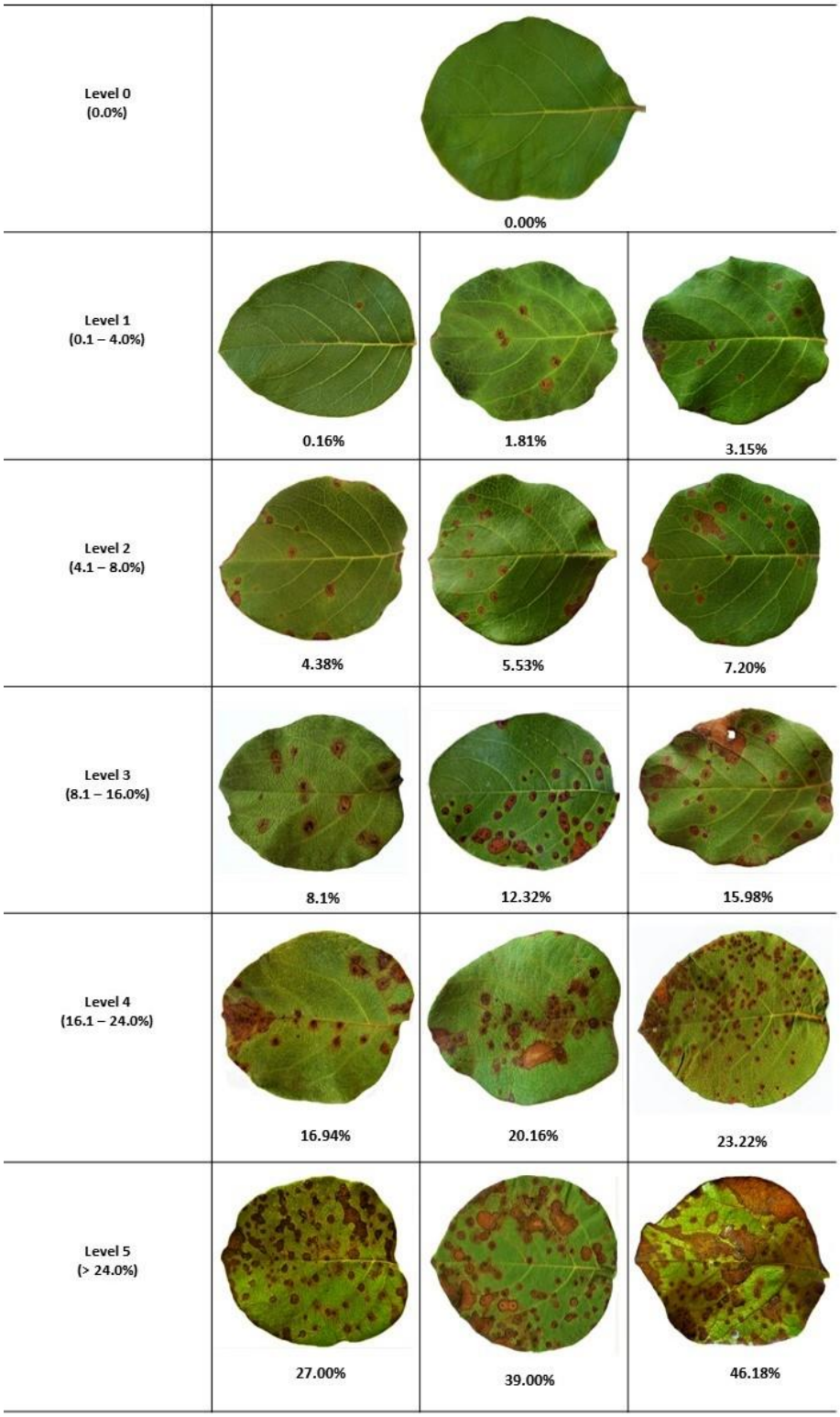

Figure 2. Diagrammatic scale elaborated with levels to quantify the entomosporiosis on quince (*Cydonia oblonga*). Numbers below each picture represent the real percentage of leaf area affected by the disease.

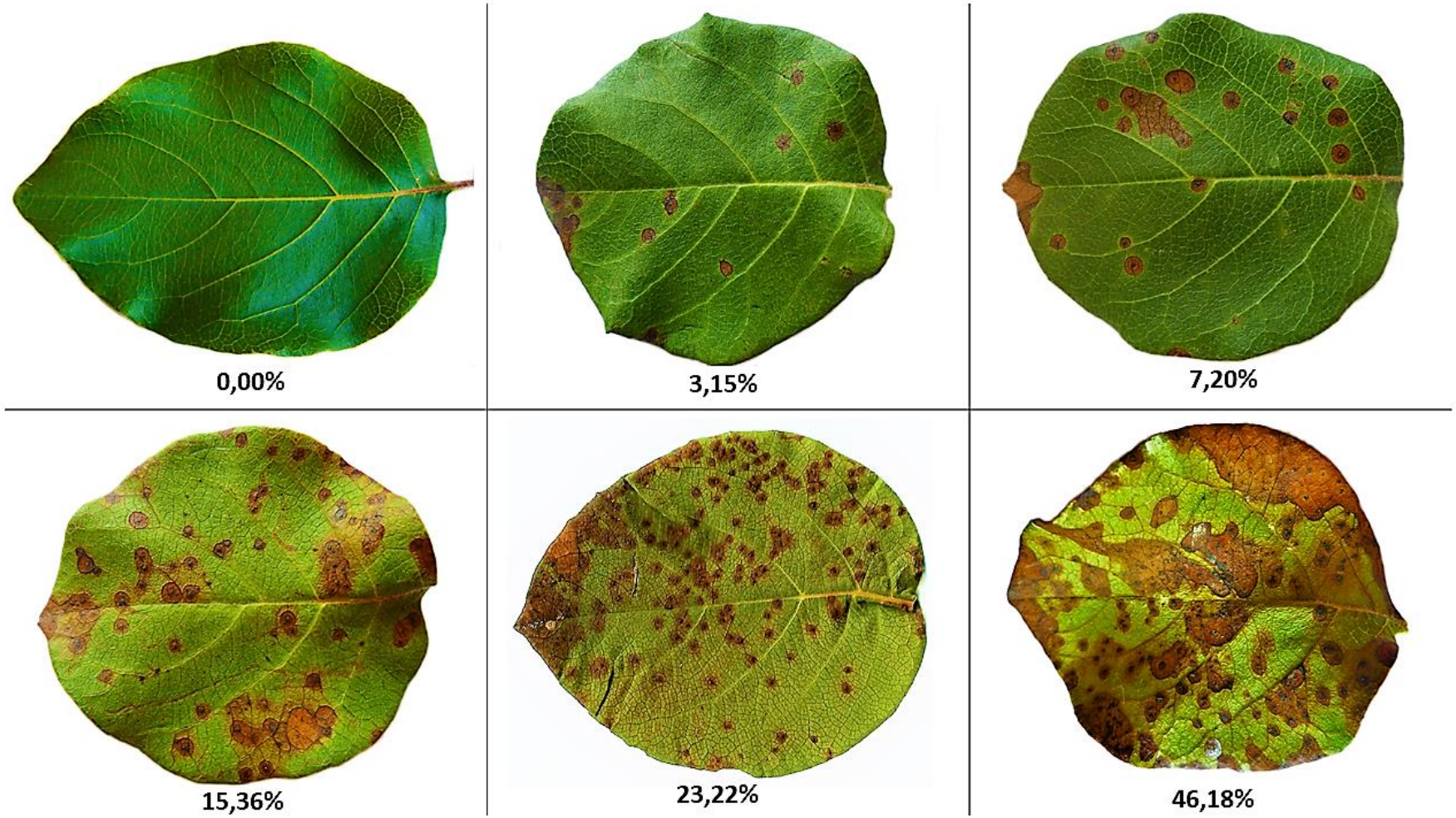

Figure 3. Diagrammatic scale without levels to quantify the severity of entomosporiosis on quince (*Cydonia oblonga*). Numbers below each picture represent the real percentage of leaf area affected by disease.

Table 1. Coefficients of visual estimates of entomosporiosis on quince leaves performed by 10 evaluators without and with the use of the diagrammatic scale with levels as determined by using Lin's statistics.

Lin's statistics	Means		
	Without scales	With scales	95% CI[l] of the difference between means
Scale with levels			
Scale shift [a]	1.26	0.92	-0.132; 0.470
Location shift [b]	0.08	-0.28	-0.359; 0.564
Bias correction [c]	0.97	0.96	-0.244; 0.056
Correlation coefficient [d]	0.48	0.89	**-0.618; -0.087**
Concordance coefficient [e]	0.46	0.86	**-0.648; -0.179**
Sacale without levels			
Scale shift [a]	1.26	0.85	-0.066; 0.540
Location shift [b]	0.08	-0.19	-0.261; 0.619
Bias correction [c]	0.97	0.97	-0.246; 0.056
Correlation coefficient [d]	0.48	0.90	**-0.638; -0.109**
Concordance coefficient [e]	0.46	0.88	**-0.666; -0.199**

[a] Scale shift coefficient relative to the perfect match (1 = perfect match between *x* and *y*).
[b] Location shift coefficient relative to the perfect match (0 = perfect match between *x* and *y*).
[c] Bias correction factor (*Cb*) measures how much the best-fit line deviates from 45°. No deviation from the 45° line occurs when C*b* = 1. C*b* is a measure of accuracy calculated from scale shift and location shift coefficients.
[d] Pearson's correlation coefficient measures precision (*r*).
[e] Lin's concordance coefficient (ρ_c) that combines precision and accuracy to measure the agreement with the true values.
[f] Confidence intervals (CI's) were based on *t*-test (P = 0.05). Bold numbers represent significant differences.

Pearson's correlation coefficient (*r*), is another precision measurement and showed great improvement with the use of the diagrammatic scales. Without the scales its mean was 0.48. When the scales were used, the coefficient means were 0.89 and 0.90 for the scale with levels and without levels, respectively (Table 1). Overall, the use of the diagrammatic scales improved disease severity assessments (Table 1; Figure 4).

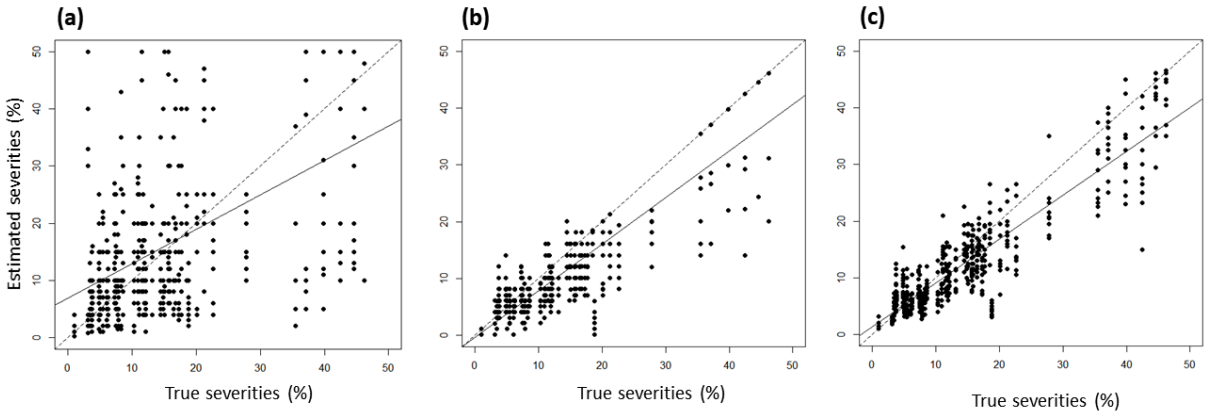

Figure 4. Relationship between true and estimated entomosporiosis severity on quince without the use of the diagrammatic scales (a) and with the use of the diagrammatic scales with levels (b) and without levels (c) for 50 diseased leaves. The solid line represents the best-fitting line, whereas the dotted line is the concordance line, which represents perfect agreement between true and estimated severities (slope of 1, intercept of 0).

Intra-evaluator reliability (repeatability) had high values when using the diagrammatic scales. The LCC (ρ_c) means were 0.85 and 0.89 with the aid of the scales with and without levels, respectively (Table 6). Interevaluator reliability (reproducibility) was tested by intraclass correlation coefficients (ICC), which was P = 0.34 without any scale and P = 0.88 and P = 0.89 for the assessments using the scale with and without levels, respectively (Table 4).

Table 4. Coefficients of determination (R^2) of linear regression equations between evaluators matched in pairs of visual estimates of entomosporiosis by 10 evaluators.

Evaluators	B	C	D	E	F	G	H	I	J
Without scale									
A	0.69	0.71	0.51	0.007	0.56	0.016	0.025	0.8	0.011
B		0.58	0.4	0.007	0.41	0.007	0.003	0.64	0.007
C			0.55	0.06	0.65	0.1	0.05	0.68	0.08
D				0.74	0.1	0.77	0.58	0.01	0.77
E					0.06	0.83	0.73	0.003	0.86
F						0.11	0.11	0.51	0.12
G							0.59	0.01	0.88
H								0.01	0.69
I									0.008
Scale with levels									
A	0.67	0.88	0.86	0.92	0.84	0.89	0.79	0.85	0.89
B		0.77	0.81	0.70	0.84	0.76	0.50	0.50	0.69
C			0.94	0.91	0.91	0.89	0.66	0.79	0.92
D				0.88	0.90	0.91	0.68	0.78	0.91
E					0.91	0.87	0.68	0.83	0.90
F						0.85	0.60	0.76	0.85
G							0.75	0.75	0.89
H								0.61	0.79
I									0.81
Scale without levels									
A	0.90	0.91	0.90	0.90	0.80	0.88	0.90	0.84	0.87
B		0.94	0.88	0.87	0.84	0.89	0.87	0.79	0.88
C			0.87	0.88	0.85	0.89	0.89	0.83	0.90
D				0.84	0.76	0.84	0.86	0.86	0.85
E					0.89	0.86	0.89	0.82	0.90
F						0.81	0.80	0.76	0.83
G							0.87	0.77	0.90
H								0.84	0.88
I									0.83

Assessment	Intraclass correlation coefficient, P (95%)[a]
Without scale	0.34 (0.23-0.47)
Scale with levels	0.88 (0.83-0.92)
Scale without levels	0.89 (0.84-0.93)

[a] Interevaluator reliability shows the reproducibility of visual estimates of entomosporiosis severity by 10 evaluators.

Linear Regression

The linear regression is another method used to validate the scales. The effectiveness of the elaborated diagrammatic scales was confirmed again with this method. Regression

analyses showed that estimates increased significantly when comparing the assessments done without and with the use of the scales. When the assessments were done without any scale, the hypothesis β0 = 0 was rejected by six evaluators and the hypothesis β1 = 1 was rejected by four evaluators (Table 5). With the aid of both scales the hypothesis β0 = 0 was rejected by only one evaluator and the hypothesis β1 = 1 was accepted by all the evaluators. These results show that the assessments done with the scales improved the accuracy. The variation in the coefficient of determination (R^2) varied from 0.42, without using the scales, to 0.73 and 0.82 with the scale with and without levels, respectively (Table 5).

Table 5. Linear regression coefficients of visual estimates of entomosporiosis on quince leaves performed by 10 evaluators without and with use of the diagrammatic scales.

	Coefficients								
				Using the diagrammatic scales					
	Without scale			Scale with levels			Scale without levels		
Evaluators	$\beta 0^a$	$\beta 1^b$	R^{2c}	$\beta 0^a$	$\beta 1^b$	R^{2c}	$\beta 0^a$	$\beta 1^b$	R^{2c}
A	1.48^{ns}	1.82^{ns}	0.83	-1.14^{ns}	0.88^{ns}	0.85	2.24^{ns}	0.75^{ns}	0.83
B	2.64^{*}	0.24^{ns}	0.65	0.55^{ns}	0.68^{ns}	0.67	2.22^{ns}	0.64^{ns}	0.87
C	1.5^{ns}	1.01^{ns}	0.79	-2.51^{ns}	0.91^{ns}	0.86	0.78^{ns}	0.79^{ns}	0.86
D	2.89^{ns}	0.89^{ns}	0.51	0.02^{ns}	0.86^{ns}	0.84	1.80^{ns}	0.78^{ns}	0.77
E	16.38^{*}	0.09^{*}	0.01	-0.31^{ns}	0.94^{ns}	0.9	3.78^{*}	0.87^{ns}	0.88
F	-3.45^{ns}	0.74^{ns}	0.58	-3.62^{*}	0.91^{ns}	0.87	-0.85^{ns}	0.95^{ns}	0.88
G	17.36^{*}	0.17^{*}	0.02	1.40^{ns}	0.84^{ns}	0.84	1.04^{ns}	0.80^{ns}	0.86
H	13.24^{*}	0.14^{*}	0.02	1.97^{ns}	0.64^{ns}	0.68	1.60^{ns}	0.66^{ns}	0.87
I	3.60^{*}	0.80^{ns}	0.82	-0.46^{ns}	0.76^{ns}	0.78	0.08^{ns}	0.81^{ns}	0.8
J	13.21^{*}	0.12^{*}	0.01	-0.22^{ns}	0.78^{ns}	0.86	0.81^{ns}	0.70^{ns}	0.88
Mean	...	...	0.42	...	...	0.82	...	...	0.85

* Significant intercept (β 0) or slope (β 1) values where the null hypothesis (β 0 = 0 or β 1 = 1) was rejected according to the *t*-test (P = 0.05).
ns Non-significant intercept (β 0) or slope (β 1) values where the null hypothesis (β 0 = 0 or β 1 = 1) was accepted according to the *t*-test (P = 0.05).
[a] Intercept coefficient (β 0) of the regression equations.
[b] Slope coefficient of the line (β 1) of the regression equations.
[c] Determination coefficient values (R^2) for the regression analyses.

The residual estimates are given by subtracting the true severity from the estimated severity. With this value it is possible to know if the estimated severities were over or underestimated. Minimum and maximum residual values for all evaluators without the scales were - 36.18 and 46.87, respectively. Using the scales these values were -28.41 and 9.21 with

the scale with levels, and -27.48 and 9.64 with the scale without levels. There is a trend towards overestimation without the use of scales and of underestimation with the use of the scales (Figure 5).

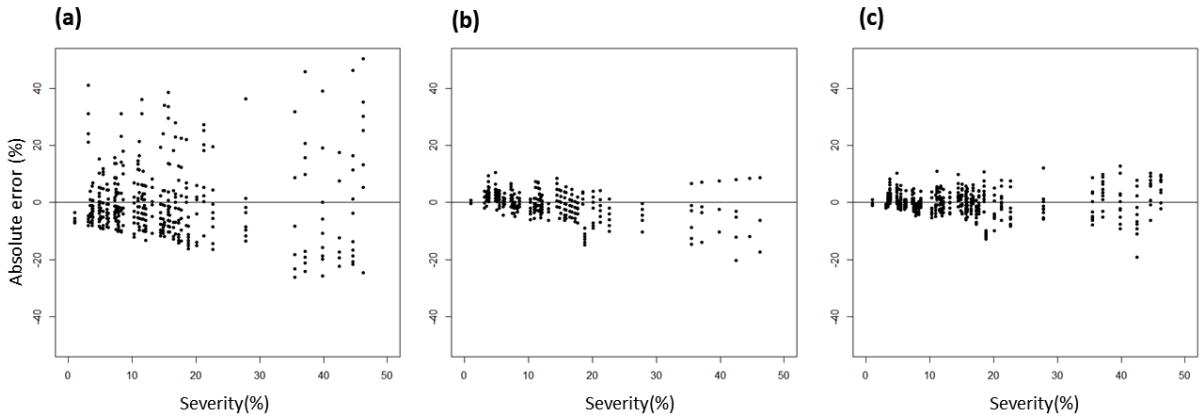

Figure 5. Distribution of residual estimates obtained from 10 evaluators. Distribution of residuals (estimated severity – true severity) of entomosporiosis estimates without a diagrammatic scale (a) and with the diagrammatic scale with (b) and without (c) levels.

The repeatability of the evaluations was calculated by linear regressions between the first and the second estimates done by the same evaluators using the scales. The scales with and without levels had the null hypothesis $\beta 0 = 0$ rejected for only one of the evaluators, whereas the hypothesis $\beta 1 = 1$ was accepted for all of them. The mean of the coefficient of determination (R^2) was higher when using the scale without levels ($R^2 = 0.84$) as compared to the scale with levels ($R^2 = 0.76$) (Table 6).

The reproducibility of each scale was tested by comparing the assessments done by pairs of evaluators in all possible combinations. Without using the scales the values of R^2 ranged from 0.003 to 0.88%, with an average of 0.36%. In evaluations of the scale with levels the R^2 value ranged from 0.50 to 0.91%, with an average of 0.80; whereas it ranged from 0.76 to 0.94% for the scale without levels, with an average of 0.86% (Table 4).

Table 6. Linear regression coefficients in comparison with two evaluations of entomosporiosis severity with the diagrammatic scale with and without levels performed by the same evaluators and Lin concordance correlation coefficient to measure the intra-evaluator reliability (repeatability).

	Coefficients							
	Scale with levels				Scale without levels			
Evaluators	$\beta 0^a$	$\beta 1^b$	R^{2c}	LCC[d]	$\beta 0^a$	$\beta 1^b$	R^{2c}	LCC[d]
A	1.65^{ns}	0.90^{ns}	0.75	0.87	0.48^{ns}	0.93^{ns}	0.88	0.93
B	1.21^{ns}	0.99^{ns}	0.9	0.94	2.21^{ns}	0.90^{ns}	0.87	0.92
C	1.8^{ns}	0.90^{ns}	0.85	0.92	1.99^{ns}	0.98^{ns}	0.73	0.84
D	0.47^{ns}	0.90^{ns}	0.81	0.9	0.98^{ns}	0.74^{ns}	0.74	0.82
E	0.57^{ns}	0.96^{ns}	0.89	0.94	1.10^{ns}	0.90^{ns}	0.82	0.91
F	2.17^{ns}	0.74^{ns}	0.76	0.86	-0.57^{ns}	0.99^{ns}	0.91	0.95
G	1.83^{ns}	0.93^{ns}	0.86	0.92	1.04^{ns}	1.04^{ns}	0.88	0.92
H	5.45^{*}	0.72^{ns}	0.5	0.68	3.46^{*}	0.77^{ns}	0.79	0.87
I	3.03^{ns}	0.86^{ns}	0.5	0.68	1.13^{ns}	0.94^{ns}	0.87	0.93
J	0.79^{ns}	1.17^{ns}	0.76	0.81	-2.50^{ns}	1.49^{ns}	0.87	0.81
Mean	…	…	0.76	0.85	…	…	0.84	0.89

* Significant intercept (β 0) or slope (β 1) values where the null hypothesis (β 0 = 0 or β 1 = 1) was rejected according to the t-test (P = 0.05).
[ns] Non-significant intercept (β 0) or slope (β 1) values where the null hypothesis (β 0 = 0 or β 1 = 1) was accepted according to the *t*-test (P = 0.05).
[a] Intercept coefficient (β 0) of the regression equations.
[b] Slope coefficient of the line (β 1) of the regression equations.
[c] Determination coefficient values (R^2) for the regression analyses.
[d] Lin's concordance coefficient (ρ_c) that combines precision and accuracy to measure the agreement with the true values.

Comparison between the diagrammatic scales

The comparison between the efficiency of the diagrammatic scales with and without levels was done for each variable obtained in the validation process through the *t*-test (P = 0.05). Accuracy and repeatability were significantly different (P<0.05) between the scales with and without levels. The scale with levels presented an intercept closer to 0 (β0 = 0.43) when compared to the scale without levels (β0 = 1.350), which means a greater accuracy. On the other hand, the scale without levels presented a better repeatability (R^2 = 0.858) than the scale with levels (R^2 = 0.802). Lin's statistics did not show any significant differences between the two scales (Table 7).

Table 7. Comparison between the results of the visual estimates of the entomosporiosis on quince leaves performed by 10 evaluators with the proposed diagrammatic scales (with or without levels) obtained by Lin's statistics and linear regression.

	Means		
Coefficient tested	Scale with levels	Scale without levels	95% CI[m] of the difference between means
Lin's statistics			
Scale shift [a]	0.908	0.840	-0.019; 0.155
Location shift [b]	-0.121	-0.197	-0.153; 0.305
Bias correction [c]	0.946	0.947	-0.038; 0.036
Correlation coefficient [d]	0.901	0.922	-0.055; 0.013
Concordance coefficient [e]	0.855	0.874	-0.070; 0.032
Linear regression			
$\beta 0$[f]	**-0.432**	**1.350**	**-3.193; -0.370**
$\beta 1$[g]	0.820	0.775	-0.047; 0.137
R^2[h]	0.815	0.850	-0.095; 0.025
Inter evaluator reliability			
R^2[i]	**0.802**	**0.858**	**-0.091; 0.020**
Intra evaluator reliability			
LCC[j]	0.852	0.890	-0.113; 0.037
R^2[k]	0.758	0.836	-0.176; 0.019

[a] Scale shift coefficient relative to the perfect match (1 = perfect match between *x* and *y*).
[b] Location shift coefficient relative to the perfect match (0 = perfect match between *x* and *y*).
[c] Bias correction factor (*Cb*) measures how much the best-fit line deviates from 45°. No deviation from the 45° line occurs when C*b* = 1. C*b* is a measure of accuracy calculated from scale shift and location shift coefficients.
[d] Pearson's correlation coefficient measures precision (*r*).
[e] Lin's concordance coefficient (ρ_c) that combines precision and accuracy to measure the agreement with the true values.
[f] Intercept coefficient (β 0) of the regression equations.
[g] Slope coefficient of the line (β 1) of the regression equations.
[h] Determination coefficient values (R^2) for the regression analyses.
[i] Determination coefficient values (R^2) for the regression analyses between evaluators matched in pairs of visual estimates of entomosporiosis by 10 evaluators.
[j] Lin's concordance coefficient (ρ_c) that combines precision and accuracy in comparison with two evaluations of entomosporiosis severity with the diagrammatic scale with and without levels performed by the same evaluators.
[k] Determination coefficient values (R^2) for the regression analyses in comparison with two evaluations of disease severity with the diagrammatic scale with and without levels performed by the same evaluators.

[m] Confidence intervals (CI's) were based on the *t*-test (P = 0.05). Bold numbers represent significant differences.

4.3.3 Resistance of quince cultivars to *D. mespili*

The Japanese cultivar was the most resistant with a final disease severity of 4.75%, whereas Rea's Mamouth with a final disease severity of 17,28% was the most susceptible to the disease (Figure 6).

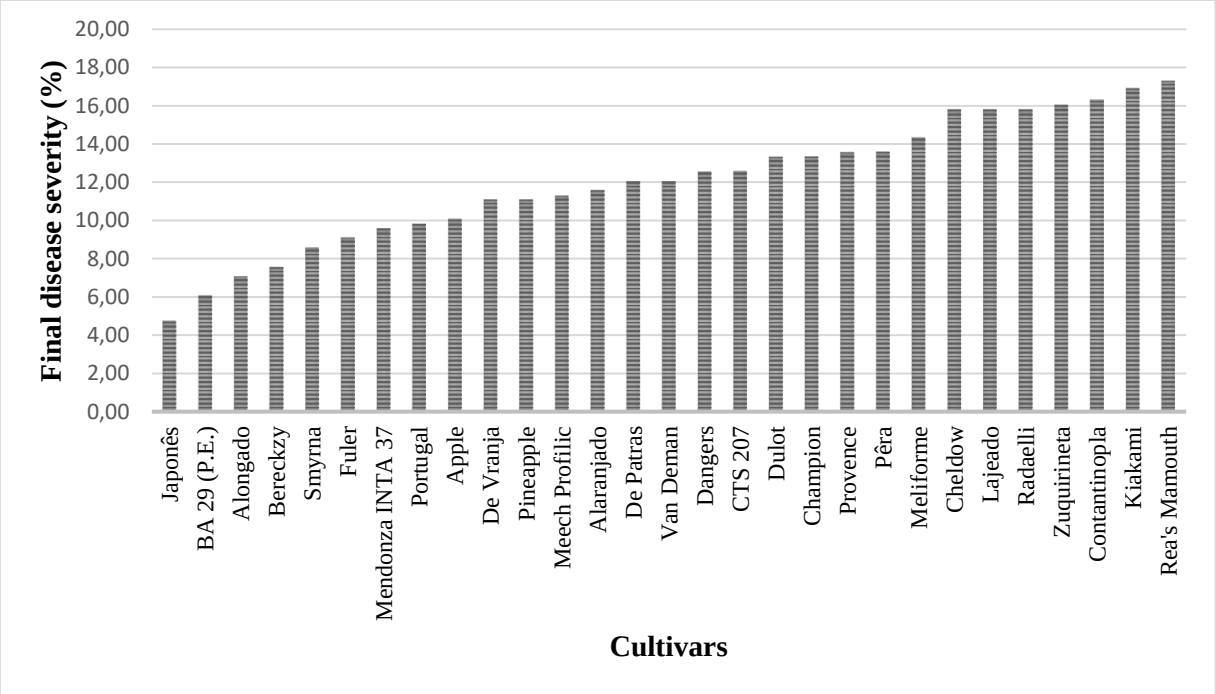

Figure 6. Final entomosporiosis severity in quince cultivars.

4.4 DISCUSSION

Different authors demonstrated the efficiency of diagrammatic scales as a tool to quantify plant diseases (Godoy et al. 1997; 2006; Angelotti et al. 2008; Dos Santos et al. 2010; Duan et al. 2015; Braido et al. 2015; De Paula et al. 2016). In this study, two diagrammatic scales to quantify entomosporiosis on quince were developed with and without levels, subsequently the scales were validated and compared. In the last part of the study, the scale with levels was used to evaluate the resistance of 29 quince cultivars.

The maximum entomosporiosis severity observed in the field (46, 18% lesioned area) expands the severity estimated by Nunes et. al (2012) on five pear cultivars (40% lesioned area). This difference may be due to some characteristic of the interactions between the fungus and each host species (Agrios 2005). The absence of leaves presenting greater severity than 46,18%

of lesioned area can be explained by disease infectious process on plant tissue. Although there are no studies regarding entomosporiosis severity on quince, it is known that the disease progress can lead to host defoliation (Pio et al. 2005).

Visual quantification of plant diseases is prone to errors that may affect its efficiency (Bock et al., 2010). The illustration of the scale is one of the contributing factors and therefore it should be as close as possible to the actual image being represented (Lazaroto et al. 2012; Damasceno et al. 2014). Entomosporiosis symptoms are rounded lesions, ranging in color from light brown to red, with small black spots in the center and are relatively easy to be observed (Pio 2005). These characteristics allowed the scales proposed in this study to be illustrated with real images of diseased leaves, which probably facilitated the assessments by the evaluators.

In this study, the validation of the scales was supported by the results obtained through Lin's statistics and linear regression. Despite this, there was a difference between the results of the use of the scales with or without levels according to the regression analysis, but not according to Lin's statistics. This happened due to the difference between the statistical methods used. No statistical method is able to perfectly quantify the acuity of a model (Lin 1989), but Lin's statistics have been considered more appropriate to validate diagrammatic scales (Del Ponte et al. 2017). Linear regression has the disadvantage of not detecting the departure from the intercept 0 and slope 1 if the data are very scattered, which can lead to the rejection of a highly reproducible model due to a small error (Lin 1989). Madden et al. (2007) presented this disadvantage in detail, which led us to consider only the results of the Lin's statisticians in this study.

A diagrammatic scale should improve the precision, accuracy and inter- and intra-evaluator reliability of assessments (Michereff et al. 2009; Ydav et al. 2013). Some of the evaluators did not show a significant difference in their accuracy in assessments with and without a scale. This can be explained by the disease symptoms, which are lesions that have a considerable size when compared with rust pustules, for example. Small lesions are more difficult to estimate precisely and accurately (Sherwood et al. 1983; Godoy et al. 1997). Larger lesions are easier to observe and estimate with less errors (Forbes and Jeger 1987). On the other hand, the precision and reproducibility of assessments were significantly higher when using the diagrammatic scales. This is because evaluations using scales for disease quantification suffer less interference from possible sources of error, making the evaluation more standardized (Custódio et al. 2011). The repeatability obtained with the use of the scales presented values

consistent with scales already validated (Braido et al. 2014; Lage et al. 2015), indicating their usefulness.

The number of illustrations on a diagrammatic scale also influences the outcome of disease quantification. (Bock et al. 2010). Del Ponte et al. (2017) recommend the use of not less than six and no more than ten illustrations per scale. Most of the validated scales were elaborated according to this recommendation: in intervals, without levels and with six illustrations (Del Ponte et al. 2017). However, the validation of scales in intervals with levels, which usually have more than ten illustrations, has increased in the last two years (Nuñez et al. 2017). This study showed no difference between the efficiency of entomosporiosis quantification when using the scale with or without levels. This result goes against the recommendation of Del Ponte et al. (2010) and may be explained by how the evaluation is performed with each type of scale. Perhaps assigning notes instead of assigning direct percentages is easier enough to nullify the influence of the recommended number of illustrations. Anyway, more studies need to be done to test this hypothesis.

This study provided the first data on the levels of resistance of quince cultivars to *D. mespili*. The severity of entomosporiosis was quantified in 29 quince cultivars using the diagrammatic scale with levels. As the two elaborate scales presented the same efficiency, the choice of the scale for this evaluation was a matter of preference. The final disease severity showed that the quince collection evaluated in this study has a variability in their level of resistance and confirmed previous observation regarding a greater resistance of the cultivar Japanese (Piot et al. 2005). A possible explanation for a greater resistance of this cultivar is the fact that it belongs to the genus *Chaenomeles* while the other cultivars belong to the genus *Cydonia*. Anyway, the result obtained through the quantification of the disease does not rule out the need for future studies. These studies will be needed to understand which factors influence this difference in resistance among cultivars and may influence the development of crop management strategies.

The elaboration, validation and comparison of the diagrammatic scales to quantify entomosporiosis severity will favor epidemiological studies and a consequent better understanding of the pathogen-host relationship and the development of alternative methods to control *D. mespili* on quince.

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