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IMPACTO DA DENSIDADE DE SEMEADURA NA INCIDÊNCIA DE MANCHA PÚRPURA EM SEMENTES DE SOJA

Impact of seed density on the incidence of purple seed stain in soybean seeds

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RESUMO

Para maximizar o rendimento das culturas, tem-se incentivado o manejo intensivo, como o ajuste da densidade de semeadura, crucial para a captação de recursos ambientais. A *Cercospora kikuchii*, um fungo comum nas regiões produtoras de soja do Brasil, especialmente em áreas com alta incidência de chuvas, pode causar perdas de até 30% na produtividade. Este estudo avaliou a influência de diferentes densidades de semeadura na ocorrência de mancha púrpura em sementes de soja e investigou se a doença afeta a qualidade das sementes, considerando vigor e germinação. Conduzido na Universidade de Passo Fundo (safra 2021/2022), o experimento utilizou um delineamento em blocos casualizados com quatro densidades de semeadura. Densidades mais altas resultaram em maior incidência da doença, mas sem impacto significativo na qualidade das sementes. A presença de *Cercospora kikuchii* em altos índices está associada a condições de alta umidade, exacerbadas por densidades de semeadura elevadas.

Palavras-chave: *Cercospora kiikuchi*. Qualidade de semente. Fitopatologia.

ABSTRACT

To maximize crop yield, intensive management has been encouraged, such as adjusting the sowing density, which is crucial for capturing environmental resources. Cercospora kikuchii, a fungus common in soybean-producing regions of Brazil, especially in areas with high rainfall, can cause yield losses of up to 30%. This study evaluated the influence of different sowing densities on the occurrence of purple spot in soybean seeds and investigated whether the disease affects seed quality, considering vigor and germination. Conducted at the University of Passo Fundo (2021/2022 harvest), the experiment used a randomized block design with four sowing densities. Higher densities resulted in higher disease incidence, but without significant impact on seed quality. The presence of Cercospora kikuchii at high rates is associated with high humidity conditions, exacerbated by high sowing densities.

Keywords: *Cercospora kiikuchi.* Seed quality. Phytopathology.

1 INTRODUÇÃO

The Soybean (Glycine max (L.) Merrill) is one of the most socially and economically important crops worldwide, playing a crucial role in agricultural production (EMBRAPA, 2022). To ensure crops with high agronomic potential, it is essential to use seeds of high physiological and sanitary quality, as well as to adopt appropriate planting practices. One of these practices is sowing density, which can significantly influence the uptake of resources by plants, such as sunlight, water and nutrients, being a determining factor in crop productivity (LOPES *et al*., 2008; PROCÓPIO *et al*., 2013). However, increasing sowing density can create a microclimate favorable to the development of diseases, such as purple spot, caused by the fungus Cercospora kikuchii (HANNA *et al*., 2008; GONZÁLEZ *et al*., 2008).

Purple spot is a disease that is increasingly prevalent in soybean crops, particularly in regions with high humidity. The presence of this fungus has been associated with significant losses in productivity, which can reach up to 30% in more severe cases (GRIGOLLI, 2015). The disease affects all parts of the plant, compromising grain filling and damaging seed quality, which may present cracks and intense coloration due to infection (SINCLAIR; BACKMAN, 1989; GALLI *et al*., 2005; SARAN, 2007).

In recent harvests, the widespread occurrence of purple spot has generated concern among producers, especially regarding the risk of discarding entire batches of seeds due to loss of quality (DORNELLES, 2021). However, research indicates that the severity of the spot does not directly affect seed vigor or germination, except when the symptoms are concentrated in the hilum region, where there is a greater chance of abnormal seedlings occurring (EMBRAPA, 2019).

In view of this scenario, this study aims to evaluate the influence of different seeding densities on the occurrence of purple spot in soybean seeds, as well as to investigate whether this disease affects the physiological quality of seeds, in terms of vigor and germination. The justification for this work is the fact that, although seeding density is a cultural practice widely used to increase productivity, its impact on the occurrence of diseases and, in particular, on seed quality is not yet fully understood. In addition, with the increasing adoption of intensive management practices, it is essential to understand how plant density can influence seed health and physiological performance, in order to guide producers in choosing the best cultivation strategies.

The article will be structured as follows: initially, a literature review will be presented on the relationship between seeding density, microclimate and the occurrence of diseases in

soybean crops. Then, the results of the experimental research will be presented, where the incidence of purple spot at different seeding densities and its relationship with seed quality were analyzed. Finally, the discussion will focus on how these results can contribute to improving management practices in soybean crops, aiming at maximizing productivity and ensuring high-quality seeds.

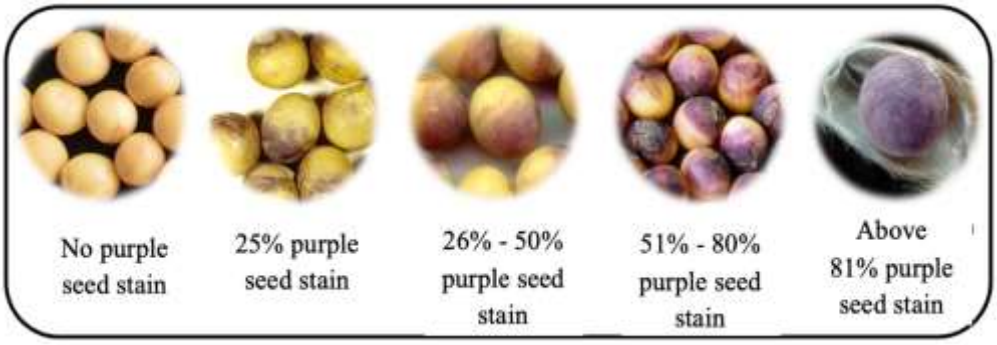
2 METHODOLOGY

The experiment was conducted in the experimental field and at the Seed Analysis Laboratory (LAS) of the University of Passo Fundo, in Passo Fundo, Rio Grande do Sul, in the 2021/2022 harvest.

The design used was randomized blocks (DBC), with 4 sowing densities: D1 – 15.7, D2 – 17.3, D3 – 14.1 and D4 – 18.8, in blocks with 5 replicates. The soybean cultivar used was BMX Zeus (55i57 RSF IPRO).

To evaluate the incidence of purple spot that occurred at each density, after harvest the seeds were analyzed and separated, according to the percentage of contamination by *Cercospora kiikuchi*, into 5 classes, following the criterion created by visual determination (Figure 1).

Figure 1 - Purple stain classes following visual determination criteria



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The first class consisted of visually healthy seeds, without stains; the second class had up to 25% of the seed coat covered by the purple stain; the third class included seeds with 26% to 50% of the seed coat affected; the fourth class comprised seeds with 51% to 80% of the seed coat compromised; and the fifth class consisted of seeds with more than 81% of the seed coat compromised.

To quantify the variables that represent the physiological quality of the seed (germination and vigor), the single-factor scheme was considered, where each density was evaluated in the five classes of severity of the purple stain.

The germination and vigor test was performed on a roll of Germitest paper, moistened with a quantity of water equivalent to 2.5 times the weight of the paper, and then kept in a Mangelsdorf germinator at 25ºC. Counts were performed after five days (to determine vigor) and after eight days for germination, according to the criteria established by RAS (BRASIL, 2009). The results were expressed as a percentage.

The results obtained were subjected to analysis of variance and the means compared by Tukey's test at 5% probability of error using RStudio software.

3 THEORETICAL FRAMEWORK

The Purple spot, caused by the fungus *Cercospora kikuchii*, is a disease that significantly affects soybean crops in several producing regions, including Brazil and the United States. The disease is characterized by the presence of purple spots on the seeds, which compromise their physiological quality, affecting germination, vigor and, consequently, the commercial value of the seeds (HARTMAN *et al*., 2015). In addition to damaging the appearance of the seeds, infection by the fungus can reduce plant performance in the field, resulting in lower productivity.

Sowing density is one of the main management factors that affect plant development and the microclimate of the crop canopy. Higher plant densities can result in a more humid environment with less air circulation, conditions that favor the development of pathogens such as *C. kikuchii*. This occurs because the proximity between plants hinders moisture evaporation and reduces the incidence of sunlight, creating a microclimate conducive to the proliferation of fungal diseases (LEE *et al*., 2013).

Pedersen and Lauer (2004) conducted a study that demonstrates how increasing seeding density can favor the occurrence of diseases in soybeans. In their research, they observed that higher densities tend to increase the incidence of purple spot on seeds, mainly due to the more favorable microclimate for the development of the fungus. This suggests that there is a direct relationship between the management of seeding density and seed health, with important implications for the final quality of the harvested material.

Seed quality is a crucial aspect in soybean production and is related to several factors, including the presence of pathogens, vigor, germination and physical purity. High-quality seeds

are essential for establishing a good crop and obtaining high yields. However, infection by *C. kikuchii* can significantly reduce this quality, negatively affecting germination capacity and seedling vigor (HENNING *et al*., 2014).

In studies on the relationship between seeding density and seed quality, it is observed that higher densities, although they can maximize yield in terms of number of plants, can compromise seed quality. The dense environment can favor the incidence of fungal diseases, such as purple spot, which directly impacts the physiological quality of the seeds. In addition, infection by *C. kikuchii* can cause damage to the seeds, such as deep spots that affect the tegument and often make the seeds unsuitable for commercialization or planting (BRADLEY *et al*., 2016).

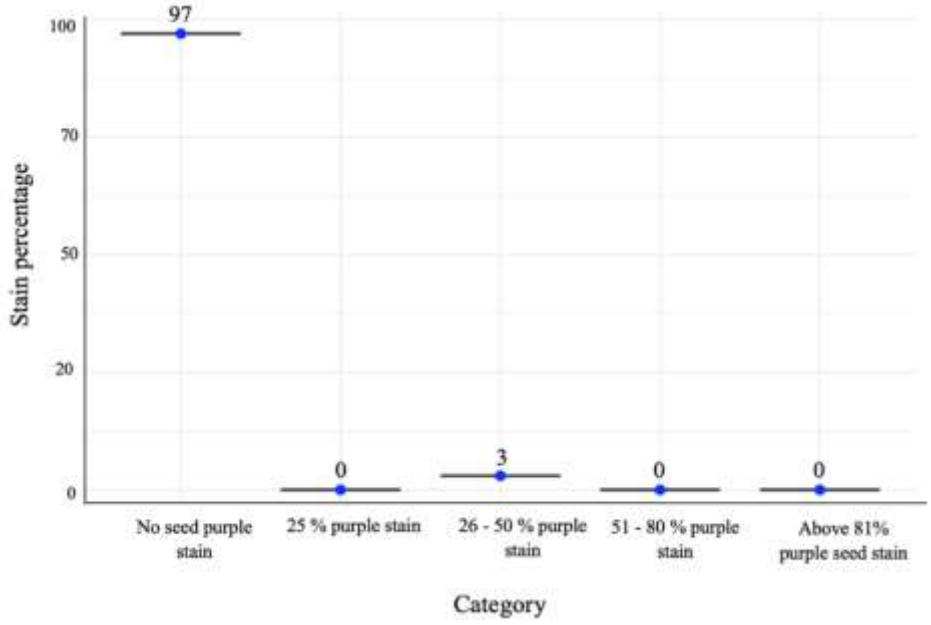
To mitigate the negative effects of seeding density on the occurrence of purple spot and seed quality, the adoption of integrated management practices is recommended. The use of resistant cultivars, associated with adequate management of plant density, can be an efficient strategy to reduce the inoculum pressure of *C. kikuchii* in areas of intensive soybean cultivation (WRATHER *et al*., 2012). In addition, seed treatment with fungicides has been widely used as a preventive measure against purple spot, improving seed health and consequently their final quality.

Therefore, managing sowing density together with other cultural practices can play an important role in reducing the incidence of purple spot and preserving seed quality. The balance between plant density, the use of resistant cultivars and the application of fungicides can result in better quality seeds with greater productivity potential.

4 DATA ANALYSIS

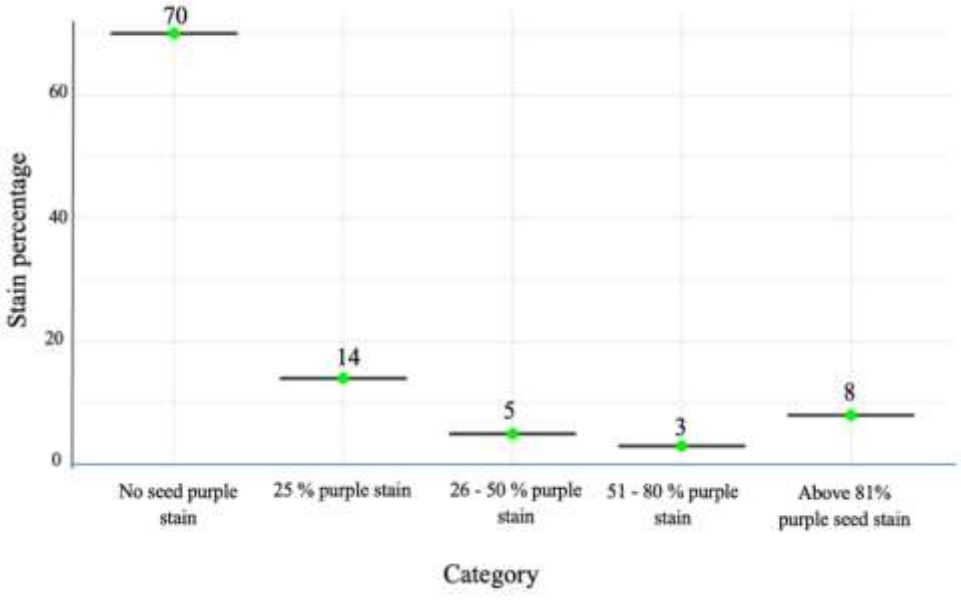
Data analysis showed a significant difference between seeding densities and the percentage of purple spot occurrence, showing that as plant density increases, there is also an increase in the presence of the disease (Figures 2, 3, 4 and 5). This relationship can be explained by the fact that higher plant densities create a favorable microclimate for the development of pathogens, due to lower air circulation and higher humidity in the crop canopy, factors that facilitate the spread and establishment of diseases (AGRIOS, 2005).

Figure 2 - Percentage of purple spot according to categories at a density of 15.7 (D1) plants per square meter



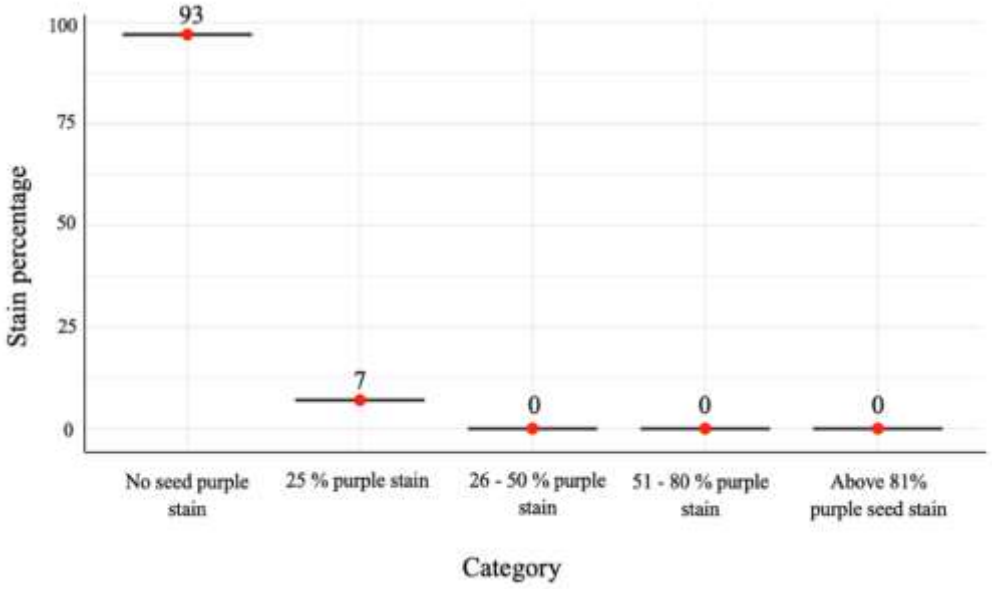
Source: Authors.

Figure 3 - Percentage of purple spot according to categories at a density of 17,3 (D2) plants per square meter



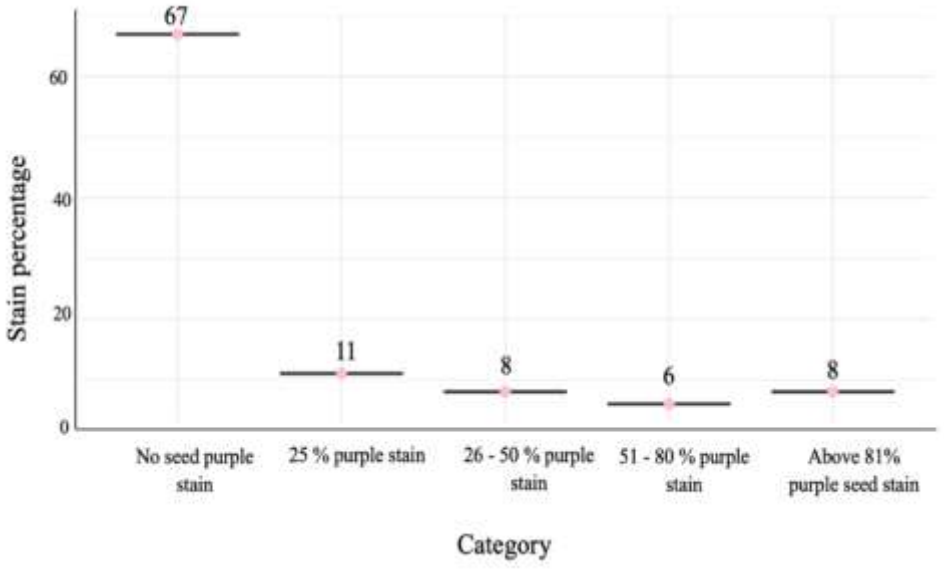
Source: Authors.

Figure 4 - Percentage of purple spot according to categories at a density of 14,1 (D3) plants per square meter



Source: Authors.

Figure 5 - Percentage of purple spot according to categories at a density of 18,8 (D4) plants per square meter



Source: Authors.

The data obtained corroborate previous studies, such as those by Oplinger and Doll (1984), which suggest that both planting date and sowing density directly affect the incidence of purple spot. Furthermore, the results are in agreement with the observations of Koenning and

Wrather (2010), who reported that plant density can influence the microclimate to the point of favoring the development of diseases, even in less favorable climatic conditions.

On the other hand, Hartman, West and Herman (2011) observed that purple spot can occur in environments with low plant density, especially in conditions of high relative humidity for long periods. This point highlights that sowing density is not the only determining factor in the incidence of the disease, with humidity being a key element in the equation.

There was no significant difference between the sowing density and the quality of soybean seeds, where the seedlings did not present low quality in relation to vigor and germination.

Table 1 - Vigor and Germination evaluated in a sample of 100 seeds (25 seeds per replicate) at a density of 15.7 (D1)

Purple seed category	Vigor (5th day)	Germination (8th day)
Class 1	89 a	91 a
Class 2	90 a	91 a
Class 3	89 a	90 a
Class 4	91 a	93 a
Class 5	89 a	90 a
CV (%)	14,5	

Treatments: Class 1 (Control, no stain); Class 2 (up to 25%); Class 3 (26% to 50%); Class 4 (51% to 80%), Class 5 (above 81%).

Table 2 - Vigor and Germination evaluated in a sample of 100 seeds (25 seeds per replicate) at a density of 17.3 (D2)

Purple seed category	Vigor (5th day)	Germination (8th day)
Class 1	89 a	92 a
Class 2	89 a	91 a
Class 3	89 a	91 a
Class 4	89 a	91 a
Class 5	89 a	90 a
CV (%)	13,7	

Treatments: Class 1 (Control, no stain); Class 2 (up to 25%); Class 3 (26% to 50%); Class 4 (51% to 80%), Class 5 (above 81%).

Table 3 – Vigor and Germination evaluated in a sample of 100 seeds (25 seeds per replicate) at a density of 14.1 (D3)

Purple seed category	Vigor (5th day)	Germination (8th day)
Class 1	90 a	90 a
Class 2	89 a	93 a
Class 3	88 a	90 a
Class 4	91 a	91 a
Class 5	89 a	91 a
CV (%)	12,1	

Treatments: Class 1 (Control, no stain); Class 2 (up to 25%); Class 3 (26% to 50%); Class 4 (51% to 80%), Class 5 (above 81%).

Table 4 - Vigor and Germination evaluated in a sample of 100 seeds (25 seeds per replicate) at a density of 18.8 (D4)

Purple seed category	Vigor (5th day)	Germination (8th day)
Class 1	89 a	91 a
Class 2	90 a	91 a
Class 3	88 a	90 a
Class 4	90 a	90 a
Class 5	90 a	90 a
CV (%)	14,6	

Treatments: Class 1 (Control, no stain); Class 2 (up to 25%); Class 3 (26% to 50%); Class 4 (51% to 80%), Class 5 (above 81%).

In soybean, it is essential that the seeds have physical integrity, high germination rates and vigor (Krzyzanowski *et al*., 2018). However, it is equally crucial that the seeds present high health, since the occurrence of diseases is a limiting factor for achieving high yields in grain production (Hamawaki *et al*., 2002).

The entry of purple spot into soybean seeds through the funiculus is a phenomenon associated with infection caused by the fungus *Cercospora kikuchii* (Hartman *et al*., 2015). The funiculus, which connects the seed to the pod, can serve as an entry point for the pathogen, allowing the fungus to colonize the pod and reach the interior of the seed, resulting in purple spots on the surface, especially in the region close to the funiculus (Yang; Navi, 2005).

Not all seeds are equally susceptible to infection by the funiculus. Susceptibility may vary depending on the genetic resistance of the cultivar, environmental conditions during grain filling, and the level of inoculum present (Wrather; Koenning, 2006). Seeds that are not directly affected by the fungus through the funiculus may present superficial or internal infections, but are generally less affected in terms of visible spots (Hartman *et al*., 2015). The presence of purple spot can negatively impact seed quality, affecting both commercial value and germination capacity (Yang; Navi, 2005).

In the case of *Cercospora kikuchii*, although coloration is a strong indicator of the presence of the pathogen, only a health test, such as the "Blotter test", can confirm the presence or absence of this pathogen in the seeds. The purple color of the seed coat makes it easier to identify the fungus, and it is only necessary to observe its growth and/or sporulation: long, hyaline, clavate, short and septate conidia are produced in fascicles and are different from sporodochial conidiophores, which are dark brown in color and, in culture medium, grow slowly, often being covered by other fungi that grow faster in conditions of moisture saturation (Goulart, 2004).

Studies indicate both positive and negative correlations, contrary to the results found, between the severity of *Cercospora kikuchii* and the germination percentage and vigor of soybean seeds (Hamawaki *et al*., 2002; Galli *et al*., 2005). For example, seeds of the BRS MA Juçara cultivar infected with C. kikuchii showed a 40% reduction in germination compared to healthy seeds. In addition to the decrease in germination potential, an increase in the incidence of dead seeds and abnormal seedlings was also observed (Bringel *et al*., 2001). However, Henning (2004) found no significant differences in germination, field emergence and grain yield between soybean seeds of the Paraná, Davis and Bossier cultivars, with 0, 5, 10, 20 and 40% purple spot.

Similarly, Câmara *et al*. (2019) reported that soybean seeds of the RK7813 cultivar, even with up to 100% purple spot, had no impact on germination or seedling emergence. The discrepancies between the results of these studies may be related not only to the variation in the level of resistance among the genotypes used, but also to the pathogen, the degree of colonization of the seed tissue and genetic variability.

Tests that assess both seed vigor and health are equally important, since more vigorous and pathogen-free seeds will produce seedlings with better field performance, especially under adverse conditions. In this context, Krzyzanowski *et al*. (2018) adopted the concept of vigor used by the Association of Official Seed Analysts (AOSA) of the USA: "They are those properties of seeds that determine their potential for rapid and uniform emergence and development of normal seedlings under a wide variety of environmental conditions."

5 RESULTS, LIMITATIONS AND FUTURE TRENDS

The results indicate that, although seeding density has a direct relationship with the incidence of purple spot, there was no significant difference in the quality of soybean seeds in terms of vigor and germination. Regardless of plant density, the seeds analyzed maintained

physical integrity and high germination rates, which is essential for good crop establishment (Krzyzanowski *et al*., 2018). However, as emphasized by Hamawaki *et al*. (2002), seed health remains a limiting factor in obtaining high yields, and monitoring diseases such as purple spot is essential.

One of the limitations of this study is the lack of variability in climatic factors, which can alter the relationship between density and disease. Studies such as that of Hartman et al. (2011) have shown that even at low densities, high humidity can promote the emergence of the disease, which could influence the results under other conditions. In addition, the genetic resistance of cultivars has not been widely explored, which is an important area for future research.

In the future, studies could explore the impact of different genotypes and the interaction between plant density and extreme weather conditions, such as prolonged high humidity or drought. Another important trend is the evaluation of integrated control methods, such as the use of fungicides and resistant cultivars, which can reduce disease incidence without compromising productivity or seed quality.

6 CONCLUSION

The present study aimed to evaluate the impact of seeding density on the incidence of purple spot in soybean seeds and its relationship with seed quality. Based on the data analyzed, it was concluded that there is a positive correlation between increased plant density and the occurrence of purple spot, corroborating the hypothesis that higher densities create a favorable microclimate for the development of *Cercospora kikuchii*.

However, even with the increased incidence of the disease at higher densities, no significant reduction in seed quality was observed in terms of vigor and germination. This suggests that, despite the presence of purple spot, the physical integrity and germination potential of the seeds were not significantly compromised, which contradicts some expectations that the disease would drastically reduce seed quality.

Thus, the objectives of the study were achieved, showing that seeding density directly influences the occurrence of purple spot, but does not significantly affect the physiological quality of the seeds. However, this study also opens space for future investigations, especially on the role of climatic and genetic factors in the dynamics of the disease, as well as on management strategies that can mitigate the effects of seeding density without compromising seed productivity and quality.

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