Detection and quantification of *Sclerotinia sclerotiorum* in soya bean seed

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Seed health is critical to ensuring a healthy yield, not only for the producer's pocket, but also for the consumer's wellbeing. However, soya bean yield and quality (protein and oil content) are being threatened by diseases caused by fungal pathogens. These include, but are not limited to, anthracnose (caused by *Colletotrichum truncatum*), charcoal rot (caused by *Macrophomina phaseolina*), leaf rust (caused by *Puccinia pachyrhizi* and/or *P. meibomiae*), *Sclerotinia* stem rot (caused by *Sclerotinia sclerotiorum*) and sudden death syndrome (caused by a complex of *Fusarium* spp).

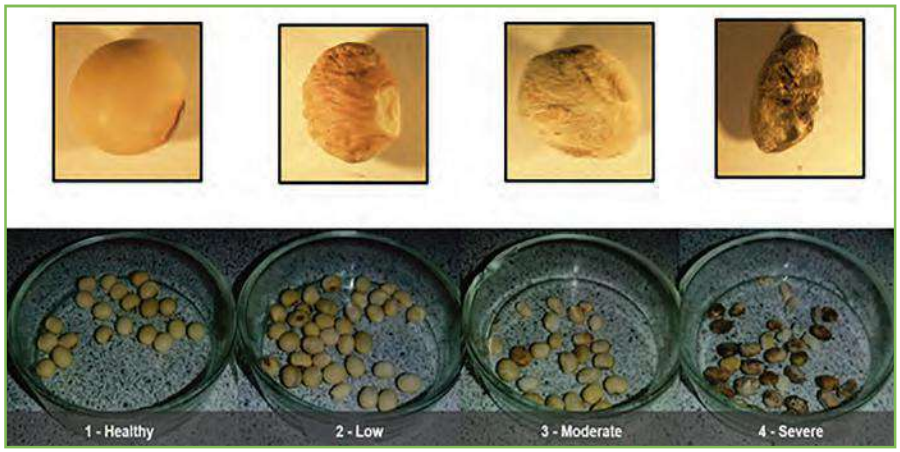
These diseases can either impact yield indirectly by influencing the onset and rate of seed germination, causing seedling death, stunted plant growth and infected pods, or directly through influencing seed quality and quantity. Additionally, *Fusarium* spp and *Aspergillus* spp are known to produce mycotoxins (secondary metabolites) which are toxic if consumed by animals and humans. Most of the aforementioned pathogens (as well as *Cercospora* spp and *Phomopsis* spp) can survive in plant debris and seed for several seasons, making these diseases an ongoing problem if not managed in a timely fashion.

To avoid the introduction of *Sclerotinia sclerotiorum* (*S. sclerotiorum*) into cropping systems, pathogen-free seed should be utilised, and certified seed should be your first defence against *Sclerotinia* (stem rot) in soya beans (Du Plessis, 2018).

A study conducted in 2021 at the University of the Free State aimed to confirm the presence of *S. sclerotiorum* in soya bean seed grown under South African conditions. Soya bean seed collected from experimental field trials from the 2019/20 and 2020/21 seasons in Delmas were randomly selected and ranked according to seed health classes. Four seed health classes were assigned and determined by seed discolouration, abnormal appearance and size: One = healthy; Two = low disease; Three = moderate disease; and Four = severe disease (*Figure 1*).

Four hundred seeds – 200 from each season – were surface sterilised to remove any superficial fungal contaminant and were plated onto a semi-selective media. The semi-selective media was initially

Figure 1: Four health classes grouped according to abnormalities of soya bean seed colour, size and general appearance.



neon blue, but in the presence of *S. sclerotiorum* a yellow halo formed around the seed, indicating the presence of the pathogen (*Photo 1*). Once a yellow halo was present, the colony was grown until sclerotia – the survival structure – had formed in order to confirm that *S. sclerotiorum* is indeed the causal organism. The germination percentage of the seed classes were also determined in this study.

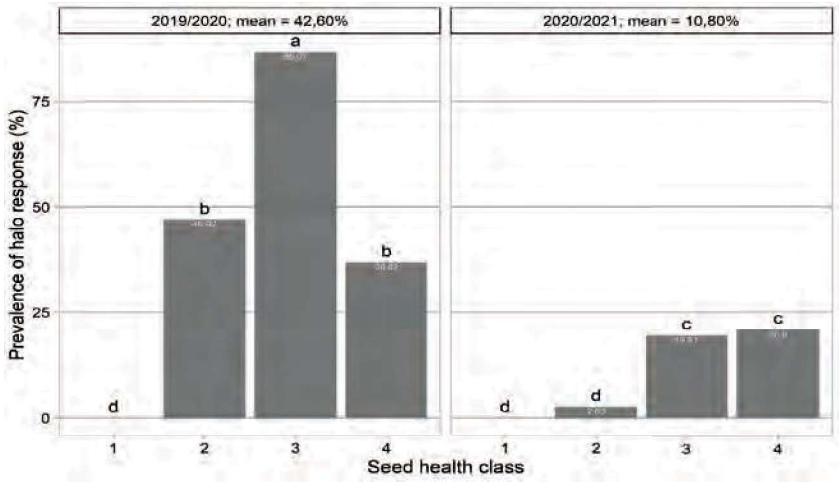
Seed included in Class 1 presented no colonies as expected, which indicated healthy seed that was not infested/ infected with pathogens. Additionally, seed from Class 4 hosted the greatest fungal colonies, indicating the greatest presence of disease-causing organisms. The germination percentage of the seed was also as expected – the most germinated among Class 1, and the least germinated among Class 4.

The seed from Class 1 had the highest average germination percentage because no pathogens were found in the seed, and seeds functioned optimally (*Figure 2*). Seed health Class 3 indicated the greatest average halo formation, indicating the presence of *S. sclerotiorum* (*Figure 2*). Although seed health Class 4 did not have the greatest halo formation, the lowest germination percentage was associated with this seed health class.

Seed within health Class 4 may have been infected by pathogens that cause more severe effects than seed infected by *S. sclerotiorum*. Seeds infected with *S. sclerotiorum* had a white 'powdery' appearance, as presented in seed Class 3. The seed was also malformed and had a reduced seed weight. Sclerotia

Photo 1: Sclerotinia sclerotiorum grown from seed health Class 3.

Figure 2: The prevalence of halo responses of fungal colonies after 48 hours' incubation on Steadman semi-selective media across two seasons.



Mean values in the graphs are the average percentage of the prevalence of halo formation of the four seed health groups; values with the same letter indicate means do not differ statistically.

formation confirmed that it is was indeed *S. sclerotiorum* within the seed.

Additionally, the average halo formation across the two seasons indicated a higher average of halo formation for the seed from the 2019/20 season than for the 2020/21 season, which indicated that there was more stem rot in the 2019/20 season than in the 2020/21 season. The amount of stem rot found in the field on the plant tissue during these seasons (38,33% for the 2019/20 season; 10,80% for the 2020/21 season) correspond with the seed health findings (*Figure 2*).

Disease management is divided into four critical strategies, namely avoidance, exclusion, eradication and protection. The tactic of disease-free seed, or acceptable thresholds, is included in exclusion. This tactic is critical to mitigating the entry or build-up of primary inoculum in production systems. However, in lieu of disease-free seed, chemical treatments can be applied to seed, a tactic of the protection strategy, to increase seedling survival.

Currently, the active ingredient fludioxonil, as well as strobilurin products such as azoxystrobin, trifloxystrobin or pyraclostrobin, are used to manage soya bean seedborne diseases.

The research group is currently busy with a master's project titled "Identification, prevalence and fungicide control of seedborne fungi associated with soya bean". The hope is that the study can contribute to identifying commonly associated soya bean seed pathogens and potential seed treatments to reduce future yield losses, as well as inoculum persistence.

As *S. sclerotiorum* was confirmed to be seedborne in seed appearing diseased, producers need to be mindful when sorting retained seed to avoid reinfection in fields. Heeding the advice of Hanlie du Plessis and making use of certified seed will reduce the risk significantly.

Scan this QR code to read the article by Hanlie du Plessis (2018) from the South African National Seed Organization.

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