UNIVERSITY OF
KWAZULU-NATAL

STUDIES ON *SCLEROTINIA SCLEROTIORUM* (SCLEROTINIA STEM ROT) ON SOYBEANS

by

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ABSTRACT

Soybeans, *Glycine max*, are an economically and strategically important crop in South Africa (SA). In order to meet local demands, large imports of soybeans are required, e.g., in the 2005/2006 soybean production period, 842 107 tonnes of oilcake were imported. Due to an increase in soybean production throughout the world, diseases that affect this crop have also increased in incidence and severity.

Sclerotinia sclerotiorum, the causal organism of sclerotinia stem rot (SSR), is an important yield limiting disease of soybeans, as well as numerous other crops. The pathogen was first reported in SA in 1979. However, it was only in 2002 that this fungus was considered a major pathogen of soybeans in SA.

The research reported in this thesis was conducted to investigate the epidemiology of *S. sclerotiorum* and examine numerous potential control methods for this pathogen, i.e., resistant cultivars, biocontrol, chemical control and seed treatments. A *S. sclerotiorum* isolate was obtained from sunflowers in Delmas, Mpumulanga, SA, in the form of sclerotia. This isolate was cultured and sent for identification and deposition in the Plant Protection Research Institute collection. This isolate, in the form of mycelia, was used for the duration of the study.

For epidemiology studies, the effect of temperature, leaf wetness duration (LWD) and relative humidity (RH) were examined for their effect on rate of pathogen development. Twenty four combinations of temperature (19°C, 22°C, 25°C and 28°C), LWD (24, 48 and 72 hr) and RH (85 and 95%) were investigated. No interaction between temperature, LWD and RH was found. Temperature alone was the only factor that affected disease development. At 22°C, the rate of pathogen development (0.45 per unit per day) was significantly higher than all other temperatures, indicating that this temperature is optimum for disease development.

Thirteen different soybean cultivars, i.e., LS6626RR, LS6710RR, LS666RR, LS555RR, LS6514RR, LS678RR, Prima 2000, Pan 626, AG5601RR, AG5409RR, 95B33, 95B53 and 96B01B, commercially grown in SA were investigated for their reaction to *S. sclerotiorum*. Prima 2000, 96B01B, 95B33 and AG5409RR were considered to be the least susceptible as they showed a significantly low rate of pathogen development (0.28, 0.28, 0.24, 0.23 per unit per day, respectively) and produced a significantly low number of sclerotia (3.03, 3.42, 3.21, 2.38, respectively). LS6626R and LS666RR may be considered most susceptible because of their significantly high rate of pathogen development (0.45 and 0.42 per unit per day, respectively) and high sclerotia production (8.16 and 7.50, respectively). Regression analysis showed a positive correlation coefficient (R^2=0.71) between rate of growth of the pathogen and number of sclerotia produced, indicating that a higher rate is associated with a higher number of sclerotia.

In vitro dual culture bioassays were performed to identify the biocontrol mechanisms of the biocontrol agents, EcoT® (a seed treatment) and Eco77® (a foliar treatment), against hyphae and sclerotia of *S. sclerotiorum*. Ultrastructural studies revealed that mycoparasitism is the probable mode of action as initial signs of hyphae of EcoT® and Eco77® coiling around hyphae of *S. sclerotiorum* were observed. Surface colonization of sclerotia by hyphae of EcoT® and Eco77® was also observed.

In vitro antagonism of EcoT® against *S. sclerotiorum* on soybean seed was performed to determine pre-emergence and post-emergence disease. There was no significant difference in percentage germination between seeds treated with EcoT® and plated with the pathogen, untreated seeds and no *S. sclerotiorum*, and the control (i.e. no EcoT® and no pathogen). However, percentage non infected seedlings from seeds not treated with EcoT® was significantly lower, suggesting that EcoT® may be successfully used as a seed treatment for the control of SSR. *In vivo* trials were performed to investigate the effect of silicon (Si) alone, and in combination with Eco77®, on the effect of the rate of disease development. Plants treated with Eco77® had a significantly lower rate of disease development (0.19 per unit per day for plants treated with Eco77® and *S. sclerotiorum* and 0.20 per unit per day for plants treated with Eco77®, *S. sclerotiorum*

and Si), compared to plants not treated with Eco77® (0.29 per unit per day for plants treated with *S. sclerotiorum* and 0.30 per unit per day for plants treated with *S. sclerotiorum* and Si), regardless of the application of Si. Similarly, plants treated with Eco77® had a significantly lower number of sclerotia (0.46 for plants treated with Eco77® and *S. sclerotiorum* and 0.91 for plants treated with Eco77®, *S. sclerotiorum* and Si), compared to plants not treated with Eco77® (3.31 for plants treated with *S. sclerotiorum* and 3.64 for plants treated with *S. sclerotiorum* and Si). The significantly lower rate of disease development coupled with a significant reduction in sclerotia showed that Eco77®, and not Si, was responsible for reducing the severity of SSR. A strong positive correlation between rate of disease development and the number of sclerotia produced (R^2=0.79) was observed.

For the investigation of various fungicides for the control of *S. sclerotiorum*, *in vitro* trials to determine the potential of three different fungicides at different rates, i.e., BAS 516 04F (133 g a.i. ha^{-1}), BAS 516 04F (266 g a.i. ha^{-1}), BAS 512 06F (380 g a.i. ha^{-1}) and Sumisclex (760 g a.i. ha^{-1}) were initially conducted. The control (non-amended PDA) had a significantly higher area under mycelial growth curve (243.0) than all fungicides tested. BAS 516 04F (at both concentrations) and BAS 512 06F completely inhibited the mycelial growth of *S. sclerotiorum*. Sumisclex inhibited the fungus by 89.07%. For *in vivo* trials, preventative treatments, i.e., BAS 516 04F (133 g a.i. ha^{-1}), BAS 516 04F (266 g a.i. ha^{-1}), BAS 512 06F (380 g a.i. ha^{-1}), curative treatment, i.e. Sumisclex (760 g a.i. ha^{-1}) and a combination preventative/curative treatment, i.e., BAS 512 06F (380 g a.i. ha^{-1})/Sumisclex (570 g a.i. ha^{-1}) were investigated. No significant difference in disease severity index (DSI) was found between fungicide treatments and the inoculated control. BAS 512 06F and BAS 512 06F/Sumisclex had significantly lower grain yields (6.09 g and 5.96 g, respectively) compared to all other treatments. There was a positive correlation coefficient (R^2=0.76), between DSI and grain yield, indicating that a high DSI is correlated with low grain yield.

Trials to evaluate the effect of commercially available and currently unregistered seed treatments for the control of *S. sclerotiorum* on soybean seeds *in vivo* and *in vitro* were performed. Seed germination tests were performed to determine if seed treatments had any negative effects on seed germination *in vitro*. All seed treatments tested, i.e., BAS 516 03F (8, 16 and 32 ml a.i. 100 kg^{-1} seed), BAS 512 00F (7.5, 15 and 32 ml a.i. 100 kg^{-1} seed), Celest XL (100, 125, 200 and 250 ml a.i. 100 kg^{-1} seed), Sumisclex (5 and 10 ml a.i. 100 kg^{-1} seed), Benomyl (150 g a.i. 100 kg^{-1} seed), Captan (240 ml a.i. 100 kg^{-1} seed), Thiulin (180 g a.i. 100 kg^{-1} seed) and Anchor Red (300 ml a.i. 100 kg^{-1} seed), showed no negative effect on seed germination. For *in vivo* trials, BAS 516 03F (16 and 32 ml a.i. 100 kg^{-1} seed), BAS 512 00F (7.5, 15 and 32 ml a.i. 100 kg^{-1} seed), Celest XL (100, 125, 200 and 250 ml a.i. 100 kg^{-1} seed), Sumisclex (5 and 10 ml a.i. 100 kg^{-1} seed), Benomyl and Anchor Red had significantly similar percent germination and percent seedling survival as the untreated/uninoculated control. These seed treatments should be recommended for the control of *S. sclerotiorum*, as they protected seed during germination and subsequent seedling development. BAS 516 03F (8 ml a.i. 100 kg^{-1} seed) should not be recommended for the control of SSR, as it gave the lowest percent germination and percent seedling survival.

The results presented in this thesis have helped to identify optimal environmental conditions for the development of *S. sclerotiorum*, which is important for the development of forecasting models for disease control. The least and most susceptible cultivars of those tested have been identified. Biocontrol using Eco77® as a foliar application showed great potential.

The effect of Si needs to be further investigated, including the testing of more frequent applications and higher concentrations. The fungicides tested in this research did not show any potential for the control of SSR. However, the spray programme tested is for the control of soybean rust (*Phakopsora pachyrhizi*), and was investigated for its potential for the control of SSR. The spray programme, fungicide application and rating scale needs to be modified, to determine the true potential of these fungicides for the control of SSR. Numerous seed treatments have shown potential for the control of seed

infection by SSR. Due to difficulties in producing ascospores, which are the primary source of inoculum for this pathogen in the field, all studies in this research were conducted with mycelia and not ascospores. The production, collection and storage of ascospores needs to be thoroughly investigated, and research conducted with ascospores.