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PROGRESS REPORT

INVESTIGATING THE ETIOLOGY AND INCIDENCE OF SOILBORNE DISEASES OF SOYBEAN IN SOUTH AFRICA

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EXECUTIVE SUMMARY

During 2012/13 commercial soybean farmers' fields were surveyed to determine the incidence of soilborne diseases of soybean. This survery was important to determine whether soilborne pathogens found in soybean in the national cultivar trials during 2010/11 and 2011/12, are also present in commercial soybean fields. The main objectives of this study during 2012/13 were to determine the incidence and severity of soilborne diseases of soybean in commercial farmers' fields, irrespective of the cultivar planted, in the major soybean production areas of the country; and to investigate the potential of all the fungal species, recovered from the previous study, as soilborne pathogens of soybean by employing pathogenicity tests on soybean seedlings under glasshouse conditions.

Soybean plants and rhizosphere soil were collected from all commercial farms that were surveyed. Plants were sampled at three growth stages (two weeks after planting, flowering and physiological maturity or seed pod filling) from twelve soybean commercial farms that represent three climatic regions (cool, moderate and warm) in five provinces of South Africa. Diseased cotyledons, hypocotyls/crowns and roots were rated for disease severity and analysed for associated mycoflora using morphological description and sequence analyses of DNA exracted from representative isolates. Soil samples collected from each area were used for glasshouse assays to determine the existence and biological nature of soilborne diseases by pasteurizing half of the soil at 83ºC and planting it with soybean seeds. In addition, pathogenicity trials with all the representative species of fungi and oomycete were conducted under glasshouse conditions. Pathogenicity and relative virulence of each isolate were determined by assessing the percentage survival of soybean seedlings, plant length and crown and root rot severity three weeks after planting.

From the survey conducted, the highest crown and root rot ratings were recorded at Brits, Bothaville and Clocolan, while Brits, Clocolan and Vryheid had the highest cotyledon rot severity. The highest crown rot severities were recorded at the third sampling time. Root rot severity was high at Clocolan throughout the season (all the three sampling times). For instance, the field in Clocolan had a seedling establishment problem with most seedlings either dead or dying, and with a root rot severity score that ranged from 2.43 to 2.46 (65% root rot on roots) on average through out the growing season. The field in Bothaville also had a root rot severity

score ranging from 2.0 at seedling stage to 2.50 (more than 50% root rot on roots) at maturity. In Koedoeskop, late in the season, dead plants were visible with charcoal rot symptoms. The fields in Greylingstad, Morgenzon and Winterton had poor seedling emergence with a number of dead plants visible and bare patches spread through the fields. However, in Kinross and Wonderfontein, both fields had poor seedling emergence with roots with more than 50% of plant roots damaged due to rot caused by soilborne pathogens.

A total of 6860 fungal isolates were recovered from cotyledons, crowns and roots. The fungal isolates were from 29 fungal genera. The most frequently isolated fungal genera and species included *Alternaria* spp., *F. equiseti, F. oxysporum, F. solani, Gliocladium* spp., *Macrophomina* sp., *Phoma* spp., *Phomopsis* spp., *Pythium* spp., *Rhizoctonia spp., Trichoderma* spp. and *Sclerotium* sp. In addition, *Sclerotinia* isolates were recovered from diseased soybean stems and crowns. The incidences of genera and species isolated were affected by area sampled, time of sampling and climate of the area sampled.

In this study, close to 60% of all isolates obtained belonged to the genus *Fusarium* of which *Fusarium oxysporum* was the dominant species and more than 40% of the isolates belonged to this species. The second most dominant species within the genus *Fusarium* was *F. solani*. *Fusarium equiseti* was also amongst the frequently isolated species within the genus. *Fusarium solani* isolates recovered in this study do not have 100% sequence similarity with the sudden death syndrome causing species (*Fusarium virguliforme* formally known as *F. solani* f. sp. g*lycines*, *F. tucumaniae, F. brasiliense* and *F. crassistipitatum*). However, some *Fusarium* isolates obtained from a consultation study, on soybean with typical sudden death symptoms, in collaboration with PANNAR on a farm in Lydenburg (Mpumalanga) were *Fusarium virguliforme*. A glasshouse trial conducted on soybean seedlings showed that the *F. virguliforme* isolates obtained in this study caused the typical sudden death symptoms. This is the first report of sudden death syndrome of soybean in South Africa.

The survey conducted in commercial fields showed that certain of the virulent pathogens are affected by the crop rotation employed. For instance, *F. graminearum* was only obtained from fields previously planted to maize and wheat, and *R. solani* AG-4 HGIII seems to be more prevalent in monoculture soybean fields. Pathogens such as *F. graminearum* and *P. rostratifingens* were also more frequently obtained

from the commercial fields than cultivar trials and in addition some *Pythium* species, such as P. *echinulatum, P. mamilatum, P. perplexum, P. spinosum, Pythium* G group, P group and T group were only found in commercial soybean fields.

Glasshouse assays revealed that the survival of soybean seedlings grown in pasteurized field soils collected from all the farms sampled significantly improved, though with varying degrees at each sampling time. The surveys conducted during the 2012/2013 growing season confirmed that the fungi reported as soilborne disease causing organisms in other countries, are found associated with diseased cotyledons, crowns and roots of soybean in South Africa, but some fungi were also reported on soybean for the first time.

Pathogenicity tests were conducted using 46 representative isolates of *Rhizoctonia* species and AGs, 109 *Pythium* isolates (12 species), 121 *Fusarium* isolates (14 species), *Macrophomina phaseolina* (24 isolates), *Sclerotinia sclerotiorum* (12 isolates), *Sclerotium rolfsii* (17 isolates), *Diaporthe phaseolorum* (10 isolates), *Phomopsis longicola* (33 isolates), *Phomopsis* sp. (4 isolates), *Phoma* spp. (17 isolates), *Alternaria* spp. (8 isolates), *Aspergillus* spp. (2 isolates), *Bipolaris* spp. (18 isolates), *Botryosphaeria* spp. (6 isolates), *Cladosporium* spp. (2 isolates), *Colletotrichum* spp. (3 isolates), *Curvularia* spp. (8 isolates), *Epicoccum* spp. (2 isolates), *Gliocladium* spp. (25 isolates), *Neocosmospora* spp. (3 isolates), *Penicillium* spp. (3 isolates), *Pyrenochaeta* spp. (8 isolates), *Rhizopus* spp. (2 isolates), *Stenocarpella* spp. (3 isolates) and *Trichoderma* spp. (31 isolates). The results showed that, the most virulent species were *Fusarium begoniae, F. graminearum, F. oxysporum, F. solani, Macrophomina phaseolina, Diaporthe/Phomopsis* spp. complex *(Diaporthe phaseolorum, Phomopsis longicola* and *Phomopsis* sp.*), Pythium aphanidermatum, P. heterothallicum, P. irregulare, P. ultimum, Rhizoctonia solani* AG-2-2 IIIB, *R. solani* AG-4 HG-III, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum.* The distribution of these highly virulent species was variable. *Fusarium oxysporum, F. solani, P. ultimum, R. solani* AG-4 HG-III and *Diaporthe/Phomopsis* spp. were found in all the areas sampled. *Macrophomina phaseolina* was found in all the areas sampled, except in Wonderfontein, while *F. begoniae* was obtained from all the areas sampled, except in Brits and Koedoeskop; *F. graminearum* was reported in Bothaville, Cedara, Clocolan, Delmas, Groblersdal, Kinross, Koedoeskop, Morgenzon, Normandien, Potchefstroom, Vryheid, Winterton and Wonderfontein; *R. solani* AG-2-2 IIIB in Brits, Clocolan, Delmas, Groblersdal, Morgenzon and Vryheid;

P. aphanidermatum in Brits, Groblersdal, Normandien, Rustenburg and Vaalharts; *P. heterothallicum* in Clocolan, Greylingstad, Kinross, Potchefstroom, Rustenburg and Vaalharts; *P. irregulare* in Brits, Cedara, Clocolan, Dirkiesdorp, Groblersdal, Kinross, Morgenzon, Normandien, Winterton, Wonderfontein and Vryheid; *Sclerotium rolfsii* in Bothaville, Brits, Clocolan, Koedoeskop, Rustenburg, Vaalharts and Villiers; and *Sclerotinia sclerotiorum* in Brits, Cedara, Delmas, Groblersdal, Kinross, Normandien, Wonderfontein and Vryheid.

During the surveys of the national cultivar trials, one cultivar (A5409RG) was planted in all the trials. The surveys conducted in commercial fields included eight (AG 6107, AG 5904, PAN 1454, PAN 1666, Silver (Argentina), PAN 358, LS 6164 and 95B53) cultivars. Since cultivars differ in resistance to certain soilborne diseases, conducting the surveys on a range of cultivars may have also allowed for obtaining at a wider range of pathogens.

This is the first extensive survey on soybean commercial farms that investigated soilborne diseases and associated pathogens of soybean in South Africa, and confirmed that the fungi reported as important soilborne pathogens in other countries, are found associated with diseased cotyledons, crowns and roots of soybean locally. Also significant is the wide distribution of some of the very virulent fungi that were reported for the first time on soybean in some of these farmers' fields. It is therefore crucial that the soybean survey conducted during the 2012/2013 planting season be repeated on commercial soybean farmers' fields in order to determine the importance of soilborne dieases in the major production areas. This will provide a solid knowledge base with regards to the current status of soilborne disease of soybean in South Africa. Furthermore, the distribution of these soilborne pathogens is extensive and warrants control measures in order to reduce the loss in soybean production. Management strategies for soilborne diseases of soybean often do not completely protect soybean when implemented alone. An integrated management approach of available control strategies delivers more sustainable and effective control against these diseases. Of the available management strategies against soilborne diseases, seed treatment, planting less-susceptible cultivars and crop rotation are among the most widely used practices.