

SOYBEAN RESPONSE TO RUST AND SCLEROTINIA STEM ROT UNDER DIFFERENT BIOTIC AND ABIOTIC CONDITIONS

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SUMMARY

- The annual increase in soybean production to meet the increasing demands is limited by environmental constraints, fungal diseases and insect pests. *Sclerotinia* stem rot (caused by *Sclerotinia sclerotiorum*) is increasing in importance both locally and globally and the wide host range challenges disease management strategies. Soybean rust (caused by *Phakopsora pachyrhizi*) is a serious yield limiting foliar disease that has increased in importance due to rapid spread over large geographic areas. Reliable and repeatable screening methods have limited the quest for stable resistance to these diseases. Genetic variability in *S. sclerotiorum* isolates need elucidation since this could assist in the identification of stable resistance. Similarly, understanding the physiological processes associated with host response to the respective pathogens could be useful in identifying new mechanisms of resistance or the timely implementing of appropriate control strategies. The absence of disease resistant cultivars, in particular to soybean rust, has necessitated extensive use of fungicides as a control method to ensure acceptable yields.
- A study was conducted to compare the reaction of experimental soybean lines to soybean rust in the greenhouse and evaluate epidemiological factors that need to be considered in field evaluations of germplasm. Greenhouse trials indicated significant differences in rust severity between lines while field data illustrated the importance of differences in relative life time at disease onset, apparent infection rate, AUDPC and relative life time at which 100% disease severity was reached. The importance of multiple evaluation criteria was emphasized. Constraints to achieving acceptable levels of disease resistance are the absence of a correlation between greenhouse and field results and repeatability of data over seasons and localities. Although lines differed in disease severity only Selection 43 showed a combination of late disease onset and slow apparent infection rate and hence a significant level of rust resistance. However, an extended study, which includes a variety of South African *P. pachyrhizi* races, is needed to determine the stability of the identified resistance.
- Yield losses associated with soybean rust in varieties from three maturity groups as well as the effects of row spacing and fungicide application on disease development were evaluated. Disease onset was closely related to host growth

stage and fungicides had little effect on delaying the disease. The apparent infection rate was higher in 45 cm, compared with 90 cm, row widths in the absence of fungicide applications while greater reductions in apparent infection rate due to fungicide applications were recorded in the wider rows. These responses were also reflected in AUDPC and yield. Differences in these variables between sprayed and unsprayed plots were more pronounced in the long maturity cultivar compared with the shorter maturity cultivars but the extent of these differences is dependent of spray efficiency. Differences in cultivar responses to the disease and row spacing do not provide adequate control and fungicide applications remain primary disease control strategy against soybean rust.

- Physiological changes associated with soybean growth stage in field trials indicated that soybean rust onset and development are determined by the activity of key pathogenesis-related enzymes. Environmental effects had a relatively small effect on disease severity once infection is initiated. Host responses to rust infection were directly related to the decline in chitinase and β -1,3-glucanase which coincided with flowering and pod formation and the subsequent rapid increase in rust severity. Plots that received fertilizer treatments indicated that chitinase, β -1,3-glucanase, peroxidase and phenol content were related to the form of nitrogen received. Higher chitinase activity, in particular, was also associated with lower disease severity.
- Evaluation of cultivars for resistance to *Sclerotinia* stem rot in the greenhouse and field failed to identify sources of resistance although lower risk cultivars were observed in field trials. Most cultivars were susceptible to *Sclerotinia* stem rot even when conditions were sub-optimal for disease development. A poor correlation between greenhouse and field data was observed. Field trials indicated that cultivars react differently to changing environmental conditions and disease potentials and high genotype x environment interactions were recorded. Data indicate that local cultivars still lack the desired level of resistance to *Sclerotinia* stem rot or stability to changing disease potentials.
- *In vitro* studies indicated that *S. sclerotiorum* is favoured by cool to moderate temperatures (20°C and 25°C) while extreme cold or hot temperatures impair growth. Molecular characterisation of isolates using AFLP analysis revealed a relatively uniform distribution with evidence of genetic diversity within and amongst isolates across the five provinces evaluated. A low, recombination frequency

suggests that the population is in a developing stage which is emphasized by isolates found on the end of the minimal spanning network. No correlation between host plant or locality and genetic similarity was observed but AFLP proved to be a valuable tool to characterize *S. sclerotiorum* isolates.

Keywords: *Sclerotinia sclerotiorum*, *Phakopsora pachyrhizi*, Sclerotinia stem rot, soybean rust, cultivar evaluation, yield loss, genetic variation, physiological changes, fungicides.

OPSOMMING

- Die jaarlikse toename in sojaboonproduksie om te voldoen aan die aanvraag word belemmer deur faktore soos omgewingsveranderinge, plantsiektes en insekplae. *Sclerotinia* stamvrot veroorsaak deur *Sclerotinia sclerotiorum* se belangrikheid neem toe en die wye gasheerreëks maak die beheer van hierdie patogeen 'n uitdaging. Sojaboonroes veroorsaak deur *Phakopsora pachyrhizi*, is 'n ernstige blaarsiekte en word aangetref in al die belangrike sojaboonproduksiegebiede wêreldwyd. 'n Beperking in evaluering van siekteweerstand is dat die metodes wat gebruik word nie herhaalbaar en betroubaar is nie. Die evaluering van genetiese variasie van *S. sclerotiorum* isolate is belangrik aangesien dit gebruik kan word om moontlike weerstandsbronne te identifiseer. 'n Beter begrip van die fisiologiese prosesse in sojaboonplante tydens infeksie kan daartoe lei dat nuwe bronne van weerstand geïdentifiseer kan word of dat beter beheermetodes geïmplementeer kan word. Die afwesigheid van weerstandbiedende kultivars, spesifiek teen sojaboonroes, lei daartoe dat chemiese beheermetodes steeds grootskaals gebruik word om aanvaarbare oesopbrengste te kry.
- 'n Studie is gedoen om die reaksie van eksperimentele lyne teen sojaboonroes in die glashuis sowel as om die epidemiologiese faktore wat belangrik is vir veldevaluering van kiemplasma te ondersoek. Glashuisproewe het aangedui dat daar duidelike verskille in die vatbaarheid vir roesinfeksie tussen lyne is. Velddata het aangedui dat lyne verskil in die relatiewe leeftyd met die aanvang van siekte, die tempo van infeksie, die area onder die siekte-ontwikkelingskurwe en die relatiewe leeftyd van die gasheer wanneer 100% siekte aangeteken is. Alle lyne was vatbaar vir sojaboonroes. Die belangrikheid van veelvuldige evalueringskriteria by die evaluering van siekteweerstand is beklemtoon. Die swak korrelasie tussen glashuis- en velddata, asook die konsekwentheid van data oor seisoene en lokaliteite, is 'n bron van kommer. Alhoewel lyne verskil het in die graad van roesinfeksie, het een inskrywing Selection 43 sekere nuttige eienskappe getoon soos latere aanvang van siekte en stadige tempo van siekte ontwikkeling. 'n Studie van *P. pachyrhizi* isolate in Suid Afrika sal egter eers gedoen moet word om 'n deeglike evaluering van kultivars of lyne te doen wat verdere navorsing regverdig.

- Opbrengsverliese veroorsaak deur roes op sojabone in drie kultivars van verskillende volwassenheidsgroeierings, sowel as die effek van ry-spasiëring en swamdoders op siekte-ontwikkeling, is geëvalueer. Die aanvang van siekte was nou verwant aan die gasheer-groeistadium en die toediening van swamdoders het 'n minimale effek op die vertraging van siekte-aanvang gehad. In blokke waar geen swamdoders toegedien was nie, was die tempo van infeksie vinniger in die 45cm rye, in vergelyking met die 90 cm rywydtes. 'n Groter afname in die tempo van infeksie is in die wyer rywydtes waargeneem, as gevolg van die toediening van swamdoder. Hierdie tendens is ook in die area onder die siekte-ontwikkelingskurwe en opbrengs waargeneem. Verskille in die veranderlikes wat gemeet is tussen die behandelde en onbehandelde blokke was die grootste vir die lang-groeiseisoen kultivar in vergelyking met die ander kultivars, maar die verskille is afhanklik van die spuitdoeltreffendheid. Data het aangedui dat verskille in die kultivars, asook rywydtes, nie afsonderlik voldoende is om siekte te beheer nie en dat die gebruik van swamdoders as beskerming teen sojaboonroes steeds gebruik moet word.
- Fisiologiese veranderinge, wat geassosieer is met spesifieke groeifases van sojaboonplante tydens veldproewe, het aangedui dat sojaboonroes ontwikkeling bepaal word deur die aktiwiteit van belangrike patogenese-verwante ensieme. Die effek van die omgewing was minimaal op die graad van siekte nadat infeksie plaasgevind het. Reaksie van die gasheerplant op roesinfeksie was direk verwant aan die afname in chitinase en β -1,3 glukanase wat ooreengestem het met blom- en peulvorming en daaropvolgende toename in roesinfeksie. Blokke wat kunsmis toedienings ontvang het, het aangedui dat chitinase, β -1,3-glukanase, peroksidase en fenool inhoud verwant was aan die vorm van stikstof wat toegedien is. 'n Hoër vlak van chitinase is geassosieer met minder siekte.
- Evaluering van kultivars teen Sclerotinia stamvrot in die glashuis kon geen bronne van weerstand identifiseer nie, alhoewel laer risiko kultivars in die veld waargeneem is. Die meerderheid van kultivars was vatbaar vir siekte selfs onder minder optimale toestande. 'n Swak korrelasie tussen glashuis- en velddata is waargeneem. Veldproewe het getoon dat kultivars verskillend reageer teenoor veranderende omgewingstoestande en siektepotensiaal. 'n Hoë genotipe x omgewingsinteraksie is aangeteken. Data het aangetoon dat die gewenste vlak van weerstand steeds ontbreek in plaaslike kultivars, asook stabiliteit van die kultivars in toestande waar siektepotensiaal wissel.

- *In vitro* studies op die groei van *Sclerotinia sclerotiorum* isolate by verskillende temperature het aangedui dat koel tot matige temperature (20°C en 25°C) groei bevoordeel teenoor baie koue of warm temperature, wat 'n inhiberende effek gehad het. Karakterisering van isolate met behulp van molekulêre tegnieke soos AFLP (geamplifiseerde fragment lengte polimorfisme) analise het getoon dat 'n relatiewe uniforme verspreiding met bewyse van genetiese diversiteit in en tussen isolate wat in vyf provinsies versamel is voorkom. Die moontlikheid dat die populasie nog besig is om te ontwikkel is gestaaf deur 'n lae rekombinasie-frekwensie en word verder beklemtoon deurdat hierdie isolate op die punte van die minimum rykwydte netwerk analise voorkom. Geen korrelasie tussen gasheerplante of lokaliteite en die genetiese ooreenkoms is waargeneem nie, maar daar is gevind dat AFLP 'n waardevolle tegniek is om *S. sclerotiorum* isolate te karakteriseer.

Sleutelwoorde: *Sclerotinia sclerotiorum*, *Phakopsora pachyrhizi*, *Sclerotinia* stam vrot, sojaboonroes, kultivar evaluering, opbrengs verlies, genetiese variasie, fisiologiese verandering, swamdoders