PHYSIOLOGY OF GROWTH STAGE-RELATED SUSCEPTIBILITY IN SOYBEAN TO RUST

Z.A. Pretorius, N.W. McLaren, A.J. van der Westhuizen and L.C. van Jaarsveld

Department of Plant Sciences, University of the Free State, Bloemfontein 9300

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EXECUTIVE SUMMARY

Soybean yield losses due to rust, caused by *Phakopsora pachyrhizi*, result from premature defoliation, early maturity and reduced seed number and mass. The extent of losses is related to time of onset, severity and yield components affected. The primary aim of this study was to monitor disease development in soybean field experiments and to relate changes in susceptibility to host physiology. Detection of changes in host physiology, in particular anti-fungal substances, and the variation within soybean populations of these fungal inhibitors, could lead to the identification and exploitation of partial rust resistance and yield stability in soybean.

Field experiments were conducted during 2003/04 and 2004/05 at the Cedara research station in KwaZulu-Natal. The trials consisted of four and seven sequential plantings, respectively, of the commercial variety LS666 (medium maturity) from early-November to mid-December at weekly intervals. These sequential plantings created environmental and growth stage variation during sampling intervals. During the season leaves were collected from plants at six growth stages, ranging between one-month-old to advanced pod-fill stage. The oldest, as well as the youngest leaves of each plant were collected and treated as separate treatments during each sampling procedure. Intercellular wash fluids (IWFs) were extracted from the afore-mentioned samples and frozen. Whole leaves were harvested as fresh plant material and stored at -74°C until analysis.

Field experiments conducted during 2005/06 at Kenilworth (Bloemfontein) and Cedara, and in 2006/07 at Cedara, consisted of two plantings of the commercial varieties LS678 and Stork, the first planting in mid-November and the second in mid-December 2005. To ensure physiological variation, leaves were collected from plants at two-weekly intervals, starting from four weeks after planting. Sampling procedure was similar to the 2003-2005 trials.

To confirm results from the 2005/06 and 2006/07 field trials, glasshouse experiments were conducted using LS678 and Stork. Sampling was done at

the same time intervals and using the same methods as in the abovementioned field trials.

In 2004, experimental techniques for extraction of pathogenesis-related (PR) proteins from leaves collected at different growth stages were optimized. Determination of the activities of the PR-proteins ß-1,3-glucanase, peroxidase and chitinase subsequently followed.

In all the trials, chitinase and ß-1,3-glucanase activities mostly increased and then decreased after flowering, indicating a possible involvement in disease resistance. These changes could also be linked to the growth and development of the host plant. Peroxidase activity of the 2004/05 trial increased after flowering, possibly due to its involvement in consecutive oxidative bursts generated by secondary rust infections later in the season. Total phenolics increased before flowering, which was followed by a decrease in content in upper and lower leaves of post-flowering plants.

In 2005/06 chitinase and ß-1,3-glucanase activity increased slightly prior to flowering (60 % relative life time [RLT] / 8 weeks after planting), before decreasing. Similar to a previous observation, this decline in PR-protein activity indicated a possible involvement in disease resistance. Peroxidase activity again increased after flowering, decreasing again as plants matured, possibly due to its involvement in consecutive oxidative bursts. Total carbohydrates fluctuated in the upper and lower leaves throughout the season with no indications of a role in disease resistance.

In the 2006/07 season, no infection of *P. pachyrhizi* occurred at Cedara. It was observed that for all the enzymes, the individual activities were much lower than in the treated and untreated infected plants of the previous seasons. Chitinase activity showed a slight increase before flowering at 60 % RLT, where after it decreased. ß-1,3-glucanase activity slightly decreased before flowering and after a brief increase, started decreasing again as the plants matured. Peroxidase activity showed a decrease before flowering but increased slightly after 65 % RLT.

Rust development was studied in the 2003/03, 2004/05 and in the 2005/06 field trials. Detection of rust in unsprayed plots ranged from 66-81 % RLT although most ranged from 71 to 74 % RLT which corresponded with late pod development and the early stages of grain development. Indications were that the detection date was closely related to host growth stage. Detection date of disease was significantly related (R^2 = 0.62P>0.05) to AUDPC and, therefore, any host component that could delay onset will contribute significantly to disease reduction. It was apparent that subsequent to onset, the rate of disease development in the various plots was similar.

An additional glasshouse experiment was done in 2006 using the cultivar PAN535 RR, treating glyphosate-resistant seedlings at the 2nd trifoliate stage with glyphosate and inoculating it with *P. pachyrhizi*. Histological investigation revealed that glyphosate inhibited colony formation up to 8 days post-inoculation (d.p.i.) on the treated leaves.

Investigation into PR-protein activities as possible factors to exploit to induce partial resistance to soybean rust does not seem viable. The enzyme activities tended to increase around the time of flowering, but the subsequent changes towards maturity suggests the enzymeconcentrations are growth-regulated rather than influenced by infection by the rust pathogen.

Although carbohydrates have been linked to host susceptibility, the levels in the trials fluctuated throughout the season, suggesting host response to the environment rather than to physiological changes brought about by disease.

This leaves phenols as a possible link between growth stage and susceptibility to rust. The results from the histological studies with Roundup®, where glyphosate inhibited colonization, supports this conclusion as glyphosate inhibits a key enzyme in the synthesis of aromatic amino acids.