Growing from Strength to Strength

18TH AUSTRALIAN RESEARCH ASSEMBLY ON BRASSICAS

Tanunda, South Australia
29 September – 2 October 2014

CONFERENCE PROCEEDINGS

GRDC
Grains Research & Development Corporation
Your GRDC working with you

Variability of Western Australian Isolates of *Sclerotinia sclerotiorum* and the Potential of Local Biological Control Agents

Baiq N. Hidayah[1, 3], **B. Dell**[1], **R. Khangura**[2]
[1]School of Veterinary and Life Sciences, Murdoch University, 90 South Street, Murdoch WA 6150 Australia, [2]Department of Agriculture and Food, Western Australia (DAFWA), [3]Balai Pengkajian Teknologi Pertanian (BPTP) NTB, Indonesia.
Email: B.Hidayah@murdoch.edu.au

ABSTRACT

Stem rot disease caused by *Sclerotinia sclerotiorum* has emerged as a serious problem for canola (*Brassica napus* L.) production in Western Australia (WA) over the past few years where crop losses can be up to 40% in the worst affected crops. The biological characteristics and pathogenicity of the pathogen in WA is poorly understood. Also the potential for local biological control agents (BCAs) to be used in the management of the disease has not been explored. This paper provides preliminary data in these fields. One hundred and forty isolates of *S. sclerotiorum* were collected from WA canola growing regions for identification of biological characteristics which include colour of mycelia, growth rate, production of sclerotia, and pathogenicity. Other fungal isolates with potential biological control activity were collected from southern regions of WA. Colour of mycelia of Sclerotinia isolates varied from white, yellowish white, greyish white, brownish white, grey, dark grey to brown. Each isolate had its 24 and 48 hour growth rate recorded after sub-culture on PDA + ampicillin medium. ANOVA showed highly significant differences between growth rates of isolates 24 and 48 hours after being sub-cultured ($P \leq 0.001$). There were significant differences in number of sclerotia produced by each isolate. Two potential fungal biological control agents were found in WA, namely isolate KEN1 and isolate MTB1. These local fungal BCAs were found to be effective in inhibiting *in vitro* both the growth and ability to produce sclerotia of *S. sclerotiorum*.

Key words: *Sclerotinia sclerotiorum*, biological control agents, Western Australia

INTRODUCTION

Canola is one of the most important oil crops worldwide. Stem rot disease caused by *Sclerotinia sclerotiorum* is a serious problem for canola production worldwide and in Western Australia (WA), over the past few years, crop losses have reached 40% (Khangura and MacLeod, 2012). Control techniques available to manage *S. sclerotiorum* mainly involve using chemical fungicides and crop rotation as no commercially available resistant cultivars of canola are available for the region. However, the uses of chemical pesticides can have negative impacts to the environment and bio-ecosystems (Rimmer et al., 2007).

Therefore, research on the variability of *S. sclerotiorum* in relation to its biological characteristics, pathogenicity, and how to control the pathogen using new and established biological control agents is very important. Biological control may be an option to control soil-borne fungal diseases on brassicas. There is increased interest in biological control encouraged by public awareness about issues related to the use of chemical pesticides. Hence, the role of biological controls agents will become more important in agricultural systems in the future. Biological control agents (BCAs) have potential to control *S.*

sclerotiorum in farming practices and a number of effective BCAs have been identified (Saharan and Mehta, 2008; Zheng et al., 2012). The rhizosphere of brassicas is an important source for likely biological control agents, as these organisms have already established themselves in the target area. Evidence that BCAs can suppress pathogens is shown by *in vitro* studies where strains of the BCAs *Pseudomonas fluorescence* and *Bacillus amyloliquefaciens* isolated from the rhizosphere of soybean controlled the growth of the pathogens *Botrytis cinerea* and *Sclerotinia sclerotiorum* (Simonetti et al., 2012). BCAs, such as some species of *Trichoderma,* have been used to control *S. sclerotiorum* on green beans (Lopes et al., 2012). Based on the evidence from many completed studies (Saharan and Mehta, 2008), BCAs are claimed to be a potential control agent of plant diseases caused by *S. sclerotiorum*.

This paper investigates the biological characteristics of WA isolates of *S. sclerotiorum* which have been collected for the past three years and presents preliminary findings on the potential of local BCAs to control stem rot caused by *S. sclerotiorum* in canola.

MATERIALS AND METHODS

Isolate collection and inoculum production of *S. sclerotiorum*

Isolates of *S. sclerotiorum* were isolated from infected canola plants across canola growing regions of WA. Sclerotia from affected plants were surface sterilized for one minute by submerging in chlorine bleach solution (1% sodium hypochlorite), rinsed three times in sterile distilled water, and plated on PDA. The plates were incubated at 22°C in the growth room for three to four days until the mycelium covered the plate.

Morphology and growth rate of *S. sclerotiorum* isolates

The morphology of fungal cultures was observed on Potato Dextrose Agar (PDA) amended with ampicillin in Petri dishes. For each isolate, the inoculum disc (1 mm diameter) was placed in the centre of the Petri dish. Petri dishes were sealed with parafilm and incubated at room temperature. The diameter of fungal colonies was measured after 24 and 48 hours and colony colour and morphology were recorded. After 2 weeks of incubation, sclerotia produced by the fungus were collected and counted.

Pathogenicity test of *S. sclerotiorum* isolates

One hundred and forty isolates of *S. sclerotiorum* were assessed for pathogenicity on Cobbler canola seedlings in a growth room at 19°C with 12h photoperiod. Ten day old seedlings were inoculated using an agar plug method (Khangura and Mian in Press). After inoculation of cotyledons, the plants were placed in a misting chamber for 48 h and were then transferred to the growth room for another 48 h. There were six seedlings in each pot and there were four replications. The pots were arranged in completely randomized design. The number of diseased plants in each pot was counted after placed in misting chamber and growth room. Plants were watered in the potting mix and overhead as needed.

Antagonistic test of potential local Biological Control Agents

Some potential biological control agents were opportunistically collected from the southern agricultural regions of WA. The isolates of BCAs were isolated from canola farms and maintained for further test. For dual culture tests, a mycelial plug (5 mm diameter) of each BCA isolate was incubated on PDA, about 1 cm from the edge of each petri dish. A mycelial

plug of *S. sclerotiorum* removed from the colony margin of a 3-day-old culture grown on PDA was placed 6 cm away from the plug of the BCA isolate in the same petri dish. Petri dishes similarly inoculated with BCAs or *S. sclerotiorum* isolates alone were used as controls. Plates were incubated in a 22ºC growth room for 3 days, and were examined after 72 h for assessment of inhibition zones between BCAs and *S. sclerotiorum* isolates. At end of the incubation period, radial growth was measured. Radial growth reduction was calculated in relation to growth of the control as follows: % Inhibition of radial mycelial growth = [(C-T)/C] × 100, where C is the radial growth measurement of the pathogen in control plates, and T is the radial growth of the pathogen in presence of BCA. After two weeks of incubation, the number of sclerotia produced by *S. sclerotiorum* in each petri dish was determined. There were three replicate petri dishes for each treatment.

Statistical Analysis

The results were subjected to Analysis of Variance using GenStat release 14.

RESULTS

Variability among the WA isolates of *S. sclerotiorum*

Colour of mycelia of each isolate varied from white, yellowish white, greyish white, brownish white, grey, dark grey, to brown. There were highly significant differences ($P \leq 0.001$) between growth rates of isolates at 24 and 48 h (Figs 1 and 2). Growth rate frequency distribution showed that growth rates between 3-4 cm after 24 hours occurred with the highest frequency (>80 isolates) (Figure 1). Further, more than 60 isolates had fully covered the 9 cm diameter petri dishes after 48 hours (Figure 2).

The number of sclerotia produced on each isolate varied and there were highly significant differences between isolates ($P \leq 0.001$). Frequency distribution showed that an average of 20 sclerotia was produced at the highest frequency (>25 isolates). However, 15 isolates did not produce sclerotia while one isolate produced 50 sclerotia (Figure 3).

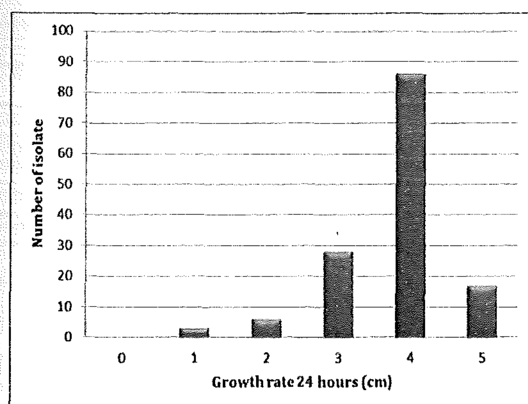

Figure 1. Frequency growth rate of *S. sclerotiorum* 24 hours after sub-culture

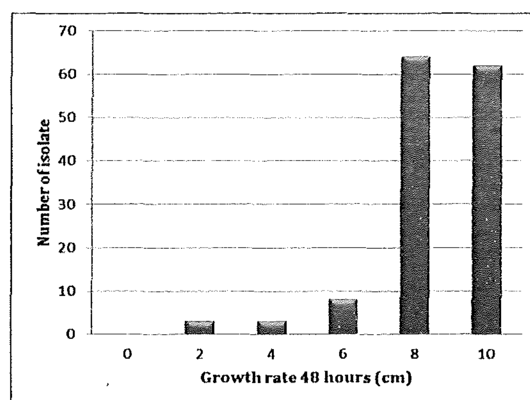

Figure 2. Frequency growth rate of *S. sclerotiorum* 48 hours after sub-culture

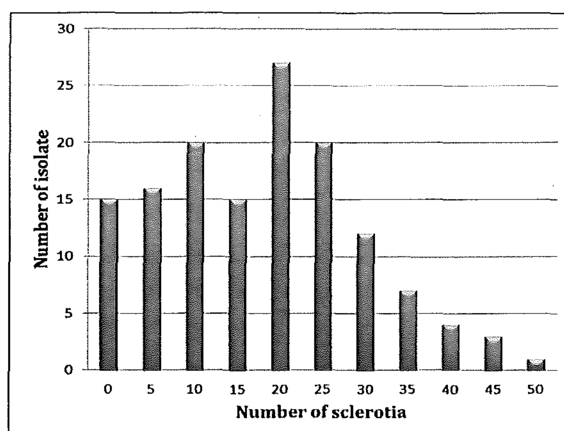

Figure 3. Frequency number of sclerotia produced by *S. sclerotiorum* two weeks after sub-culture

There was considerable variation (P≤0.001) in pathogenicity of the 140 WA isolates of *S. sclerotiorum* on canola seedlings (Figs 4 and 5). Frequency distribution showed that more than 40 isolates caused seedling mortality of only 10% after 24 hours (Figure 4). However more than 30 isolates caused 100% seedling mortality after 48 hours in the growth room (Figure 5).

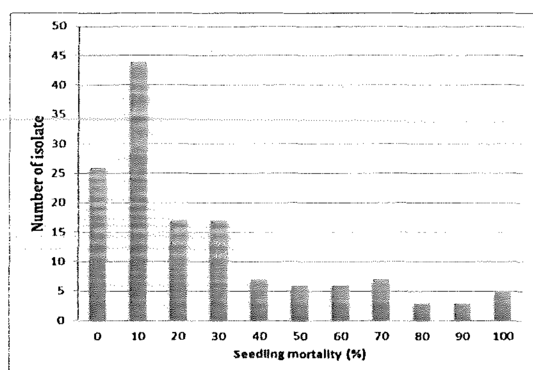

Figure 4. Frequency of seedling mortality after 48 hours in a misting chamber

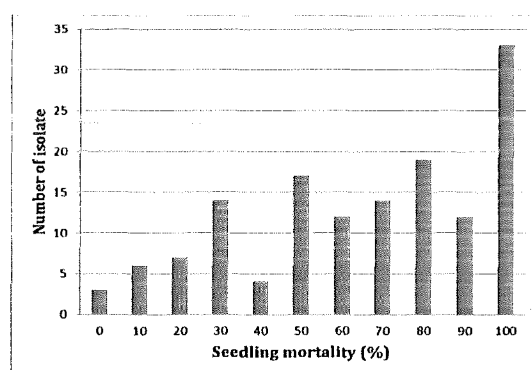

Figure 5. Frequency of seedling mortality after 48 hours in a growth room

Potential of Local WA Biological Control Agents (BCAs)

Two potential fungal biological control agents, namely isolate KEN1 and isolate MTB1 were isolated from the southern region of WA. Results showed that growth inhibition of *S. sclerotiorum* in the presence of the potential BCAs, isolate KEN1 and MTB1, was up to 54% and 55% respectively (Figure 6). The number of sclerotia produced per colony dropped from an average 28 sclerotia in the control treatment to 0.3 and 1.7 in the presence of BCA isolates KEN1 and MTB1 respectively (Figure 7).

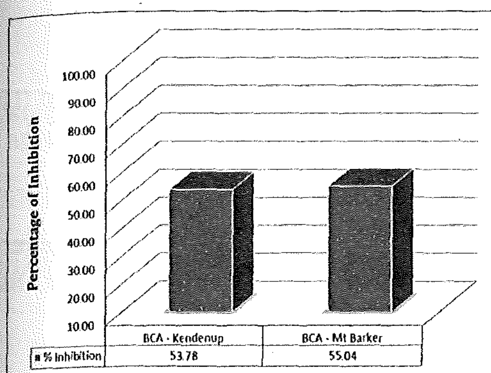

Figure 6. Percentage of growth inhibition of *S. sclerotiorum* in the presence of potential biological control agents

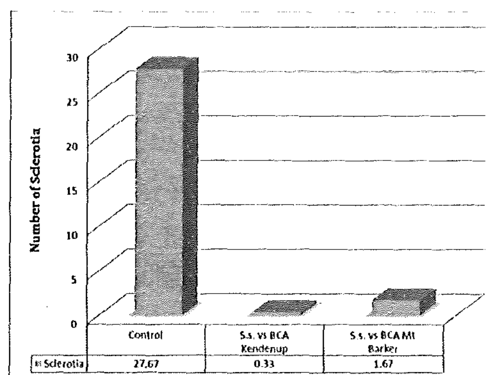

Figure 7. Number of sclerotia produced by *S. sclerotiorum* in petri dishes in the presence of biological control agents

DISCUSSION

The pathogen causing stem rot in canola, *S. sclerotiorum,* is a devastating and cosmopolitan plant pathogen (Bolton et al., 2006) that can be a serious problem for producers in WA in some years. Preliminary results show that there is considerable genetic variation among the WA isolates of *S. sclerotiorum* for mycelial colour, growth rate, number of sclerotia produced and pathogenicity on canola seedlings in laboratory conditions. *Sclerotinia sclerotiorum* is generally grown on nutrient-rich culture media where the fungus produces white or slightly grey, suppressed, aerial mycelium (Rimmer et al., 2007), but our experiments found that there was a wide range in the colour of the mycelia. Further studies on the genetic variation of the pathogen are needed including molecular approaches to define the population structure of the pathogen in WA. Significant differences in pathogenicity were observed among the 140 isolates of *S. sclerotiorum*. However, pathogenicity level of each isolate was not influenced by mycelial colour or diameter of growth rate on petri dishes. The pathogenicity of isolates should be further evaluated in field conditions.

Two BCA isolates showed potential to inhibit the growth of pathogen *in vitro* by 54% and 55%, respectively. One explanation of this growth inhibition may be that the BCAs compete with the pathogen for nutrients, however further work is needed to determine the influence of other modes of action such as myco-parasitism and antibiosis. Research is in progress in evaluating the effectiveness of the BCA isolates in managing *Sclerotinia* stem rot under controlled and field conditions.

ACKNOWLEDGEMENTS

We would like to thank Australian Centre for International Agricultural Research (ACIAR) for granting John Allwright Fellowship for PhD program to the first author at Murdoch University. Many thanks to Andrew Van Burgel, Department of Agriculture and Food, Western Australia who help in statistical analysis and M. Aberra, Department of Agriculture and Food, Western Australia, for helping with laboratory, glass house and field experiments.

REFERENCES

Bolton, M. D., Thomma, B. P. H. J., and Nelson, B. D. (2006). Sclerotinia sclerotiorum (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. *Molecular Plant Pathology* **7**, 1-16.

Khangura, R., and MacLeod, W. J. (2012). Evaluation of fungicides for managing Sclerotinia stem rot in canola in Western Australia. *In* "Seventh Australasian Soilborne Diseases Symposium", The University of Notre Dame - Fremantle - Australia.

Lopes, F. A. C., Steindorff, A. S., Geraldine, A. M., Brandao, R. S., Monteiro, V. N., Lobo Junior, M., Coelho, A. S. G., Ulhoa, C. J., and Silva, R. N. (2012). Biochemical and metabolic profiles of Trichoderma strains isolated from common bean crops in the Brazilian Cerrado, and potential antagonism against Sclerotinia sclerotiorum. *Fungal Biology* **116**, 815-824.

Rimmer, S. R., Shattuck, V. I., and Buchwaldt, L. (2007). "Compendium of brassica diseases," American Phytopathological Society (APS Press).

Saharan, G. S., and Mehta, N. (2008). "Sclerotinia Diseases of Crop Plants: Biology, Ecology, and Disease Management," Springer Science + Business Media B.V.

Simonetti, E., Hernandez, A. I., Kerber, N. L., Pucheu, N. L., Carmona, M. A., and Garcia, A. F. (2012). Protection of canola (Brassica napus) against fungal pathogens by strains of biocontrol rhizobacteria. *Biocontrol Science and Technology* **22**, 111-115.

Zheng, L., Ding, Y., Jiang, D., Fu, Y., Li, G., and Huang, J. (2012). Biocontrol efficacy of Coniothyrium minitans strain ZS-1 on Sclerotinia stem rot of oilseed rape caused by Sclerotinia sclerotiorum in field trials. *Acta Phytophylacica Sinica* **39**, 191-192.