Effect of Bacterial Antagonist Populations and Time of Application on Control of *Sclerotinia sclerotiorum* on Canola

S. Savchuk, and W. G. D. Fernando*

Department of Plant Science, University of Manitoba, Winnipeg, MB, Canada, R3T 2N2
Telephone number: (204) 474-6072, Fax: (204) 474-7528, E-mail: D_Fernando@Umanitoba.ca

ABSTRACT

Sclerotinia sclerotiorum causes stem rot of canola in many canola and rapeseed growing regions of the world. There are no known sclerotinia resistant varieties available to the grower. We have identified several bacterial strains as potential biological control agents for *S. sclerotiorum*. One such strain, *Pseudomonas* spp. isolate 41, was evaluated in the greenhouse at concentrations log 8, 6, and 4 cfu/ml using a petal inoculation technique. When bacteria were inoculated 24 h prior to ascospore inoculation complete disease suppression was observed at all concentrations. Antagonism of *Sclerotinia* ascospores by isolate 41 was also studied at the microscopic level at log 4 and 8 cfu/ml, using the same inoculation technique with random sampling of the petals at 4 different time points (0, 6, 14, and 24 h after ascospore inoculation). Significant disease suppression was observed at 6, 14 and 24 h, for both concentrations.

Key words: biological control - *Sclerotinia* – canola – *Pseudomonas* - time of application

INTRODUCTION

The importance of understanding time of application of potential biological control organisms is fundamental to the implementation of these organisms into a disease management strategy. The rapidly changing environment of the phylloplane often presents a challenge to successful colonization by antagonists, given that they can be affected by temperature, leaf wetness, and competition from other microbes, pesticide applications, insects, relative humidity, pH levels, as well as the host itself, and the host's susceptibility period.

The objectives of this study were to determine the time of application of a potential antagonist on the phylloplane, and the effect of the presence of *S. sclerotiorum* on this variability. Experiments were conducted to observe the degree of antagonism occurring at the microscopic level with different initial concentrations of the antagonist against the pathogen.

MATERIALS AND METHODS

Bacterial antagonist isolate 41 (*Pseudomonas* spp.) was used in this study. A rifampicin resistant strain of isolate 41 was derived through spontaneous mutation on rifampicin amended (150 mg l^{-1}) nutrient agar plates. Ascospores used in the experiments were produced using modifications of the protocol described by Lefol 1998.

Disease suppression by strain 41 was investigated using a petal inoculation technique. Petals were dipped into either strain 41 or ascospore suspension and placed onto potato dextrose agar plates for 24-72 h before being used to inoculate the plants. Eight different inoculation regimes were analyzed for efficacy using differential timing of application of both organisms and a completely randomized design (CRD) (Table 1). Ten plants were used for each treatment, each having 2 leaves inoculated with one petal placed into the axil of the leaf. Plants were incubated in a humidity chamber for 24 h following inoculation and then placed in the greenhouse. Disease severity ratings, were taken every other day for two weeks, and were based on a 0-10 scale where 0: no visible stem or leaf infection, and 10: >190 mm lesion, or plant death.

Ascospore germination related to bacterial population levels:

The effect of strain 41 on inhibition of ascospore germination and germ tube elongation was investigated using microscopic techniques. Petals were inoculated in the same manner as that used for the above trial. The control treatments were petals inoculated with ascospores only.

Treatments in the experiment were as follows: (1) strain 41 at log 8 cfu ml^{-1}, (2) strain 41 at log 4 cfu ml^{-1}, and (3) control. At 0, 6, 14, and 24 h, 25 petals were randomly sampled from the PDA plates and treated and stained as described in Fernando et.al. 1994. Ascospores were considered to have germinated when the germ tube was longer than the length of the ascospore itself. One hundred ascospores were randomly selected per treatment per sampling time. Germ tube length was measured using a compound light microscope. Germ tube lengths were classified into four categories as follows: 0– 40μm, 41μm - 90μm, 91μm- 180μm, and >180μm.

RESULTS

Infestation of the petals just prior to plant inoculation can be seen in Fig. 1. Disease progressed at the highest rate when the ascospores were present on the petals before the bacteria (B, D, Fig. 1), or when there were no bacteria present at all (E, A, G, Fig. 1). When bacteria were inoculated prior to or at the same time as *S. sclerotiorum* (co-inoculation), there was complete inhibition of disease (DSR = 0 at day 14). Analysis of variance of the results found there to be a significant difference ($P<0.05$) between the treatments on all of the days.

Ascospores on petals treated with bacteria exhibited significantly less germination than those growing in isolation on the petals (Fig.2). As well, there was less total growth and the germ tubes were shorter as well. Germination of ascospores on petals incubated on PDA plates was significantly inhibited in the presence of isolate RR41 at a concentration of log 8 cfu ml^{-1}, but not to the same extent at log 4 cfu ml^{-1} (Fig. 3). Near complete inhibition was observed for the higher concentration at over 80% after 24 hours, while only 63% was reported with the lower concentration at the same time point (Fig. 3). Spore germination followed a linear growth pattern over the 24-hour sampling period for petals treated with strain 41, and only reached 8% (Fig. 3).

DISCUSSION

Successful colonization by *Pseudomonas* sp. isolate 41 and effective competition with *Sclerotinia sclerotiorum* (at log 4 and 8 cfu ml^{-1}) was observed in this investigation, both macroscopically and microscopically. The superior competitive ability of isolate 41 was demonstrated in its ability to completely suppress disease development when applied as a co-inoculation treatment or prior to ascospore inoculation. In a practical sense, this could mean that a field application of antagonist could be concurrent with infection by the pathogen.

Control of ascospore germination and growth was observed when isolate 41 was applied at log 8 cfu ml^{-1}, Bacteria can be seen “eating” the ascospores (data will be shown on poster). This was further exhibited by the fact that there were considerable fewer ascospores overall on the petals at 14 and 24 h, and at 6, 14, and 24 h for the log 4 and log 8 cfu ml^{-1} treatments. Pathogens are very vulnerable at the germination stage of infection (Campbell, 1989).

In summary, isolate 41 is able to effectively colonize the canola petals for several days and effectively control disease development, even when applied to canola petals at the same time as the pathogen (in the greenhouse). This isolate thus warrants further investigation as a potential foliar biocontrol agent of *S. sclerotiorum,* as it has demonstrated adaptability and longevity in greenhouse trials, and, in previous studies in this lab, the ability to decrease infection in the field.

REFERENCES

Lefol, C. (1998) Protocol to rapidly obtain apothecia of *Sclerotinia sclerotiorum*. In: 1998 International Sclerotinia Workshop: (Nelson, B., and Gulya, T, Eds.), pp. 74.

Campbell, R. (1989). Biological Control of Microbial Plant Pathogens, 576pp. Cambridge University Press, Great Britain.

Fernando, W.G.D., Paulitz, T.C., and Watson, A.K. 1994. Biological Control 4: 125-131.

ACKNOWDEGEMENTS

Financial assistance of the Canola Council of Canada is gratefully acknowledged.

Table 1 – Time of Inoculation of *Sclerotinia* ascospores and *Pseudomonas* strain 41

Treatment	Day 1	2	3	4
A	ascospores			
B	ascospores		bacteria	
C	bacteria	ascospores		
D		ascospores	bacteria	plant inoculations
E		ascospores		
F		bacteria	ascospores	
G			ascospores	
H			bacteria and ascospores	

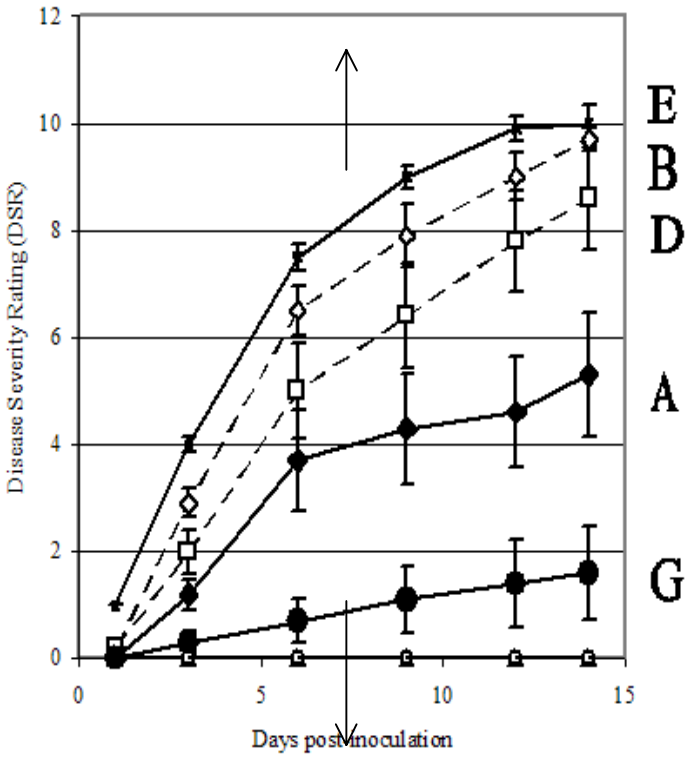

Figure 1 - Disease Severity over Time

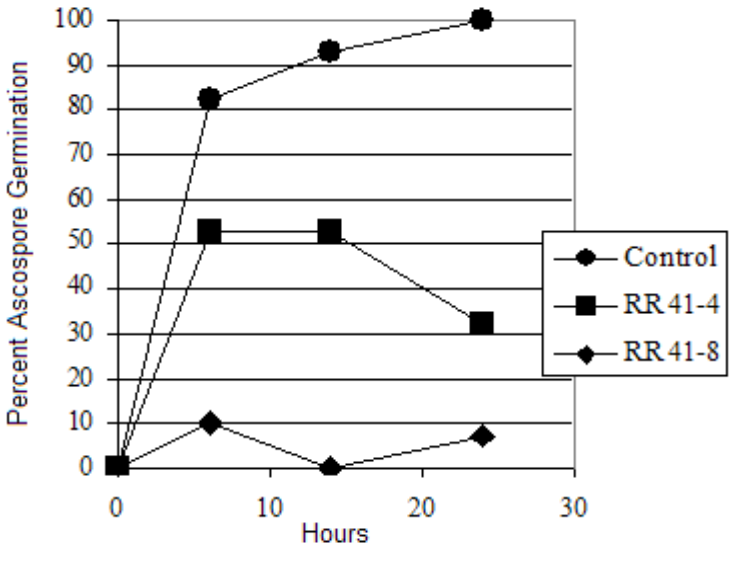

Figure 2 - Ascospore Germination over Time (hrs)