Suppression of preemergence damping-off of canola, caused by *Rhizoctonia solani*, by rhizobacteria

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Summary. *Pre-emergence damping-off and brown girdling root rot caused by* Rhizoctonia solani *are important diseases of canola/rape seed in western Canada. Annual yield losses in excess of 20-30% have been reported in several infected fields. This paper reports the results of large-scale experiments conducted under commercial conditions. Field plots were established in Saskatoon, Regina and Melfort, Sask., from 1990 to 1993 to evaluate the potential of seven bacterial strains as seed treatment to increase the healthy stand of canola cv. Westar grown in* R. solani *infested fields. The bacteria were formulated either in sterile peat or in a liquid carrier and applied to seed just before planting. Bacterized seed (log 5-6 cfu seed*$^{-1}$*) were mechanically planted in replicated field plots artificially infested with* R. solani. *Final healthy stand was measured 30 days after planting. Two strains (63-49 and U-14) were the most effective and also consistent in their efficacy irrespective of the field or year tested compared to non-bacterization controls.*

Introduction

Pre- and post-emergence seedling damping-off or brown girdling root rot of canola/rape (*Brassica napus*) seed crops caused by *R. solani* are important diseases in western Canada (Kaminski and Verma, 1985). Canola grown fields in Alberta, particularly in the Peace River region have been severely affected, at times as high as 80-100% loss with annual yield losses in excess of 20-30% (Sippell *et al.*, 1985). Cultural control or resistant cultivars are currently unavailable for this disease. Though chemicals have potential in suppressing the disease, their use is becoming less acceptable from an environmental point of view. The development of resistance to many fungicides by major pathogens, and public concern over chemical fungicides in foods and the environment have created interest in alternative methods of disease control. Biological control of soil-borne diseases of agricultural crops has emerged as a promising option. Moreover, biological control is a desirable strategy for control of damping-off diseases since breeding for resistance for many of the damping-off diseases has been unsuccessful (Reddy, 1991). The object of this study was to test the efficacy of select pseudomonads under extensive field trials infested with *R. solani* in order to bring the microbials towards commercialization as a seed treatment.

Materials and Methods

Rhizobacterial strains used in this study are listed in Table 1. Bacterial strains were isolated from diverse soils and root regions (Kloepper *et al.*, 1986, 1988). Field trials were conducted from 1990-1993 on canola cv. Westar in Saskatchewan. Experimental sites maintained by Agriculture Canada in Saskatoon, Regina and Melfort were chosen for this study. In 1990, experiments were conducted at one site (Saskatoon) and in the other three years experiments were set up at three locations. Basically, there were four treatments in each experiment included: i) canola seed treated with select bacterial strain and planted in *R. solani* infested plot (infested at 2-3 cm depth); ii) canola seed not treated with bacteria and planted in *R. solani* infested; iii) canola seed treated with Vitavax RS (carbathion/thiram/ lindale; Uniroyal Chemical Ltd, Elmira, Ontario) and planted in *R. solani* infested plot and iv) untreated canola planted in a plot without *R. solani* infestation. There were 5 bacterial strains tested in 1990 and 1991, 6 in 1992 and 3 in 1993 as seed treatments for their efficacy to enhance seedling stand establishment. In 1990 bacteria formulated in sterile peat and in 1991-1993 bacteria formulated in a liquid inoculum were used to treat the seed before planting. This procedure yielded log 5-6 cfu seed^{-1}. Treatments at each site were replicated 8 times. Each replicate plot was 6 m x 3 m, 5 experimental rows, and were separated by 2 guard rows planted with winter canola. There were 300 seeds planted per each 6 m row. Experimental field plots were artificially infested with *R. solani* grown on barley grain. Treatment plots were arranged in a randomized complete block design. Final healthy stand data was taken from 3 centre rows 1 m length per replication at 30 days after plating to asses the efficacy of the bacteria in all the sites. The data was analyzed by ANOVA.

Table 1. Bacterial strains used in this study.

Strain	Identification	Source of isolation
63-49	*Pseudomonas fluorescens*	Canola rhizosphere-Winnipeg
G1-3	*Pseudomonas fluorescens*	Soil-Arctic
U-14	*Pseudomonas fluorescens*	Cotton field soil-Mississipi
44-9	*Coryneform group*	Canola roots
17-114	*Pseudomonas fluorescens*	Corn field soil-Yellowknife
63-28	*Pseudomonas fluorescens*	Canola field-Winnipeg
L-114	*Enterobacter agglomerans*	Labrador

Results

The influence of seed bacterization on canola stand establishment grown in a *R. solani* infested site at Saskatoon in 1990 is shown in Figure 1. Strain G1-3 and Vitavax RS significantly enhanced the healthy stand of canola compared to nonbacterized control. These increases ranged from 28% (G1-3) to 44% (Vitavax RS) increase in stand above the untreated control. The results from the field trial conducted during 1991 at three locations are shown in Figure 2. Among five strains tested only 2 strains significantly enhanced the stand establishment in 2 out of 3 sites tested. Strain U-14, significantly enhanced the stand at Saskatoon (65% increase over control) and Melfort (62% increase over control) sites, whereas strain L-114, significantly increased the stand at Regina (78% increase) and Melfort (56% increase) sites. Canola seed treated with Vitavax significantly enhanced the stand at the 3 sites tested. Another 2 strains also increased the stand but is not significant. Canola stand enhancement influenced by the rhizobacterial strains tested during 1992 field trials is shown in Figure 3. Strains 63-49 and U-14 and Vitavax significantly increased the stand establishment at the 3 locations tested, whereas strain G1-3, at Regina site, strain L-114 at Saskatoon and Regina sites and strain 63-28 at Regina and Melfort sites significantly enhanced the stand compared to nonbacterized control. Overall, strains U-14 and 63-49 consistently increased the stand irrespective of the sites tested during 1992.

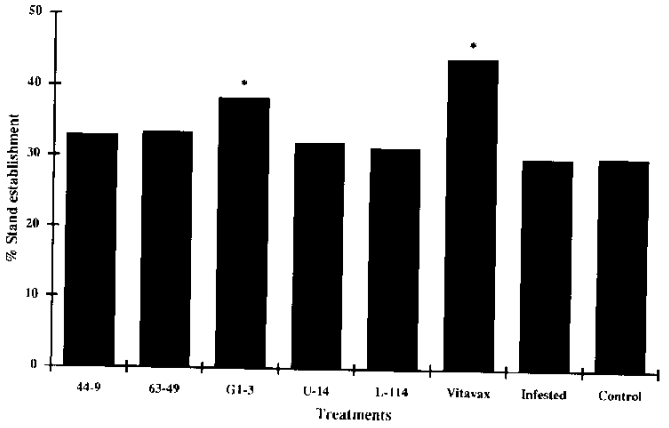

Figure 1. Influence of rhizobacteria on canola stand establishment grown in *Rhizoctonia solani* infested field (Saskatoon) in 1990. Percent stand was measured 30 days after planting. Percent stand is the mean of 8 replications per treatment. Control plots were contaminated with *R. solani.* *Significantly different from control (Pathogen infested, nonbacterized) at P = 0.05.

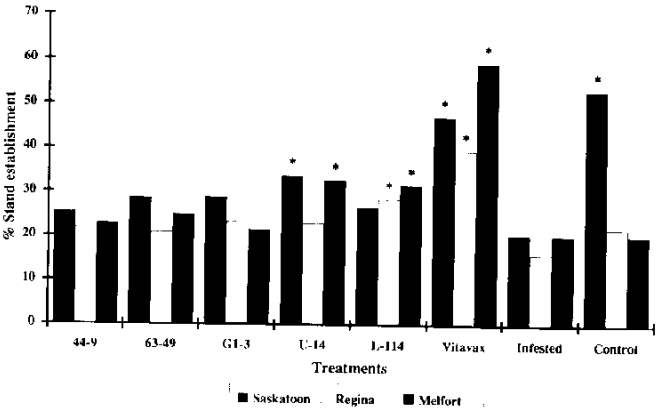

Figure 2. Influence of rhizobacteria on canola stand establishment grown in *Rhizoctonia solani* infested fields of Saskatchewan in 1991. Percent stand was measured 30 days after planting. Percent stand is the mean of 8 replications per treatment. *Significantly different from control (Pathogen infested, nonbacterized) at P = 0.05. Control plots at Regina and Melford were contaminated with *R. solani.*

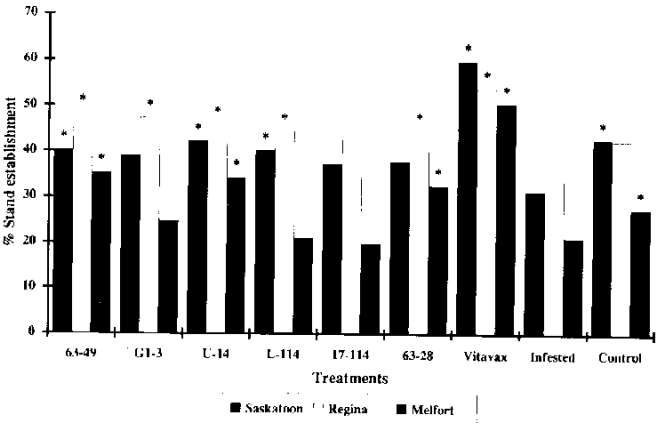

Figure 3. Influence of rhizobacteria on canola stand establishment grown in *Rhizoctonia solani* infested fields in 1992. Percent stand was measured 30 days after planting. Percent stand is the mean of 8 replications per treatment. *Significantly different from control (Pathogen infested, nonbacterized) at P = 0.05. Control plots at Regina and Melford were contaminated with *R. solani.*

Discussion

Earlier studies in the greenhouse over the years we found several of the strains significantly increased canola root and shoot dry weights and also suppressed the preemergence damping-off of canola caused by *R. solani.* Some of these strains has been shown to enhance emergence of canola and soybeans (Kloepper *et al.*, 1986) and to improve yield of canola grown in low or no disease field sites (Kloepper *et al.*, 1988). Current results showed that the canola seed treated with rhizobacteria resulted in effective stand establishment when tested under commercial growing conditions with a very high disease pressure. There was a positive trend (more than 10%) for strains U-14 and 63-49 in all the trials. These strains also increased canola seed yield at an average of 5% over control (not significant). Concurrent studies on root colonization by strains marked with antibiotic resistance indicate strains U-14 and 63-49 persist in the canola rhizosphere throughout the growing season. The majority of strains tested here were antagonistic to *R. solani* in PDA.

All the strains evaluated in this study grew in agar media amended with high concentrations of Vitavax RS and (or) captan (Zablotowicz *et al.*, 1992). Strains that survived on Vitavax treated seed also exhibited good root colonization on canola. Our studies also showed that these bacteria when introduced onto Vitavax treated seed significantly increased the canola stand grown in *R. solani* infested field site (results are not shown). The ability of these strains to enhance stand establishment of canola treated with Vitavax RS under disease pressure by *R. solani* suggests that the effects of disease control by fungicides can be combined and are additive. However, the

final assessment of potential for combining bacteria with fungicides on seeds requires extensive field testing. It is concluded that seed bacterization with select pseudomonads can result in a suppression of damping-off of canola grown in *R. solani* infested western Canadian soils.

References

Kaminski, D. A. and Verma, P. R. (1985). Cultural characteristics, virulence and *in vitro* temperature effect on mycelial growth of *Rhizoctonia* isolates from rape seed. *Canadian Journal of Plant Pathology* 7, 256-261.

Kloepper, J. W., Scher, F. M., Laliberte, M. and Tipping, B. (1986). Emergence-promoting rhizobacteria: description and implications for agriculture. In *Iron, Siderophores and Plant Diseases*. Ed. T. R. Swinburne. pp. 155-164. Plenum Publishers, New York.

Kloepper, J. W., Hume, D. J. Scher, F. M., Singleton, C., Tipping, B., Lalibert, E. M., Frauley, K., Kutchen, T., Simonson, C., Lifshitz, R., Zalesha, F. and Lee, L. (1988). Plant growth-promoting rhizobacteria on canola (rape seed). *Plant Disease* 72, 42-46.

Reddy, M. S. (1991). Biological control of plant diseases. In *Biological Control of Pests in Canada*. Ed. A. S. McClay. pp. 33-42. Alberta Environmental Centre, Alberta, Canada AECV91-P1.

Sippel, D. W., Davidson, J. G. N. and Sadasivaiah, R. S. (1985). Rhizoctonia root rot of rapeseed in the Peace River region of Alberta. *Canadian Journal of Plant Pathology* 7, 184-186.

Zablotowicz, R. M., Press, C. M., Lyng, N., Brown, G. L. and Kloepper, J. W. (1992). Compatibility of plant growth promoting rhizobacterial strains with agrichemicals applied to seed. *Canadian Journal of Microbiology* 38, 45-50.