Use of Rhizobacteria as Biological Control Agents of Downy Brome. S.M. Boyetchko and K. Mortensen. Agriculture Canada, Research Station, Regina, SK.

### Introduction

Downy brome (Bromus tectorum L.) is a winter annual grass which is rapidly spreading across southwestern Saskatchewan. This weed competes effectively for space, water and nutrients and its roots are capable of growing at low temperatures. Lack of selective herbicides and effective cultural control methods in spring and winter wheat and fall rye have led to the increased problem in controlling downy brome.

Rhizobacteria have been shown to suppress seed germination and root growth of several plant species. These deleterious rhizobacteria are able to produce metabolites, such as phytotoxins, which may impair plant development. Therefore, these microorganims may be used as potential biological herbicides to control target weed species such as downy brome.

The objectives of this study were to test potential rhizobacteria to control downy brome and to evaluate their effects on winter wheat and other grassy weeds and cereal crops.

#### Materials and Methods

Bacteria were isolated from downy brome infested field soils and 762 isolates were used in a bioassay to test their effects on growth of downy brome. Cell-free culture filtrates (CFCF) from bacterial cultures were incorporated into agar and used in a plate bioassay to test metabolites produced by the bacteria separately from the bacteria themselves. Seeds of downy brome were placed at one end of the bioassay plate and placed in a dark incubator at 15 C. After 10 days, germination and root growth were recorded.

Those isolates having a deleterious effect on downy brome were tested against winter wheat (Triticum aestivum L. cv. Norstar) using a tube bioassay. CFCF from bacterial cultures was incorporated into agar and pipetted into microcentrifuge tubes. One seed of winter wheat was placed into each tube, grown at 15 C in the dark for 7 days, and germination and root growth were recorded.

Selected isolates which were deleterious to downy brome were also tested against wild oats and green foxtail using the plate bioassay previously described.

Selected rhizobacterial isolates were used in the tube bioassay, previously described, to test their effects on winter wheat, spring wheat, fall rye and spring barley.

A preliminary greenhouse experiment was set up to test the effect of 3 concentrations of isolates 811 and 813 on root and shoot

growth of downy brome in soil. Plants were grown in Spencer-Lemaire rootrainers, each treatment comprised of 30 pre-germinated plants. Each seed was placed in a rootrainer and received either 0,  $10^2$ ,  $10^3$ , or  $10^4$  bacterial cells. Several concentrations were used to determine the optimum level of bacteria necessary to reduce growth of downy brome in soil. Plants were harvested after 4 weeks, and root and shoot dry weights were recorded. The experiment was replicated 3 times.

### Results and Discussion

Nine rhizobacterial isolates significantly reduced germination and root growth of downy brome when compared to the control (Table 1). Isolates 237 and 811 showed the greatest potential at suppressing growth of downy brome.

Isolates 811 and 813 had no deleterious effect on winter wheat while isolate 189 had some detrimental effect to root development of this crop (Table 2). Since these isolates had the least deleterious effect on winter wheat, they were selected as potential biocontrol agents for downy brome.

From the original rhizobacterial isolates showing deleterious effects on downy brome, four isolates also reduced germination and root growth of wild oats (Table 3). Isolate 811, which suppressed growth of downy brome (Table 1) but not winter wheat (Table 2), also suppressed growth of wild oats.

Four isolates were effective at reducing root growth but not germination of green foxtail (Table 4). Isolate 189 was deleterious to downy brome (Table 1) and green foxtail while isolate 821 effectively suppressed growth of wild oats (Table 3) and green foxtail, but not downy brome.

Isolates 189, 811, and 813, which had little or no deleterious effect on Norstar winter wheat, were tested on other cereal crops. Some cultivars experienced a reduction in germination and/or root growth while growth of other cultivars was increased (Table 5). Isolate 189 had the greatest deleterious effect on root growth of all cultivars tested.

Preliminary greenhouse tests revealed that isolate 811 is effective at reducing shoot growth of downy brome in soil when 10<sup>2</sup>, 10<sup>3</sup>, or 10<sup>4</sup> bacteria are applied to the plant, while isolate 813 is not able to reduce shoot growth at these bacterial concentrations. Root growth of downy brome is not reduced by either isolate at these concentrations.

### Summary

Three rhizobacterial isolates reduced germination and root growth of downy brome while showing little or no deleterious effect to winter wheat. Therefore, these isolates show some potential as

biocontrol agents for downy brome.

Several isolates show some potential for controlling wild oats and green foxtail. Three isolates were capable of promoting growth of some cultivars, while other cultivars experienced a reduction in germination and root growth by the bacteria. This indicates that host specificity occurs among the rhizobacterial isolates tested.

Preliminary greenhouse tests indicate that isolate 811 is effective at reducing shoot growth of downy brome in soil. Further tests are required to determine the optimum concentrations of isolates 189 and 813 for control of downy brome under soil conditions, as well as methods of applying these bacteria in the field.

Table 1. Germination and early root growth of downy brome on agar containing cell-free culture filtrate of rhizobacterial isolates.

Isolate No.	Germination (%)	Root Length (mm)	Root length (% of control)
Control	58.9	28.4	100.0
178	32.2	5.2	18.3
189	42.2	10.1	35.6
237	13.3	0.5	1.8
295	21.1	7.0	24.6
584	31.1	4.9	17.3
750	35.6	9.4	33.1
811	15.6	0.7	2.5
812	35.6	6.2	21.8
813	32.2	7.3	25.7

Table 2. Germination and early root growth of winter wheat on agar containing cell-free culture filtrate of rhizobacterial isolates.

Isolate No.	Germination (%)	Root Length (mm)	Root length (% of control)
Control	94.2	49.5	100.0
178	88.3	10.1	20.4
189	94.2	34.0	68.7 *
237	86.7	30.8	62.2
295	82.5	17.1	34.5
584	88.3	30.0	60.6
750	94.2	29.9	60.4
811	88.3	44.9	90.7 *
812	95.0	27.7	56.0
813	92.5	41.9	84.6 *

Table 3. Germination and early root growth of wild oats on agar containing cell-free culture filtrate of rhizobacterial isolates.

Isolate No.	Germination (%)	Root Length (mm)	Root length (% of control)
Control	57.8	38.1	100.0
243	37.8	16.5	43.3
751	33.4	9.8	25.7
811	40.0	12.3	32.3
821	43.3	4.7	12.3

Table 4. Germination and early root growth of green foxtail on agar containing cell-free culture filtrate of rhizobacterial isolates.

Isolate	Germination (%)	Root Length (mm)	Root Length (% of control)
Control	98.9	25.4	100.0
189	96.7	4.8	18.9
216	96.7	10.9	42.9
484	100.0	10.3	40.6
821	95.6	11.9	46.9

Table 5. Germination and early root growth of cereal crops on agar containing cell-free culture filtrate of rhizobacterial isolates.

Crop	Isolate No.	Germination (% of control)	Root length (% of control)
Readymade <sup>1</sup>	189	95.6	32.9
<b>≟</b>	811	100.0	64.7
	813	121.6	83.6
$Prima^2$	189	92.3	30.6
	811	96.1	67.7
	813	84.5	82.0
Musketeer <sup>2</sup>	189	164.2	53.5
	811	149.9	97.7
	813	100.0	102.5
Katepwa³	189	69.2	39.7
	811	92.3	103.9
	813	88.5	111.8
Genesis³	189	95.3	24.4
	811	100.0	92.0
	813	128.6	94.6
Bonanza⁴	189	82.2	35.3
	811	92.9	73.2
	813	92.9	86.9
Harrington⁴	189	80.7	38.8
_	811	92.3	81.4
	813	92.3	122.9

<sup>&</sup>lt;sup>1</sup> Winter wheat; <sup>2</sup> Fall rye; <sup>3</sup> Spring wheat; <sup>4</sup> Spring barley

Table 6. Growth of downy brome in soil treated with 2 rhizobacterial isolates at concentrations of 0, 102, 103, or 104 colony-forming units per plant. Plants were grown in the greenhouse for 4 wks with 16-h days at approximately 20 C.

Isolate	Concentration	Root Dry Wt <sup>a</sup> (mg plant <sup>-1</sup> )	Shoot Dry Wt <sup>b</sup> (mg plant <sup>-1</sup> )
811	0	39.1	94.2
	10 <sup>2</sup>	33.9	61.8 **
	10 <sup>2</sup> 10 <sup>3</sup> 10 <sup>4</sup>	43.7	71.3 **
	10 <sup>4</sup>	36.2	73.4 **
813	0	24.0	56.5
	$10^{2}$	26.9	59.2
	$\begin{array}{c} 0 \\ 10^2 \\ 10^3 \end{array}$	23.4	65.4
	10 <sup>4</sup>	27.2	55.5

<sup>\*\*</sup> Statistically lower than the noninoculated control at the 0.05 level using LSD.

<sup>&</sup>lt;sup>a,b</sup> Mean of 30 plants per treatment, 3 reps per treatment

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