

**Organic Sector Market Development Initiative (OSMDI)
The Canadian Wheat Board**

**Interim Report
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Project Title:

An organic option for broadleaved weed control in cereals using a microbial bioherbicide

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Executive Summary

Phoma macrostoma is a fungal bioherbicide being developed to control broadleaved weeds in turfgrass. A study was undertaken to determine if the bioherbicide could be used to control broadleaved weeds that are important in western Canadian agriculture. The objectives in this project were to: a) determine what common broadleaved weeds in wheat and barley can be controlled by the *Phoma* bioherbicide, b) determine the least effective rate of the bioherbicide for control of Canada thistle using a pre-emergent and post emergent application, and c) determine the least effective rate of the bioherbicide for control of wild mustard. The bioherbicide was able to control dandelion (68%), field bindweed (60%), annual sow thistle (97%), and wild mustard (82%). The least effective rate for Canada thistle was 0.7X the standard agricultural rate and for wild mustard it was 1.0X. There was also some reduction in perennial sowthistle, smart weed, Canada thistle, false cleavers, hemp nettle, and brassica volunteers but reductions were only from 25-50%. The bioherbicide had no effect on stinkweed, lambs quarters, and wild oat. Weed control of the various species was site dependent which was likely due to different moisture conditions and weed pressure. Soil moisture is a key requirement for the bioherbicide to work. Yet the bioherbicide was able to survive in dry soil for nearly 4 weeks before favorable conditions occurred and then it provided effective control of late-emerging wild mustard seeds. The bioherbicide worked best to control emerging seedlings and was less effective on well established weeds using a single application. Post-emergent application of the bioherbicide granules was more difficult when the crop was established. It is necessary to get even distribution of the bioherbicide over the surface otherwise control becomes more variable. To make the bioherbicide work better, there needs to be more work done on the application method to get even distribution of the product, the time of bioherbicide application relative to weed emergence, the impact of environmental conditions on efficacy, and multiple trials at several sites to assess the consistency of response for specific weeds.

Introduction

Phoma macrostoma is fungus that is being developed as a microbial bioherbicide for control of multiple broadleaved weeds. The strain under development is a naturally-occurring, indigenous micro-organism that was isolated from Canada thistle plants in Melfort, SK. It causes photobleaching and root inhibition in susceptible plants when broadcast to soil as a granule. Host range studies have shown that the bioherbicide affects several plant species in Asteraceae, Brassicaceae, and Leguminosae, but has no affect on Graminae (i.e. wheat, barley, forage grasses) or Linaceae (flax). Environmental fate studies have shown that the fungus declines to non-detectable levels within a year and the bioherbicide has no carryover effects in the year following application. AAFC has been working with The Scotts Company for several years to register the bioherbicide for broadleaved weed control in turfgrass and other non-food use plants in Canada and the USA. *Phoma macrostoma* may have other potential uses such as in agriculture for controlling broadleaved weeds in cereal crops and for organic production systems. But data must be collected to demonstrate the breadth of the weeds controlled, efficacy and safety on crops used for feed and food purposes. There were three objectives in this project: a) determine what common broadleaved weeds in wheat and barley can be controlled by the *Phoma* bioherbicide, b) determine the least effective rate of the bioherbicide for control of Canada thistle using a pre-emergent and post emergent application, and c) determine the least effective rate of the bioherbicide for control of wild mustard.

Methods and Materials

Experiment 1: Demonstration of agriculutral weed species controlled by the bioherbicide

Paired test plots (3 x 1 m²) compared untreated plots to bioherbicide-treated plots using a randomized design with 5 replicates. A single application rate (1.6X the standard agricultural rate) was broadcast to the soil surface after sowing the plots with wheat cv. Osler at 2 bu/ac placing seed 5 cm deep with 0.2m row spacing. The plots were evaluated for the weed species present, weed counts by species, weed cover and weed biomass (end of season). The experiment was conducted at Melfort and Prince Albert, SK. Tests were initiated on May 26-27, 2009. Data were analyzed using ARM software for analysis of variance and presented as mean ± standard error.

Experiment 2: Determine the least effective rate for control of Canada thistle with a pre-emergent and post-emergent application of the Phoma bioherbicide

The bioherbicide was broadcast to 3 x 1 m² plots seeded with barley (cv. Metcalf at 2 bu/ac placing seed 5 cm deep with 0.2m row spacing) at five bioherbicide rates of application (0, 0.7X, 1.0X, 1.3X, and 1.7X the standard agricultural rate) in a Canada thistle nursery at AAFC, Saskatoon. The crop was sown on May 26, 2009 which was one week prior to the pre-emergent application on June 2, 2009. The post-emergent application was applied on June 26, 2009 after the emergence of Canada thistle. Data were collected on weed count, weed cover, and weed biomass (end of season). The experimental design was a randomized complete block with 4 replications. Data were analyzed using ARM software for analysis of variance and presented as mean ± standard error.

Experiment 3: Determine the least effective rate for control of wild mustard with a pre-emergent application of the Phoma bioherbicide

The bioherbicide was broadcast to 1 m² plots seeded with wild mustard at varying rates of application (0, 0.7X, 1.0X, 1.3X, and 1.7X the standard agricultural rate) prior to weed emergence. Wild mustard was sown at the rate of 218 seeds/m seeding 3 rows spaced 25 cm apart on May 25, 2009 and the bioherbicide was applied the same day. A standard herbicide check (2,4-D ester at the rate of 560 g ai /ha in 110 L/ha) was applied at the 4 leaf stage on June 18, 2009. Data were collected on weed count, weed cover, photobleaching symptoms and weed biomass (end of season). The experimental design was a randomized complete block with 4 replications. Data were analyzed using ARM software for analysis of variance and presented as mean ± standard error.

Results

Experiment 1: Demonstration of agricultural weed species controlled by the bioherbicide

At Melfort, the wheat crop was well established and showed no signs of injury caused by the bioherbicide. The growing conditions were relatively dry which was not favorable to the establishment of the bioherbicide and also resulted in low weed populations. However, the bioherbicide did provide weed control against some species (Table 1). The bioherbicide reduced dandelion by 68% and field bindweed by 60% relative to the untreated control. The separation between treated and untreated plots with sowthistle and smartweed were too small to show any significant differences. Overall the weed control was low to moderate at 54%.

At Prince Albert, this site had more moisture. Crop establishment was uneven and poor due to problems with seeding equipment. The weed population was very high, but the species were not evenly distributed resulting in large variation between some plots. There was little to no photobleaching symptoms on susceptible weeds which is normally an indicator that the bioherbicide is working. Overall there was little control of weeds at this site except for annual sow thistle which was reduced by 96% (Table 1). There was 40-50% reduction in Canada thistle, false cleavers, and hemp nettle; brassica volunteers were reduced by 27%. There was no control of dandelion, stinkweed, lambs quarters, field bindweed, wild oat, and smart weed.

In conclusion, the bioherbicide controlled dandelion, field bindweed, and annual sow thistle at one of two sites. There was also some reduction in perennial sowthistle, smart weed, Canada thistle, false cleavers, hemp nettle, and brassica volunteers at one site. Weed control of the various species was site dependent which was likely due to different moisture conditions and weed pressure at the two sites. The bioherbicide had no effect on stinkweed, lambs quarters, and wild oat.

Table 1. The % weed control (mean and standard error) of various naturally occurring weed species at the end of season resulting from the application of the bioherbicide relative to the untreated control.

Location	Weed	Fresh weight g Bioherbicide	Fresh weight g No bioherbicide	% Biomass reduction
Melfort	Dandelion	27.3 ± 10.4	80.3 ± 28.0	68
	Sow thistle perennial	0.1 ± 0.1	3.5 ± 2.7	50
	Smart weed	0.5 ± 0.5	1.2 ± 0.8	24
	Field bindweed	0.0 ± 0.0	8.6 ± 4.7	60
	Mean all weeds	52.8 ± 18.6	116.3 ± 45.6	54
Prince Albert	Canada thistle	6.5 ± 5.2	11.7 ± 7.8	45
	False cleavers	100.4 ± 21.7	168.2 ± 36.9	40
	Dandelion	50.3 ± 26.9	19.2 ± 8.0	0
	Stinkweed	178.2 ± 71.4	65.8 ± 8.2	0
	Lambs quarters	1037.6 ± 213.1	1049.0 ± 253.4	1
	Hemp nettle	20.7 ± 11.0	38.8 ± 17.7	47
	Brassica	78.2 ± 30.3	107.3 ± 49.2	27
	Field Bindweed	135.4 ± 28.6	91.3 ± 24.3	0
	Wild oat	9.3 ± 9.31	7.5 ± 4.7	0
	Smart weed	15.1 ± 5.9	8.2 ± 4.0	0
	Sow thistle annual	4.4 ± 3.2	124.3 ± 51.6	97
	Mean all weeds	1636.3 ± 333.9	1724.8 ± 325.4	5

Experiment 2: Determine the least effective rate for control of Canada thistle with a pre-emergent and post-emergent application of the Phoma bioherbicide

The spring of 2009 in Saskatoon was very dry with no rains until the third week in June. Barley was sown on May 26 but did not start to emerge until July 8 and crop establishment was uneven and poor. The pre-emergent bioherbicide treatment was applied June 2 and there was some thistle starting to emerge. The post-emergent treatment was applied June 26 after weed emergence. There were no photobleaching symptoms on weeds until June 24 (corresponding to precipitation that fell on June 21) and by July 7 the symptoms were strong; these symptoms are an indicator that the bioherbicide has started to grow, but the soil at the site was still quite dry. Dry soil is not favorable to bioherbicide establishment and its efficacy. There were never any photobleaching symptoms on the crop.

The emergence of Canada thistle was slow. In the pre-emergent trial, there were no thistle plants by 24 days after application (DAA), by 58 days there were 1-4 plants per plot, but by 83 days there were 5-18 plants per plot. There was no difference in the number of plants /plot at 58 days but by 83 days two bioherbicide rates (0.7X and 1.0X) reduced the number of plants per plot and the biomass (Table 2). In the post-emergent trial, there were 9-22 plants/plot by 21 days after application and 6-17 plants/plot by 61 days. The 1.7X bioherbicide rate reduced the number of plants by 67% relative to the untreated control. The bioherbicide treatments reduced foliar biomass by 40-70% depending on the rate. However, dose dependency was not clearly

demonstrated. This could have been due to difficulties in getting even coverage of the bioherbicide on the entire plot area and due to high plot to plot variability in the natural weed stands.

In conclusion, the both pre-emergent and post-emergent applications of the bioherbicide reduced the number of Canada thistle plants and foliar biomass. The level of control was moderate in the range of 60-75%. The least effective rate was 0.7X.

Table 2. The effect of 5 bioherbicides rates on the number of Canada thistle plants during the season, % weed control relative to the untreated plot at the end of the season, foliar biomass and % biomass reduction at the end of season

Application method	Bioherbicide agriculture rate	Number of plants/plot July 16 (58 DAA)	Number of plants/plot Aug 24 (83 DAA)	% Weed control 83 DAA	Fresh weight/plot g	Biomass reduction %
Pre-emergent (0 DAA= June 2)	1.7 X	2.1 ± 0.8	13.5 ± 6.5	28	182 ± 122	0
	1.3 X	3.9 ± 1.3	15.0 ± 5.5	17	203 ± 166	0
	1.0 X	0.6 ± 0.3	4.7 ± 2.4	74	48 ± 27	71
	0.7 X	2.4 ± 1.0	6.7 ± 3.5	63	44 ± 22	74
	0.0 X	4.2 ± 1.3	18.0 ± 0.0	0	167 ± 42	0
Post-emergent (0 DAA= June 26)	Bioherbicide agriculture rate	Number of plants/plot July 16 (21 DAA)	Number of plants/plot Aug 26 (61 DAA)	% Weed control 61 DAA	Fresh weight/plot g	Biomass reduction %
	1.7 X	9.0 ± 5.5	5.7 ± 3.2	67	32.7 ± 25.8	62
	1.3 X	16.0 ± 4.2	12.0 ± 3.6	29	51.5 ± 19.5	40
	1.0 X	21.7 ± 14.3	12.7 ± 7.7	26	39.8 ± 34.0	54
	0.7 X	16.7 ± 6.4	8.7 ± 5.6	49	26.1 ± 21.9	70
	0.0 X	17.0 ± 4.5	17.0 ± 5.5	0	85.9 ± 28.8	0

Experiment 3: Determine the least effective rate for control of wild mustard with a pre-emergent application of the Phoma bioherbicide

It was a dry spring at the Scott, SK. Seeding and the bioherbicide application were completed on May 25 and the weeds emerged on June 1. The site received 20 mm of irrigation the day after seeding to aid in establishment of the wild mustard and bioherbicide. The conditions remained dry and there were few photobleaching symptoms, indicating that the bioherbicide was not working. In mid June, significant precipitation fell and a second flush of wild mustard emerged on June 24. These newly emerging plants had photobleaching symptoms. The first flush was harvested on June 24. The second flush was harvested on July 30.

The bioherbicide had no effect on the first flush of wild mustard. The bioherbicide plots were similar to the untreated control and these were different than the standard herbicide for the % weed cover. For all other parameters (number of plant/plot, % weed control, foliar biomass, and % biomass reduction) all treatments were the same (Table 3). The second flush of wild mustard was significantly reduced by all bioherbicide treatments and the standard herbicide relative to the untreated control for all parameters measured. The 1X rate of bioherbicide or greater gave the same level of control as the standard herbicide. The 0.7X rate of bioherbicide gave moderate weed control at 57%.

In conclusion, when there is sufficient soil moisture, the bioherbicide can control wild mustard (average 82% control at 1X rate or greater) similar to the standard herbicide (92% control). When soil moisture is not adequate, the bioherbicide can remain in the soil until the conditions become favorable and then act as a pre-emergent to control the flush of late germinating seedlings.

Table 3. Bioherbicide effects on % weed cover, number of plants/plot, % weed control, foliar biomass, and % biomass reduction relative to a standard herbicide treatment and the untreated control.

Weed emergence	Treatments	% Weed cover	Number of plants/plot	% Weed control	Foliar biomass g	% Biomass reduction
First flush June 1 (0 DAA= May 25; harvest 30 DAA= June 24)	1.7 X Bioherbicide	81 ± 2.1	393 ± 56	0	105 ± 10	11
	1.3 X Bioherbicide	88 ± 3.1	417 ± 47	0	141 ± 20	0
	1.0 X Bioherbicide	85 ± 2.3	508 ± 108	0	105 ± 14	11
	0.7 X Bioherbicide	83 ± 3.9	221 ± 27	39	106 ± 16	10
	2,4-D Herbicide	28 ± 1.6	363 ± 41	0	72 ± 5	39
	Untreated	86 ± 2.8	362 ± 74	0	118 ± 7	0
	Treatments	% Weed cover	Number of plants/plot	% Weed control	Foliar biomass g	% Biomass reduction
Second flush June 24 (0 DAA= May 25; harvest 65 DAA = July 29)	1.7 X Bioherbicide	11 ± 2.5	14 ± 1	92	31 ± 9	77
	1.3 X Bioherbicide	9 ± 3.6	25 ± 14	86	16 ± 8	88
	1.0 X Bioherbicide	12 ± 3.1	19 ± 3	89	27 ± 8	80
	0.7 X Bioherbicide	20 ± 4.8	18 ± 5	89	57 ± 20	57
	2,4-D Herbicide	3 ± 1.2	12 ± 3	93	11 ± 3	92
	Untreated	53 ± 5.6	180 ± 46	0	132 ± 22	0

Summary

In conclusion, the bioherbicide was able to control dandelion (68%), field bindweed (60%), annual sow thistle (97%), and wild mustard (82%). There was also some reduction in perennial sowthistle, smart weed, Canada thistle, false cleavers, hemp nettle, and brassica volunteers but reductions were only from 25-50%. The bioherbicide had no effect on stinkweed, lambs quarters, and wild oat. Weed control of the various species was site dependent which was likely due to different moisture conditions and weed pressure. Soil moisture is a key requirement for the bioherbicide to work. Yet the bioherbicide was able to survive in dry soil for nearly 4 weeks before favorable conditions occurred and then provided effective control of emerging wild mustard seeds. The bioherbicide worked best to control emerging seedlings and was less effective on well established weeds using a single application. Post-emergent application of the bioherbicide granules was more difficult when the crop was established. It is necessary to get even distribution of the bioherbicide over the surface otherwise control becomes more variable. To make the bioherbicide work better, there needs to be more work done on the application method to get even distribution of the product, the time of bioherbicide application relative to weed emergence, the impact of environmental conditions on efficacy, and multiple trials at different locations to assess the consistency of response of specific weeds.

Outputs:

- a) Demonstration at the AAFC Scott Field Day, July 15, 2009 with 275 producers in attendance.
- b) Demonstration at Saskatchewan Provincial Weed Tour, July 16, 2009 with 30 weed scientists in attendance
- c) Demonstration to the Prairie Canola Agronomic Research Steering Committee, July 2009 with 20 producers and researchers in attendance.
- d) Demonstration at the AAFC Melfort Field Day, July 22, 2009 with 80 producers in attendance.
- e) Interim report to OSMDI, CWB

Activities for Next Reporting Period

- a) Abstract and Poster Presentation at CWSS
- b) Report to OACC, Prairie region
- c) Oral presentation to Saskatchewan Agriculture and Food, Organic Producers and Regional Specialists on October 27, 2009 (30 people by invitation)
- d) Information Bulletin to SAF Organic Newsletter, October 2009
- e) Final report to OSMDI

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