Fungal pathogens as biological control agents for herbicide resistant pigweeds and waterhemp

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Introduction

Common waterhemp *(Amaranthus rudis)* and pigweeds *(Amaranthus spp.)* have become major problems in pumpkin *(Cucurbita pepo) and* soybean *(Glycine max)* production. Management of these weeds is a challenge because of the development of herbicide resistance to several herbicide families. The need for alternative weed control methods has increased since there are limited options for the control of these weeds. In an effort to control these weeds, the use of fungal pathogens *(Microsphaeropsis amaranthi)* and *(Phomopsis amaranthicola)* as biological control agents for herbicide resistant waterhemp and pigweeds was investigated in Havana and Macomb, IL. These organisms are ideal for use as biological control for waterhemp and pigweeds because they are easily cultured and are host specific to weeds in the family *Amaranthaceae.* The fungal organisms need moisture on the leaf surface to infect the weeds. Dew can increase the ability of a pathogen to infect. Irrigation was evaluated to see if it caused a difference in the performance of the organisms ability to infect and control waterhemp and pigweeds.

Objective

The goal of this project was to evaluate the effects of two fungal pathogens as biological control agents of waterhemp and pigweeds in irrigated and non-irrigated pumpkin and soybean crops.

Methods

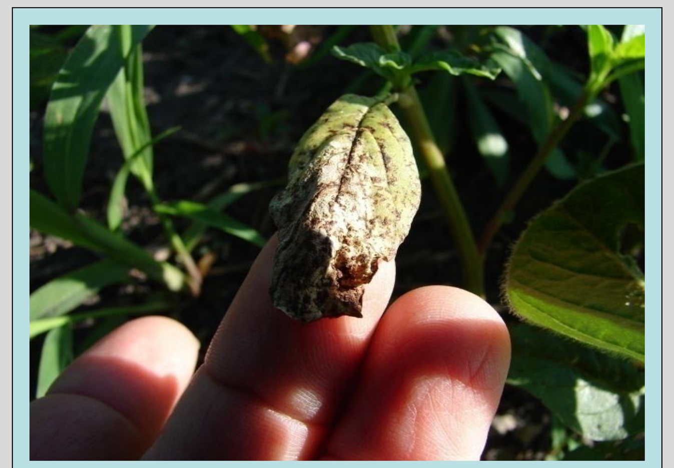
Field trials were performed over two years in Havana and Macomb, IL to evaluate the effectiveness of two organisms in irrigated and non-irrigated pumpkin and soybean plots. The fungal pathogens were grown on a V8 agar. After inoculation the organisms were incubated in a growth chamber for 14 days at 25 º C with a 12-h light-dark cycle. After incubation conidia were harvested and collected to prepare the spore suspension. The concentration of conidia was measured with a hemacytometer. The treatments included spore suspensions of each fungal pathogen alone, a mixture of both pathogens, and sequential treatments with either halosulfuron-methyl (Sandea® Herbicide) in pumpkin or glyphosate (Roundup® Herbicide) in soybean. The concentrations of spore suspensions in 2008 in Havana and Macomb, IL were *M. amaranthi* (1.5×10^6 conidia.ml^{-1}), *P. amaranthicola* (4×10^6 conidia.ml^{-1}), and a mixture of *M. amaranthi* and *P. amaranthicola* ($1.5\times10^6+4\times10^6$ conidia.ml^{-1}, respectively). The concentrations of spore suspensions in 2009 in Havana and Macomb, IL were *M. amaranthi* (1.9×10^6 conidia.ml^{-1})*, P. amaranthicola* (2.2×10^6 conidia.ml^{-1}), and a mixture of *M. amaranthi* and *P. amaranthicola* ($1.9\times10^6+2.2\times10^6$ conidia.ml^{-1}, respectively). The spore suspensions were applied at the 4-6 leaf growth stage of the weeds in a lecithin and vegetable oil formulation at 163 L ha^{-1} in 2008 and 326 L ha^{-1} in 2009. In 2008, treatments were applied using a hand held spray bottle at 163 L ha^{-1}. In 2009, treatments were applied using a CO_2–pressurized backpack sprayer with a hand-held boom having six 8002VS Teejet nozzles calibrated to deliver the treatments at 326 L ha^{-1} at 15 psi. The effectiveness of the bioherbicides was evaluated at 6 and 14 days after application for their effect on disease incidence, disease severity, percent weed control, and weed dry weight. Disease severity was evaluated on a scale of 0-5 (0 = no disease 5 = plant mortality). Total yield was taken in October, 2009 and was not included. In 2008, soybean plots were set up as a randomized complete block design with four reps. The irrigated plot in Havana, IL was in a location where it received central-pivot irrigation and the non-irrigated plot was located on the other end of the field. In 2009, a split block design was utilized; the four blocks were split into irrigated and non-irrigated sections.

The Pathogens

Ma = *Microsphaeropsis amaranthi*

Pa = *Phomopsis amaranthicola*

Weed mortality from Sandea followed by Ma Treatment



Symptoms of infection on leaf of weed from pathogens

Disease lesions on leaves from Ma and Pa Mix Treatment

Table 1. The impact of bioherbicide treatments on weed weight and percent weed control in soybean plots in Havana and Macomb, IL.

Treatment	Weed Weight (g)					Weed Control (%)[e]		
	Havana[a]		July 2009[d]			Havana	Macomb[f]	
	2008		Havana	Macomb				
	NON IR	IR				July 2009[g]	7/2/09	7/8/09
Ma	0.534 BC[b]	0.141 A	0.475 B	0.557 B		0 D	12 E	12 E
Pa	0.411 BC	0.399 A	0.650 B	0.452 B		0 D	0 F	0 F
Ma Pa Mix	0.373 BC	0.327 A	0.507 B	0.459 B		0 D	0 F	0 F
Roundup fb[c] Ma	0.424 BC	0.282 A	0.275 B	0.315 B		89 A	38 A	53 A
Roundup fb Pa	0.113 C	0.363 A	0.285 B	0.424 B		76 C	18 D	27 D
Roundup fb Ma Pa mix	0.118 C	0.166 A	0.112 B	0.312 B		82 B	31 B	38 C
Roundup	1.223 BC	0.443 A	0.143 B	0.330 B		84 B	26 C	41 B
Untreated Control	2.544 A	0.555 A	3.285 A	1.349 A		0 D	0 F	0 F
LSD (P= 0 .05)	1.305	0.311	0.665	0.298		3	5	3
CV	2.093	2.086	2.120	2.064		2	2	2
Mean	0.747	0.336	0.716	0.525		41	16	21
Prob (F)	0.001	0.132	<.0001	<.0001		<.0001	<.0001	<.0001

[a]Means are separated by irrigation because of a significant interaction between treatment and irrigation.
[b]Means followed by the same letter in a column are not significantly different at (P<0.05).
[c]fb stands for followed by.
[d]Means are presented for treatment across date and irrigation because there was no significant interaction.
[e]Weed control data were square root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means.
[f]Means are separated by date for % weed control because of a significant interaction between date and treatment.
[g]Means are presented for treatment across date because there was no significant interaction.

Table 2. The impact of bioherbicide treatments on weed weight and percent weed control in pumpkins in Macomb, IL.

Treatment	Weed Weight (g)			Weed Control (%)[cd]			
	2008	2009		6/16/08	6/25/08	7/2/09	7/9/09
Ma	0.159 B[a]	0.311 B		19 DE	5 BC	0 C	0 C
Pa	0.225 B	0.273 BCD		19 CDE	3 C	0 C	0 C
Ma Pa Mix	0.198 B	0.259 BCD		10 E	19 AB	0 C	0 C
Sandea Alone @ 1oz/A	0.262 B	0.210 CD		53 A	18 A	0 C	16 B
Sandea fb[b] Ma	0.229 B	0.271 BCD		51 A	14 AB	8 B	18 B
Sandea fb Pa	0.199 B	0.211 CD		35 ABC	15 AB	8 B	24 A
Sandea fb Mix	0.137 B	0.292 BCD		40 AB	14 AB	8 B	24 A
Ma fb Sandea	0.166 B	0.188 D		28 BCD	18 A	10 A	25 A
Pa fb Sandea	0.150 B	0.275 BCD		35 AB	18 A	0 C	0 C
Mix fb Sandea	0.145 B	0.247 BCD		39 AB	13 AB	8 B	25 A
Untreated Control	0.510 A	0.875 A		0 F	0 C	0 C	0 C
LSD (P= 0 .05)	0.209	0.095		2	0.209	2	2
CV	2.042	2.028		2	2.042	2	2
Mean	0.216	0.303		30	0.216	3	12
Prob (F)	<0.0001	<0.0001		<0.0001	<0.0001	<0.0001	<0.0001

[a]Means followed by the same letter in a column are not significantly different at (P<0.05).
[b]fb stands for followed by.
[c]Weed control data were square root transformed to meet assumptions of normality or homogeneity of variance; data shown are untransformed means.
[d]Means are separated by date for % weed control because there was a significant interaction between date and treatment.

Results

Soybean

In 2008, weed dry weights in non-irrigated plots were significantly lower in all treatments than the untreated control. In 2009, weed dry weights were significantly lower in all treatments than the untreated control. Soybean plots showed the best weed control in treatments with Roundup® herbicide followed by Ma and were significantly higher than all treatments including Roundup® herbicide alone and the untreated control. Results show a reduction in weed biomass when treated with one or both of the fungal organisms compared to the untreated control treatment.

Pumpkin

In 2008, weed dry weights were significantly lower in all treatments compared to untreated control. In 2009, weed dry weights were lowest in the treatment with Ma followed by Sandea® herbicide but it was not significant. On 6/16/08 weed control was significantly higher in treatments with fungal organisms than the untreated control. On 6/25/08 weed control was significantly higher in all treatments except Ma and Pa alone than the untreated control. In 2009, weed control was significantly higher in treatments with Ma followed by Sandea® herbicide than treatments with Sandea® herbicide alone and the untreated control.

Disease

The results for disease incidence and disease severity are not shown. The bioherbicide organisms caused high levels of disease incidence and showed a range from 1 to 4.4 for disease severity in the plots treated with the fungal pathogens. The rates for disease severity were not as high as sought after and were lower in plots that were irrigated.

Discussion

Increased weed control was present in treatments when the bioherbicides were used in sequence with the chemical herbicide; this shows the potential for their use together. In Havana IL, results for weed control were not taken in 2008 because of the low weed pressure throughout the field. The areas within each plot with the most weeds were chosen to evaluate. In 2009, the field was seeded for increased consistent weed pressure. Due to the high weed pressure in the pumpkin plot in Macomb, IL no treatment gave exceptional weed control in either year. Even with the high weed pressure the organisms caused infection on the weeds as there was a high level of disease incidence and lower weed weight than the control treatment. The effect of irrigation on weed dry weight was not as good in the irrigated treatments compared to the non-irrigated treatments; this may have been influenced by the irrigation because the water might have washed some of the bioherbicide organisms off the weeds. Irrigation is important because the organisms need a period of dew to have enough moisture to infect the weeds. There may have been more infection on the irrigated weeds if irrigation was provided after a longer period of time instead of directly after application of the organisms.

Conclusion

These two fungal organisms show potential for use as a biological control agent for controlling herbicide resistant weeds in the genus *Amaranthus.* The development of these organisms as bioherbicides may improve crop yields by controlling problematic weeds. In order to manage herbicide resistant waterhemp and pigweeds alternative control methods such as these biological control agents should be further researched.

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