Northern New York Agricultural Development Program
2015-2016 Project Report

Breeding Alfalfa Varieties with Resistance to Alfalfa Snout Beetle

Project Leader(s):
Cornell University School of Integrative Plant Science, Plant Breeding and Genetics Section, 523 Bradfield Hall, Ithaca, NY:

- Don Viands, 607-255-3081, drv3@cornell.edu
- Jamie Crawford, 607-255-5043, jln15@cornell.edu
- Julie Hansen, 607-255-5043, jlh17@cornell.edu

Cornell University Department of Entomology, 4144 Comstock Hall, Ithaca, NY:

- Elson Shields, 607-255-8428, es28@cornell.edu
- Antonio Testa, 607-591-1493, at28@cornell.edu

Collaborator(s):

- Michael Hunter, Cornell Cooperative Extension Regional Field Crops Specialist
- Joe Lawrence, Cornell University PRO-DAIRY Dairy Forage Systems Specialist

Cooperating Producers:

- Haskell and Tim Yancey, 7981 Long Pond Road, Croghan, NY 13327-2508
- Gary Berrus, Limestone Ridge Farm, 9373 Delles Road, Lowville, NY 13367

Background:
Alfalfa snout beetle (snout beetle or ASB), *Otiorhychus ligustica*, is the most destructive insect pest of alfalfa in Northern New York (NNY). Alfalfa snout beetle is currently infesting nine counties in New York State and has been identified in Canada across the St. Lawrence River from NNY. This pest causes severe yield and stand losses on alfalfa by larval feeding on alfalfa roots. The Cornell alfalfa breeding program (led by D.Viands, J. Hansen, and J. Crawford) and Cornell entomology program (E. Shields and A. Testa) have been cooperating to develop a two-pronged approach to control alfalfa snout beetle on alfalfa: 1) identify and incorporate resistance genes (breeding for resistance) into alfalfa varieties adapted to northeastern USA, and 2) identify and establish NNY biological control organisms. Application of both of these insect control strategies in tandem are necessary to reduce alfalfa snout beetles to sub-economic numbers.

We continue to use the greenhouse screening method developed by E. J. Shields and A. Testa with Hatch Act funds and long-term grant support from the farmer-driven Northern New York Agricultural Development Program, to identify and select alfalfa plants that indicate resistance. Screening more than 30,000 seedlings annually, we have completed up to 14 cycles of selection in several alfalfa populations.

Impacts from Past Research:
Progress is being accomplished in selection for alfalfa with resistance to snout beetle. In previous reports, we have reported significant progress toward ASB-resistance in plants from selection under controlled greenhouse conditions. The greenhouse experiments are being complemented by field experiments in Northern NY.

The first alfalfa variety released from this ASB-resistance breeding program, Seedway 9558 SBR, is now being used by growers in NNY. Seedway 9558 SBR has shown moderate resistance to alfalfa snout beetle both when tested in the greenhouse and in the field. Our goal is to continue to develop resistant varieties that have higher levels of resistance to alfalfa snout beetle and to test the most advanced breeding lines in field trials in NNY, both with and without nematode application. We believe higher levels of resistance are achievable and would provide more effective control. Furthermore, we have dug survivor plants in fall 2014 (Nov 4, Adams; Nov. 5 Lowville) from advanced breeding lines in field trials in alfalfa snout beetle-infested areas for the purpose of adding resistance and agronomic adaptation genes to the alfalfa snout beetle-resistant germplasm populations.

Methods:
Alfalfa Snout Beetle Capture in NNY for Storage in Coolers at Cornell:
On April 21, 2016, alfalfa snout beetles were collected by Tony Testa and Gary Berrus at the Berrus farm, Lowville, Lewis County, NY. Tony collected additional adult beetles on May 6, 2016, because the first batches collected were very lethargic and there was concern about their ability to survive in the coolers at Cornell until late summer, when eggs are needed for inoculation.

Alfalfa Selection for Resistance to Alfalfa Snout Beetles:
The alfalfa populations for screening for resistance to snout beetle were planted in greenhouses at Cornell in late June 2016. The seedlings were inoculated with alfalfa snout beetle eggs on August 17. After inoculating each tub with eggs, the tubs of alfalfa were left to grow in the greenhouse for another 6-8 weeks. Then, each alfalfa seedling in the tub was dug and washed to see the level of root scarring from the alfalfa snout beetle larvae. The seedlings that had no root feeding damage from the alfalfa snout beetle larvae were selected and grown in the greenhouse until flowering and pollinated to produce seed.

Alfalfa Population Evaluation Trial at Croghan:
In 2016, the trial planted at Croghan (Belfort area), NY, Lewis County, on the Yancey farm in 2014 was harvested for the second production year. The trial was planted to test

alfalfa populations developed for resistance to alfalfa snout beetles. Sixteen populations were planted in a 6-replicate trial in 2014. This trial was not treated with nematodes. Next to this trial, the same sixteen populations were planted in a 3-replicate trial that was treated with nematodes in the seeding year. The two side-by-side trials (9 replicates) were sprayed with herbicide each spring and sprayed with insecticides to treat potato leafhoppers in 2016. The 6-replicate trial was harvested by the Cornell Forage Project in 2015 and 2016. The 3-replicate nematode trial was harvested by the Yancey family as part of their normal forage operation.

The replicated plot trial in NNY at Haskell and Tim Yancey's farm was sprayed with herbicides on March 30. This trial was harvested twice for yield in 2016 on June 14 and July 27. On November 2, 2016, 3 foot by 3 foot sections from 3 replicates in the trial not treated with nematodes and 2 replicates in the trial with nematodes applied were dug. A subset of the populations in the trials were dug for root ratings. The populations that had plants dug from plots were:

- Seedway 9558 cycle 0 and cycle 9, and Seedway 9558 SBR cycle 7;
- NY9117 cycle 0 and cycle 9; and
- Guardsman II.

The plants dug were scored on a scale of 1 to 5 where 1 is no feeding damage by alfalfa snout beetle and 5 is a dead plant. The number of plants by score class were counted. The plants from each dug plot area were weighed. The average weight per plant was computed from the plot weight divided by total number of plants.

Results:

Alfalfa Snout Beetle Capture and Alfalfa Selection for Resistance to Alfalfa Snout Beetles

When the alfalfa snout beetles were taken out of storage, the alfalfa forage that the beetles eat to encourage egg laying was very drought stressed and the beetles would not eat this alfalfa. Many beetles died before discovering some irrigated alfalfa that they would eat. Thus egg collection was low in 2016 even though there were more than enough beetles collected in the spring. Even though more adults were used for egg collection in 2016, the number of eggs collected were not sufficient to inoculate all the greenhouse tubs of plants, so one population was not inoculated this summer. From the greenhouse selection protocol, five alfalfa populations were advanced one more generation this year so that some populations have been selected up to 14 cycles.

Alfalfa Population Evaluation Trial at Croghan – Yield (Table 1):

The 2016 growing season was defined by drought conditions in New York State. Much of Lewis County was in moderate drought conditions from July 5 to November 5 (http://droughtmonitor.unl.edu/MapsAndData/MapArchive.aspx). In 2015, total yield over two harvests was 2.09 tons per acre and in 2016 total yield over two harvests was 1.92 tons per acre. The correlation between 2015 and 2016 total season yield was 0.94, thus the alfalfa populations in the yield trial had similar relative yield performance over the two years. Similar to 2015, four of the seven selected populations were in the high yielding group (Seedway 9558 SBR, Potato leafhopper resistant (PLH) Asb cycle 9, ASB Cross, NY9117 Asb cycle 9).

Table 1: 2016 and 2-Year Total Yields from 2014 Alfalfa Trial planted at Croghan NY on a field infested with Alfalfa Snout Beetle. Alfalfa populations include ones selected for resistance to Alfalfa Snout Beetle for 7 to 9 cycles (Asb7 to Asb9).

BOLD = within one LSD of the highest yielding entry.

	All 6 Replicates		Replicates # 1,2,3					Replicates # 4,5,6				
			2016			2-Yr.	2-Yr.	2016			2-Yr.	2-Yr.
Pedigree	2-Yr. Total	2-Yr. Rank	Har. 1	Har. 2	Total	Total	Rank	Har. 1	Har. 2	Total	Total	Rank
	T/A		--- tons/acre ---					--- tons/acre ---				
OVR Base	3.56	15	1.45	0.71	2.16	4.39	15	0.85	**0.58**	1.44	2.72	15
OVR Asb9	3.17	16	1.29	0.65	1.94	4.00	16	0.72	0.50	1.23	2.34	16
PLH Base	4.04	9	**1.58**	0.59	2.18	**4.82**	7	**1.01**	0.51	**1.50**	3.25	10
PLH Asb9	4.09	8	1.53	0.71	2.25	**4.81**	8	**1.03**	0.47	**1.51**	3.36	7
ReSelect Sar Base	4.11	7	1.48	0.77	2.24	**4.88**	6	**1.02**	**0.60**	**1.62**	3.34	8
ReSelect Sar Asb9	3.79	12	1.48	0.74	2.21	4.57	13	0.93	**0.58**	**1.51**	3.00	12
Seedway 9558	3.91	11	1.44	0.76	2.20	4.65	10	**0.97**	**0.58**	**1.55**	3.18	11
Seedway 9558 Asb7	4.35	3	1.54	0.71	2.25	**5.06**	3	**1.14**	0.55	**1.70**	**3.64**	3
Seedway 9558 Asb9	4.19	5	**1.58**	**0.85**	**2.43**	**5.00**	5	**0.97**	**0.60**	**1.58**	3.37	6
NY9117	4.60	1	**1.81**	**0.86**	**2.67**	**5.34**	1	**1.10**	**0.66**	**1.76**	**3.86**	1
NY9117 Asb9	4.52	2	**1.79**	**0.90**	**2.69**	**5.28**	2	**1.16**	**0.59**	**1.75**	**3.76**	2
MIII Asb9	3.73	14	1.45	0.70	2.15	4.51	14	0.91	0.54	1.44	2.95	13
Curculio Asb8	3.74	13	1.47	0.72	2.19	4.63	12	0.84	**0.56**	1.39	2.85	14
ASB cross	4.30	4	**1.67**	0.75	**2.42**	**5.04**	4	**1.07**	**0.58**	**1.65**	**3.56**	5
Guardsman II	4.17	6	1.55	0.75	2.30	**4.76**	9	**1.13**	**0.59**	**1.72**	**3.58**	4
N-R-GEE	3.97	10	1.52	0.74	2.26	4.63	11	**1.00**	**0.59**	**1.59**	3.31	9
Trial Mean			1.54	0.74	2.28	4.77		0.99	0.57	1.56	3.26	
F-test			2.54 *	3.38 **	2.71 **	2.49 *		3.46 **	1.81 ns	2.75 **	7.20 **	
LSD (0.05)			0.22	0.10	0.30	0.59		0.19	0.10	0.26	0.45	
CV (%)			8.6	8.2	7.8	7.3		11.5	10.7	9.8	8.1	
Lattice Efficiency (%)			672	391	599	500		123	148	120	153	

Alfalfa Population Evaluation Trial at Croghan – Root Damage (Table 2):

The trial at Croghan was severely damaged by alfalfa snout beetles (Photos 1, 2). By November of the second production year, most of the alfalfa plants in the trial were dead. There were stems on the dead plants, indicating that the plants had regrown after the second harvest in early August prior to dying from root feeding by alfalfa snout beetles later in the fall. Late fall plant digging from alfalfa snout beetle yield trials has worked well in the past for evaluating alfalfa populations for field survival under alfalfa snout beetle infestations. Early plant death may have been due to drought stress in 2016. Many large alfalfa snout beetle larvae were observed while digging the alfalfa roots (Photo 3).

The total weight of plants dug from the plots inoculated with nematodes and the not-treated plots were not significantly different: 280 grams vs. 294 grams respectively (Table 2). There were fewer plants dug from the nematode-treated section (total number of plants, number of plants scored 5 (dead), number of plants scored 4), but these differences were not statistically significant. The average weight per plant was:

4 grams for the no-nematode trial, and

7 grams for the nematode-treated plot.

This difference was statistically significant. The plants in the nematode-treated section were about twice the size of the plants in the non-nematode treated section. The plant density was about half in the nematode-treated area so less plant-to-plant competition may be the reason for the larger plants in the nematode-treated area.

In future trials, the nematode-treated areas should have similar harvest treatment so that results are not confounded by harvest method. Since the section of the field treated with nematodes was harvested with larger equipment, the results of this study are not clear because the results could be due the difference in the harvesting method and or due to nematode application. Futhermore, the nematode-treated area was planted to compare root damage with and without nematodes, and the plants were so severely damaged by alfalfa snout beetles in both treated and not treated areas that the comparison did not lead to clear conclusions.

The yield data from this trial was very useful for determing which alfalfa populations to continue developing for resistance to alfalfa snout beetle. The root damage data from the trial was not as useful as in the past due to very fast plant death after second harvest in 2016. The severe drought in 2016 impacted the trial results.

Another trial is needed to take a detailed look at alfalfa snout beetle root feeding damage on alfalfa treated with nematodes compared to alfalfa not treated with nematodes.

Table 2: Weight, number and scoring of plants dug from the 2014 alfalfa snout beetle trial, Yancey Farm, Nov. 2, 2016, Croghan, NY. Plants were dug from a 3 foot by 3 foot area of each plot for 6 alfalfa populations in trials treated with and without nematodes.

Variable Measured	No Nematode Trial	Nematode-Treated Trial	P-value
Weight of plants dug (grams)	294	280	0.87
Number of plants dug (#)	74	37	0.09
Number of plants dug - score 5	73	32	0.06
Number of plants dug – score 4	2	5	0.08
Weight per plant (g)	4	7	0.01 **

Alfalfa snout beetle (ASB) root score 5: dead plants, 4: plants with taproots longer than 4 inches.

Conclusions/Outcomes/Impacts:

From the results of several field experiments, progress is being made in selection for alfalfa with resistance to alfalfa snout beetle. The first variety released from this ASB-resistance breeding program, Seedway 9558 SBR, is now being used by growers in NNY. This cultivar has moderate resistance to alfalfa snout beetle and, when used in combination with the entomopathogenic nematodes protocol developed by Dr. Elson Shields' Northern New York Agricultural Development Program-funded ASB solution research, should provide some control against this destructive insect. Our goal is to continue to develop resistant varieties that have higher levels of resistance to alfalfa snout beetle and to continue to test the breeding lines developed in field trials in NNY. We

believe that higher levels of resistance are achievable and would provide more effective control.

From the trial at Croghan, it is clear that under some circumstances, the alfalfa snout beetle can overcome both the alfalfa resistance and the nematode biocontrol. It is not certain what these circumstances are but it is likely due to some aspect of drought and overly abundant populations of alfalfa snout beetles. Based on this 2016 trial, the Yancey family, having seen the alfalfa snout beetle adults and larvae and the alfalfa plant root damage, will implement ASB best management practices, including crop rotation, resistant varieties, and beneficial nematodes. The beneficial nematodes take time to get substantively established in NNY fields.

Alfalfa snout beetle-resistant alfalfa with a moderate level of resistance, under very high snout beetle pressure, can currently be overwhelmed by larvae root feeding damage. We were not able to assess the staying power of alfalfa snout beetle-resistant alfalfa in the trials at Yancey's because we dug the alfalfa roots after the roots had very extensive damage from snout beetle larvae.

Outreach:

The progress in developing alfalfa snout beetle-resistant alfalfa was shared at the 2016 Seedsmen's Field Day on July 7, 2016, in Ithaca, NY; at Cornell Cooperative Extension In-Service Meeting on November 1, 2016; and at the Annual Meeting with Seedway on Dec. 20, 2016. YouTube videos of alfalfa snout beetle emergence are available.

Next Steps:

Although significant breeding progress has been accomplished, we believe that much higher levels of resistance will be needed and can be achieved through further breeding efforts. Thus selection and breeding work need to continue to develop alfalfa with lower root damage score. Additional field experiments are needed to determine the benefits (higher yield and less root feeding damage) of higher levels of resistance under actual growing conditions and climate as these plant populations are developed. Also of interest is to concentrate on combining resistances to alfalfa snout beetle and potato leafhoppers into new cultivars of alfalfa.

Acknowledgments:

Northern New York Agricultural Development Program, Cornell University Agricultural Experiment Station Hatch Funds, NE1010 Multistate Research Funds, Seedway, and Allied LLC.

Reports and/or articles in which results of this project have been published:

- *Yields of Seedway 9558 SBR on fields that are not infested with alfalfa snout beetle* - *New York Forage Legume and Grass Cultivar Yield Trials Summary for 2016 – Season Totals*. J. Hansen, D. Viands, R. Deubler, J. Crawford, J. Schiller, R. Crawford, Department of Plant Breeding and Genetics, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY 14853;

https://plbrgen.cals.cornell.edu/research-extension/forage-project/ny-forage-yield-results

For More Information:

- Dr. Donald R. Viands, Cornell University Section of Plant Breeding and Genetics, 607-255-3081; drv3@cornell.edu; http://plbrgen.cals.cornell.edu/people/profiles/viandsdonald.cfm
- Dr. Julie L. Hansen, Cornell University Section of Plant Breeding and Genetics, 607-255-5043; jlh17@cornell.edu
- Gary Berrus, Limestone Ridge Farm, 9373 Delles Road, Lowville, NY 13367
- Haskell and Tim Yancey, 7981 Long Pond Road, Croghan, NY 13327-2508