Northern NY Agricultural Development Program
2013 Project Report

Breeding Alfalfa Cultivars with Resistance to Alfalfa Snout Beetle

Project Leader(s):

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Collaborator(s):

- Doug Shelmidine; Sheland Farms; 12043 Co. Rt. 79, Adams, NY 13605.
- Gary Berrus; Limestone Ridge Farm; 9373 Delles Road, Lowville, NY 13367.
- Mike Hunter, CCE Educator, Jefferson County
- Joe Lawrence, Lowville Farmers Coop agronomist, Lewis County.

Cooperating Producers:

- Jefferson County: Alfalfa snout beetles were collected along roads.
- Jefferson County: Alfalfa trial harvested in 2013 on land owned by Doug Shelmidine, Sheland Farms in Adams, NY
- Lewis County: Alfalfa trial planted and harvested in 2013 on land owned by Gary Berrus, Limestone Ridge Farm in Lowville, NY.

Background:

Alfalfa snout beetle (ASB), *Otiorhychus ligustica*, is the most destructive insect pest of alfalfa in Northern New York (NNY), and is continuing to spread. Alfalfa snout beetle is currently infesting nine NNY counties and has invaded Canada across the St. Lawrence River. Otherwise, there is no other known infestation of this insect in North America.

This pest causes severe yield and stand losses on alfalfa by larval feeding on alfalfa roots. New infestations are often mistaken for winter injury since the majority of plants die after the last harvest and before spring growth. With other introduced insect pests, two combined strategies have been effectively used to reduce the insect populations to

manageable levels. These strategies are 1) identify and incorporate resistance genes into alfalfa cultivars adapted to NNY (breeding for resistance) and 2) identify and establish in NNY biological control organisms from the native home of ASB.

None of the alfalfa cultivars grown in northern USA during the 1990s appeared to be resistant when grown on a field heavily infested with ASB. In 1998 at Watertown, NY, the perennial *Medicago* core collection and other germplasms were evaluated for resistance/tolerance to root feeding damage by ASB by visually rating individual plants with a score from 1 to 5 (1 = no root damage, 5 = dead plant). The ASB damage score for 173 plant populations ranged from 3.7 to 4. This variability suggested that resistance genes may exist at a low level in a few populations. Therefore, we initiated recurrent selection to increase the level of resistance in the most resistant populations.

Because of the time-consuming and unreliable nature of field screenings, a greenhouse screening method was developed by E. J. Shields and A. Testa with funding from the NNY Agricultural Development Program. With this greenhouse screening method, the ASB population pressure can be controlled by the number of eggs applied uniformly to each flat and by the length of time that the larvae are allowed to feed on the alfalfa roots. Thus, plants with a low level of resistance can be selected and, over many cycles of selection, the frequency of resistance genes can be increased in several alfalfa populations. The first cycle of selection was completed on several plant populations in 2003, and selection has continued at the rate of one cycle per year. Screening an average of 20,000 seedlings annually, we have completed up to eleven cycles of selection in seven alfalfa populations.

Replicated field trials were established in ASB-infested fields in 2008, 2009, 2011 and 2013. This field research has allowed comparison of cycles 0 and cycles 4 to 9 in several alfalfa populations to determine if the breeding efforts translate into differences in forage yield, plant stand, and root damage ratings in farmers' fields where ASB populations exist.

Methods:

Breeding for Alfalfa Snout Beetle Resistance

About 4,000 alfalfa snout beetle adults were collected from NNY in early spring by Tony Testa and his crew for use in the greenhouse screening procedure. Alfalfa seedlings were inoculated with eggs collected from the beetles. About 35 days after inoculation, plants with the least injury were selected, and combined within populations to produce seed for the next cycle of selection. A total of about 16,000 plants were evaluated for resistance in 2013.

Field Evaluation of Alfalfa Snout Beetle Resistance in Alfalfa Populations

In each field trial, the most advanced alfalfa populations in the ASB breeding program are evaluated. This on-going evaluation is needed to identify the most promising populations for cultivar release. On a grower's (Doug Shelmidine) field that is naturally infested with ASB in NNY, a plot trial was established (2011) and harvested for the second production year (2013) to determine selection progress on alfalfa populations

developed after 4 cycles of selection. In the fall, alfalfa plants were dug from the 2011 plot trial and root feeding damage was evaluated. On November 5, 2013, all of the plants in an area of 1.5 feet x 1 foot at each end of plots in two replicates of the 2011 trial were dug. The plants were washed in the field and transported to Ithaca for scoring. Plants were rated from 1 to 5 where 1 was little to no root feeding damage and 5 was severe root feeding damage. In addition, the number of stems per plant and number of plants in the area dug were counted and analyzed. Following digging in the trial from 2 replicates, six replicates of the trial remain intact for harvesting in 2014.

A plot trial was planted in Lowville on May 1, 2013. The cooperator, Gary Berrus, of Limestone Ridge Farm, prepared the field for planting. The alfalfa populations planted in this trial were advanced selections (up to cycle 9, Appendix - Table 1) of seven populations and the corresponding unselected populations (cycle 0). The trial was sprayed for weed and potato leafhopper control, and the first forage growth was cut off on July 18. On September 5, the trial was harvested for yield.

Results:

Yield – 2011 Trial in Adams: In 2012 and 2013, the trial was harvested three times each year (June, July, August). For the August harvest in both 2012 and 2013, yields of the alfalfa populations planted in the trial (entries) were not statistically different from each other (F-test was not significant). Thus the August harvests were not included in the summary yield table and statistics (Table 1).

The curculio resistant population did not show improved yield after 4 cycles of selection for ASB resistance. It is unknown why selection did not improve yields, but the population may be inbred, may be susceptible to diseases, or may not have genetic variation for ASB resistance. Furthermore, selection for resistance to curculio does not at this time seem to be related to resistance to ASB.

The Seedway 9558 population showed significant increases in yield between the unselected base population and cycle 4, and again between cycle 4 and cycle 7. Seedway 9558 cycle 7 is now commercially available as Seedway 9558 SBR. The cycle 8 population was lower in yield than cycle 7, however, only enough synthetic generation 1 seed was available to plant 4 replicates, thus the performance of cycle 7 is not directly comparable to the other populations planted with synthetic generation 2 seed in 8 replicates.

The ASB-Cross population was one of the three populations that had high yield in this trial. Interestingly, ASB-Cross is a cross of Seedway 9558 SBR and Curculio resistant cycle 6. The performance of this population for yield is similar to Seedway 9558 SBR. Guardsman II was also one of the three populations that had high yield, even though it has not been selected for ASB resistance.

Of the remaining populations that were included in this trial from synthetic generation 1 seed and limited number of replicates, the population with resistance to potato leafhoppers is the most promising based on yield.

Table 1: Yields of alfalfa populations on field infested with alfalfa snout beetle.

Sown on May 25, 2011
Alfalfa Snout Beetle Trial - Adams

Alfalfa Population	Number Rep-licates	2012 6-Jun	2012 11-Jul	2012 Total 2 Har.	2013 4-Jun	2013 12-Jul	2013 Total 2 Har.	2 YR Total
		- - - - - tons per acre dry matter - - - - -						
Curculio Resistant	8	2.19	0.89	3.08	2.52	1.50	4.02	7.10
Curculio resistant-Asb4	8	2.12	0.82	2.94	2.42	1.45	3.87	6.81
Seedway 9558-Asb0	8	2.12	0.90	3.01	2.48	1.56	4.04	7.05
Seedway 9558-Asb4	8	2.23	0.94	3.17	2.61	1.56	4.17	7.34
Seedway 9558-Asb7	8	2.34	1.00	3.33	2.69	1.61	4.30	7.63
Seedway 9558-Asb8	4	2.26	0.90	3.15	2.60	1.55	4.15	7.30
ASB-Cross	8	2.36	0.92	3.29	2.60	1.64	4.24	7.53
Guardsman II	8	2.28	1.05	3.32	2.66	1.65	4.31	7.63
MIIIxPrr-Asb7	2	2.15	0.81	2.96	2.47	1.34	3.81	6.76
MIIIxPrr-Asb8	2	1.87	0.80	2.67	2.21	1.44	3.65	6.32
OVR-DisR-Asb7	2	2.26	0.90	3.16	2.36	1.62	3.97	7.13
OVR-DisR-Asb8	2	2.05	0.74	2.79	2.43	1.46	3.89	6.68
PLH-Asb7	4	2.26	1.02	3.28	2.52	1.43	3.94	7.22
	Average	2.19	0.93	3.12	2.55	1.55	4.10	7.22
	F-entries	5.15 **	3.63 **	3.81 **	4.00 **	3.48 **	6.34 **	7.25 **
	CV(%)	6.3	11.3	6.5	4.9	6.6	3.9	3.8
	LSD(.05)	0.14	0.11	0.20	0.13	0.10	0.16	0.28

AsbX: X = number of cycles of selection

Note: 2012 yields were low due to drought.

Root Ratings and other plant characteristics – 2011 Trial in Adams

The alfalfa populations selected for 7 or 8 cycles ranged in percent resistance from 26% to 38%, whereas the original unselected populations ranged from 13% to 17% resistance. Similarly the average severity index (1 is least amount of root damage and 5 is maximum root damage) for 7 or 8 cycles of selection ranged from 2.9 to 3.3, and the original unselected population ranged from 3.4 to 3.7. Thus, selection for resistance to ASB in the greenhouse resulted in alfalfa populations that have improved resistance in fields with high populations of ASB.

In addition to root feeding damage, we documented the number of plants dug from the 1.5 ft^2 plot areas and the number of stems per plant. It was interesting that the number of plants per unit area of plot did not vary significantly among the alfalfa populations. Note that the average number of plants per square foot is 22, so the plant density in the plot trial was very high in the fall of 2013. There was a statistically significant difference among the populations for average number of stems per plant, however this was not

related to ASB resistance; rather it was related to the plant stand, such that areas with lower plant density had more stems per plant. It is of interest to continue measuring above ground plant characteristics so that in the future it may be possible to replace digging plants from plots with other less destructive measurements.

Table 2: Root ratings from field, Fall 2013, Trial planted in Adams in 2011 (n= 4).

Alfalfa Population	Average Severity Index[1]	Percent Resistance	Avg. No. Stems per Plant	Avg. No. Plants per 1.0 sq ft.
		%	#	#
Seedway 9558-Asb8	2.9	38	2.9	22
MIIIxPrr-Asb7	3.0	35	2.2	22
OVR-DisR-Asb7	3.2	29	3.2	16
Seedway 9558-Asb7	3.2	26	2.7	24
PLH-Asb7	3.3	26	4.5	16
Curculio resistant-Asb4	3.3	24	2.8	24
ASB-Cross	3.4	22	2.8	24
Seedway 9558-Asb4	3.5	18	2.6	20
Guardsman II	3.5	17	2.8	22
Curculio Resistant	3.5	17	2.4	22
ASB	3.4	14	2.5	26
Seedway 9558-Asb0	3.7	13	2.6	22
p value	0.070	0.001	0.014	0.429
CV	8.8	44.6	22.1	19.3
LSD.0.05	0.4	10	1.0	8

[1] Average Severity Index: 1= least amount of root damage to 5 = most root damage.

Yield – 2013 Trial in Lowville:
As is common in seeding year alfalfa trial results, differences among the populations were small, ranging in this trial from 1.74 to 2.15 tons per acre dry matter (Appendix, Table 2). Interestingly, the group that was statistically in the top yielding group included all of the Seedway 9558 populations. Also, the base populations on average yielded more than the cycle 9 populations. This may be due to the lower seed quality of the cycle 9 populations. The cycle 9 seed had some damage from seed chalcid whereas the cycle 0 seed did not.

Conclusions/Outcomes/Impacts:

From the results of several field experiments, progress is being accomplished in selection for alfalfa with resistance to ASB. The first cultivar released from this breeding program is Seedway 9558 SBR and this cultivar has moderate resistance to ASB. This level of resistance, in combination with the nematodes that have been released by Dr. Elson Shields' research project, should provide some control against this insect. However, our goal is to continue development of resistant cultivars that have high resistance to ASB.

We believe that higher levels of resistance are achievable and would provide more effective control in the long term.

Outreach:

Alfalfa snout beetle resistance breeding and accomplishments were presented at the Seedsmen's Field Day in July and at the Extension In-Service Meeting in November. Also in NNY, a presentation was made to growers at a meeting in Belleville on March 13, 2013.

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. This work, however, is the first indication that the breeding research might have a significant payoff for the farming community that has been struggling with ASB for all these years. Also, additional field experiments are needed to determine the benefits (higher yield and less root feeding damage) of higher levels of resistance as these plant populations are developed.

Acknowledgments:

CUAES Hatch Funds, NE1010 Multistate Research Funds, Seedway and Allied LLC.

Reports and/or articles in which results of 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 2013 – 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
http://plbrgen.cals.cornell.edu/cals/pbg/programs/departmental/forage/foragetest.cfm

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Photos: See separate file at www.nnyagdev.org, Field Crops, Crop Pests, Alfalfa Snout Beetle

Appendix:

Table 1: Alfalfa Snout Beetle Resistant Experimental Alfalfa Populations for testing in yield trials.

Pop. No.	Background of Advanced ASB resistant germplasm	comparison
1201	Cornell Potato leafhopper experimental	Cycle 0 vs 9
1202	Seedway 9558	Cycle 0 vs 4,7,9
1203	Oneida VR advanced population	Cycle 0 vs 9
1204	Alfalfa Snout Beetle selection from Peck's	Cycle 0 vs 9
1205	Magnum III advanced population	Cycle 0 vs 9
1206	Curculio Resistant selection	Cycle 0 vs 8
1207	ReSelect Saranac population	Cycle 0 vs 9

Table 2: Yield of the alfalfa populations in the seeding year.

2013 ASB Trial - Lowville NY

Sept. 5, 2013 one harvest in seeding year.

Alfalfa Population	Yield 9/5/13 T/A	*=yielded in top group
Seedway 9558 Base	2.05	*
Seedway 9558-Asb4	2.09	*
Seedway 9558 Asb7 = Seedway 9558 SBR	2.11	*
Seedway 9558 Asb9	2.15	*
Oneida VR Base	2.05	*
Oneida VR Asb9	1.95	
PLH Base	1.80	
PLH Asb9	1.81	
MIII Base	2.13	*
MIII Asb9	1.85	
ReSelect Saranac Base	1.74	
ReSelect Saranac Asb9	1.85	
NY9117 Base	2.23	*
NY9117 Asb9	1.95	
Curculio Resistant	2.00	*
Curculio Asb8	1.93	
Average of Base Populations	**2.00**	
Average of Selected Populations (cycle 8 or 9)	**1.93**	
Trial Mean	1.98	
F-test	2.57**	
LSD (0.05)	0.25	
CV (%)	12.9	

First trial forage was cut off on July 18, 2013 and not weighed for yield.