Northern NY Agricultural Development Program
2012 Project Report

Breeding Alfalfa Varieties with Resistance to Alfalfa Snout Beetle

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Collaborator(s):

- Doug Shelmidine; Sheland Farms; 12043 Co. Rt. 79, Adams, NY 13605.
- Chuck Burnett, Seed producer, Nampa, ID
- Mike Hunter, CCE Jefferson County; Joe Lawrence, former CCE Lewis County Educator

Cooperating Producers:

Lewis County: Alfalfa snout beetles were collected along the roads.

Jefferson County: The alfalfa trials harvested in 2012 were on land owned by Doug Shelmidine, Sheland Farms in Adams, 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 varieties 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 varieties 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 several 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 more than 30,000 seedlings annually, we have completed up to ten cycles of selection in several alfalfa populations.

In fall of 2006, an experiment was conducted under controlled greenhouse conditions to determine progress from selection. Averaged across alfalfa populations, root damage visually scored on a 1 (no root damage) to 5 (severe root damage) basis was 3.46 for the base populations (Cycle 0), 3.35 for Cycle 1, 3.23 for Cycle 2, and 3.09 for Cycle 3. One population had a difference of more than a whole scoring unit between Cycles 0 and 3 This trend was significant and suggested that more improvements could be made by further selection. These data were the first indication that progress from selection could be made in increasing resistance to ASB. Since then, we have continued selecting to further increase the resistance levels in our alfalfa populations most adapted to the Northeast USA.

Replicated field trials were established in ASB-infested fields in the spring of 2008, 2009, and 2011. This field research has allowed comparison of Cycles 0 and 4 in three 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. The 2008 trial had poor establishment and was not harvested for yield.

Methods:

Breeding for Alfalfa Snout Beetle Resistance

With Hatch funds from the Cornell University Agricultural Experiment Station, about 4,000 alfalfa snout beetle adults were collected from NNY 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 later interpollinated to produce seed for the next cycle of selection. A total of about 30,000 plants were evaluated.

Field Evaluation of Alfalfa Snout Beetle Resistance in Alfalfa populations

All of this research was accomplished with Hatch funds through the Cornell University Agricultural Experiment Station. On a grower's (Doug Shelmidine) field that is naturally infested with ASB in Northern NY, a plot trial was established in 2009 and harvested for the third production year (2012) to determine selection progress on alfalfa populations in the earlier cycles of selection. Alfalfa plants were dug from the 2009 plot trials in the fall and root feeding damage was scored. On October 25, 2012, about 40 plants were dug from each plot in 7 replicates of the 2009 trial. 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.

On the same farm, a plot trial was established in 2011 to evaluate performance of alfalfa populations more advanced in the breeding program. First production year forage yield was collect in 2012. Two or three more years of data are needed to determine resistance levels, forage yield, and longevity of these plant populations in the field.

Seed Production

NNYADP funds this year were used to produce seed on alfalfa populations for establishing a new field experiment to determine field resistance on our more advanced breeding populations. Seed on plants selected in the most recent cycle of selection was produced at Cornell in indoor cages using bumble bees. This seed was sent to a cooperator in Caldwell, ID, to produce higher quantities of seed on 11 alfalfa populations in field cages. Plants were pollinated by leafcutter bees.

Results:

Breeding for Alfalfa Snout Beetle Resistance

The 10th cycle of recurrent selection was completed on seven alfalfa populations to increase the level of resistance to alfalfa snout beetle (ASB) under controlled greenhouse conditions.

Field Evaluation of Alfalfa Snout Beetle Resistance in Alfalfa populations

In the 2009 trial (Table 1 Appendix) over the three production years, one of the three Cycle 4 populations yielded significantly higher than its corresponding Cycle 0 population (Cycle 4 :13.31 vs. Cycle 0 : 11.70 tons/acre), two of the Cycle 4 populations yielded significantly higher in 2012 (Cycle 4 : 2.28 vs. Cycle 0 : 2.05 tons/acre averaged). Due to the drought in 2012, the yields at harvests 2 and 3 were variable and not representative of the alfalfa populations' performance.

The average root feeding damage score was 3.65, so after four years in the field, the plants were severely damaged by ASB. Although the statistical variability among the alfalfa populations for root feeding damage was not significant, the two populations selected for six cycles of resistance to ASB had the least amount of feeding damage. Thus

higher forage yields were associated with lower levels of ASB larvae feeding damage on the plant roots. In the future, root feeding damaged should be assessed 2 or 3 years after planting, not after 4 years when the extent of damage is severe.

A second trial was planted in Adams in 2011. Yield results from this trial after one production year confirm that the alfalfa populations developed by selecting for resistance to ASB feeding damage for 4 to 7 cycles have higher yield in fields with natural infestations of ASB (Table 2 Appendix). The three populations that had the highest yield were NY1010, NY1003 and NY1011. The NY1010 population is the next cycle of selection beyond NY0907 which was the highest yielding population in the 2009 trial.

Seed Production

Forty to 70 grams of seed were produced in greenhouses at Cornell on seven cycle 8 and 9 alfalfa populations selected for resistance to ASB (Table 1). Our cooperator in ID planted some (5 grams) of this seed and produced 1.4 to 3.2 pounds of seed of the next generation (including some of the original populations before selection). This seed will be used to establish a replicated plot trial next spring to determine progress from selection on more recent selection cycles.

Table 1:

Pop. No.	Pedigree of Advanced ASB resistant germplasm	pounds of seed
1201	(9810C x 9810A)PLHPrrPLHoutPerPrr2AnAsb9	2.2
1202	Seedway 9558(02-16)-Asb9	1.4
1203	[Oneida VR-(AnPrr)(ApPrr) + Oneida VR-AnAp(ApPrr)]ApPrrBwAsb9	2.2
1204	Alfalfa Snout Beetle selections-Asb9	2.1
1205	(Magnum III-AnVw x Prr Evaluation-PrrAn)VwAnPrrAnAsb9	3.0
1206	Curculio Resistant-Asb8	2.1
1207	RS Population-PrrAnFwVwPrrAnVwPrrAnVwPrrAnAsb9	3.2

Note: In addition to the list above, seed also was produced on some of the original, unselected populations for comparison in future field experiments.

Conclusions/Outcomes/Impacts:

Our initial field and greenhouse experiments indicate that progress is being accomplished from selection for alfalfa resistance to ASB. Enough progress has been made to attract interest by the seed industry, resulting in release of our first variety Seedway 9558 SBR. Seed will be available to growers in NNY for spring 2013 planting. 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. Seedway 9558 SBR should have at least moderate levels of resistance. We believe, however, that even 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 three workshops in collaboration with Testa and Shields (raising nematodes). The workshops

were March 6 at Copenhagen, March 14 at Malone, and March 15 at Canton. A poster titled 'Breeding for Resistance to Alfalfa Snout Beetle' was presented at the international conference (North American Alfalfa Improvement Conference) in July at Ithaca. Also, at the Seedsmens Field Day in July and at the Extension In-Service Meeting in November, an update about ASB resistant alfalfa was presented.

Next steps if results suggest continued work is needed in the areas of research, demonstration and/or education.

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/articles in which results of project have already been published.

Poster NAAIC July 10-12 2012. Ithaca NY Breeding for Resistance to Alfalfa Snout Beetle

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Appendices

Table 1: Yields of the 2009 Alfalfa Snout Beetle Alfalfa trial at Doug Shelmidine's Farm

Seeded May 13, 2009

Populations*	Pedigree	Total Season Yields				ASB Root Score		Total Yield as % of Asb0	
		2010 (4 Har.)	2011 (2 Har.)	2012 (1 Har.)	Total	1=best; 5 worst	Rank	Total	2012
		- - - tons per acre dry matter - - -							
Seedway 9558	Seedway 9558 -Asb0	7.03	2.74	1.93	11.70	3.78	7		
NY0703	Seedway 9558-Asb4	7.86	3.24	2.21	13.31	3.73	6	114	114
NY0907	Seedway 9558-Asb6	7.90	3.36	2.24	13.50	3.54	2	115	116
NY9117	ASB selections-Asb0	7.66	3.16	2.17	12.99	3.62	4		
NY0704	ASB selections-Asb4	7.40	3.01	2.34	12.75	3.61	3	98	108
NY0905	ASB selections-Asb6	7.46	3.05	2.19	12.68	3.50	1	98	101
NY0316	Curculio Res.-Asb0	7.35	2.91	2.12	12.39	3.64	5		
NY0710	Curculio Res.-Asb4	7.14	2.97	1.99	12.09	3.84	8	98	94
	Trial Mean	7.47	3.03	2.12	12.63				
	F-test	6.33 **	6.05 **	11.22 **	7.50 **				
	LSD (0.05)	0.35	0.20	0.11	0.58				
	%CV	4.7	6.7	5.1	4.5				
	Efficiency	162%	180%	258%	192%				

Adjusted Means by Block effects, 8 replicates

*Averages (4 replicates) for NY0905 and NY0907 adjusted for block effects:

NY0905 ASB -Asb6	7.46	3.05	2.19	12.68	3.50	1	98	101
NY0907 9558-Asb6	7.90	3.36	2.24	13.50	3.54	2	115	116

In 2011, the trial was harvested 3 times, but yields were variable at the third harvest.

In 2012, the trial was harvested 3 times, but yields were variable at the second and third harvests.

Table 2: Yield results of trial planted at Sheland Farms to test alfalfa snout beetle resistant alfalfa.

Sown on May 25, 2011

Population	Pedigree	6-Jun	11-Jul	Total Season	% of Trial Mean
Seedway 9558	Seedway 9558-Asb0	2.12	0.89	3.01	97
NY0703	Seedway 9558-Asb4	2.23	0.94	3.17	102
NY1010	Seedway 9558-Asb7	2.33	1.00	3.33	107
NY0316	Curculio Resistant	2.22	0.88	3.10	99
NY0710	Curculio resistant-Asb4	2.15	0.84	2.98	96
NY1035	ASB Cross	2.20	0.89	3.09	99
NY1003	PLH-Asb7	2.30	0.95	3.25	104
NY1001 2 reps	MIIIxPrr-Asb7	2.23	0.81	3.05	98
NY1107 2 reps	MIIIxPrr-Asb8	1.96	0.80	2.76	88
NY1011 2 reps	OVR-DisR-Asb7	2.35	0.91	3.25	104
NY1102 2 reps	OVR-DisR-Asb8	2.13	0.74	2.88	92
	Trial Mean	2.19	0.93	3.12	
	F-entries	5.31 **	5.47 **	3.72 **	
	LSD(.05)	0.14	0.10	0.20	
	CV(%)	6.4	10.5	6.5	

Severe drought in 2012, third harvest not reported.