Northern NY Agricultural Development Program
2008-2009 Project Report

Project Title: Breeding Alfalfa Varieties with Resistance to Alfalfa Snout Beetle

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Cooperating Producers: Doug Shelmidine, John Peck and Howard Keefer, Jefferson County

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.

Alfalfa snout beetle was introduced from Europe into the Port of Oswego during the middle to late 1800's in a ship ballast. Alfalfa snout beetle was first discovered as a problem around 1930 after alfalfa was introduced into Oswego County. 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 acceptable alfalfa varieties (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 suggests 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.

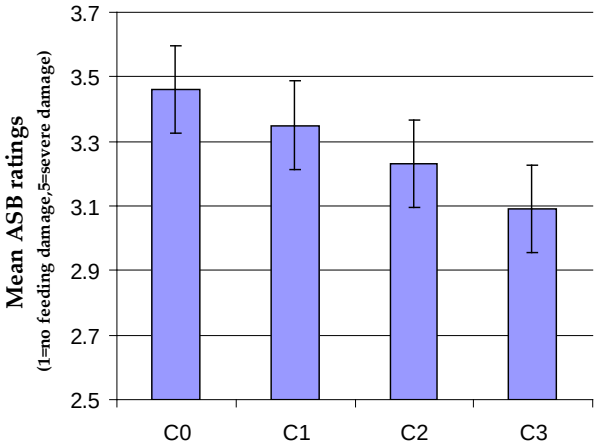
Because of the time-consuming and unreliable nature of field screenings, a greenhouse screening method was developed by E. J. Shields and A. Testa with funding from the NNY Agricultural Development Program. With this greenhouse screening method, the ASB population pressure can be controlled by the number 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 in 11 populations in 2003 and selection has continued at the rate of one cycle per year.

An experiment was completed in the fall of 2006 under controlled greenhouse conditions to determine progress from selection. Significant progress was realized through three cycles of recurrent phenotypic 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, 3.35 for Cycle 1, 3.23 for Cycle 2, and 3.09 for Cycle 3 (see Figure 1 below). One population had a difference of more than a whole scoring unit between Cycles 0 and 3. This trend is significant and suggests that more improvements could be made by further selection. These data are the first indication that progress from selection can be made in increasing resistance to ASB.

Seed was produced in pollination cages in Idaho in 2007 in order to have enough seed of the advanced generation alfalfa selection for use in establishing plot trials in ASB-infested fields during spring 2008 and 2009 at Sheland Farms. This field research will allow comparison of Cycles 0 and 3 (as well as Cycle 5 in the 2009 trial) 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. Because of the unreliable nature of insect infestation in field trials, trials established in two different years are necessary for conclusions about increased resistance.

Figure 1: Progress in breeding alfalfa snout beetle resistant alfalfa – results from a greenhouse evaluation. Root Rating was from 1 (no damage) to 5 (severe damage).



Cycle of selection and Root rating			
CO	C1	C2	C3
3.57	3.13	2.96	2.

Methods:

Breeding for Alfalfa Snout Beetle Resistance

The number of alfalfa snout beetles collected for use in the greenhouse screening procedure was about 10,000 in 2009. Three groups of alfalfa seedings were inoculated with eggs collected from the beetles. Around 35 days after inoculation, plants with the least injury were selected and seed produced for the next cycle of selection. Since 2003, a total of more than 160,000 plants have been screened for resistance to ASB. About 25,000 plants were evaluated in 2009.

Field Evaluation of Alfalfa Snout Beetle Resistance in Alfalfa populations

The trial seeded in 2008 was harvested for yield on June 11th. Since not all of the plots had acceptable plant stands, only 36 of the 108 plots at the trial site were used for the data analysis. The plots included in the yield analysis were the ones with a plant stand of at least 60%.

For the ASB root damage rating, 2580 plants were dug from 36 plots on October 26th. From each of 4 plots per entry, plots were sampled at the two ends of the plots separately. For each of eight replicates, the range of plant numbers dug was from 24 to 61, with an average of 36. Plants were dug and washed at the field site, and then roots were rated on a 1 to 5 scale at Cornell (ASB root damage score system: 1= no root feeding, 5=severe root feeding).

Results:

Breeding for Alfalfa Snout Beetle Resistance

During this past year, we completed the sixth or seventh cycle of selection for resistance in 11 alfalfa populations. In addition, an egg dosage study was completed to determine the best egg dosage rate and optimum time to select plants following inoculation. From this study it was determined that the optimum egg dose and plant selection time, was the same as has been used since 2003.

The average yield of the first harvest was 2.43 tons of dry matter per acre and differences among the alfalfa populations were not statistically significant. The Curculio Resistant Cycle 0 population was the lowest yielding trial entry and this may indicate inbreeding depression.

Two of the three populations evaluated showed improvements in root ratings; i.e., the Cycle 4 or 5 had a lower root damage rating than the Cycle 0. A comparison of Cycle 0 and Cycle 4 or 5 for these two populations (ASB selections and Seedway 9558) was 0.25 or 9% less root damage and was statistically significant a $P<0.05$.

Field Evaluation of Alfalfa Snout Beetle Resistance in Alfalfa populations (Table 1)

Table 1: Yield and alfalfa snout beetle root damage rating of alfalfa populations selected for alfalfa snout beetle resistance – a field trial at Adams, NY .

Population No.	Pop. Description	2009 Yield (6/11)	Yield as % of OVR	Root Damage Rating
		T/A		
NY0316	Curculio Res.Cycle 0	2.28	95	2.87
NY0710	Curculio Res.Cycle 4	2.38	99	3.01
NY9117	ASB selections Cycle 0	2.48	103	2.93
NY0704	ASB selections Cycle 4	2.60	108	2.71
NY0803	ASB selections Cycle 5	2.51	105	2.69
Seedway 9558	Seedway 9558 Cycle 0	2.44	102	2.84
NY0703	Seedway 9558 Cycle 4	2.36	98	2.59
Oneida VR	Check	2.40	100	2.90
Guardsman II	Check	2.46	103	2.93
	Trial Mean	2.43		2.84
	F-test	0.87 ns		1.24 ns
	LSD (.05)	0.29		0.36
	CV(%)	8.2		11.0

Other harvests in 2009 were not taken due to limited road access to trial.

Plants were dug on October 26, 2009. Ratings: 1= no ASB damage, 5=severe damage.

Conclusions/Outcomes/Impacts: From this first, preliminary field trial of ASB resistant alfalfa, two of three populations tested had lower root damage ratings (9% lower) compared to the unselected populations Figure 2, Appendix). From a duplicate trial planted in 2009 that has uniform and acceptable plant stands, yield and ASB root damage rating data will be collected in 2010 to confirm these trial results. However, field results were positive enough that the seed companies we collaborate with did not want to wait for further confirmation of these results. Instead, they wanted the first step in seed production to begin. So this spring we will send seed of our most advanced and most adapted alfalfa experimental populations to Idaho so that the handful of seed we are producing in the Cornell greenhouses can be increased to 2 to 4 pounds of seed for possible variety release.

Outreach: Varieties are not yet available for producers to use on their farms. Outreach for this project included a booth at the 'Corn Silage Management Field Day' on September 10, 2009. Additionally a poster was designed for display at the six NNY County Extension offices. These posters were delivered to the Extension Educators by January 27, 2010.

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

For 'adequate' field resistance, E. Shields and D. Viands agree that the root feeding damage score for an alfalfa snout beetle resistant alfalfa variety ultimately should be in the range of 1.5 to 2.1. The most resistant alfalfa in the 2008 trial had a root damage score of 2.6. Thus selection and breeding work need to continue to get alfalfa with this low root damage score. However, this work is the first indication that the breeding work may have a real payoff for the farming community that has been struggling with alfalfa snout beetle for all these years. Also, field studies will need to begin to determine the best deployment strategy for the combination of resistant varieties and biological control.

Acknowledgments: This project was supported by funding from the NY Farm Viability Institute.

Reports and/or articles in which the results of this project have already been published.

NNY Spring Roundup of Alfalfa Snout Beetle Begins;
Development of Two Control Methods for Crop Pest Progressing
http://www.nnyagdev.org/press-releases/2009/press-04-28-09.htm

Published on May 2, 2009, Page F5, Watertown Daily Times
SNOUT BEETLE ROUNDUP BEGINS

Published on May 19, 2009, Page B1, Watertown Daily Times
ALFALFA STANDS UP TO PEST

Person(s) to contact for more information (including farmers who have participated:

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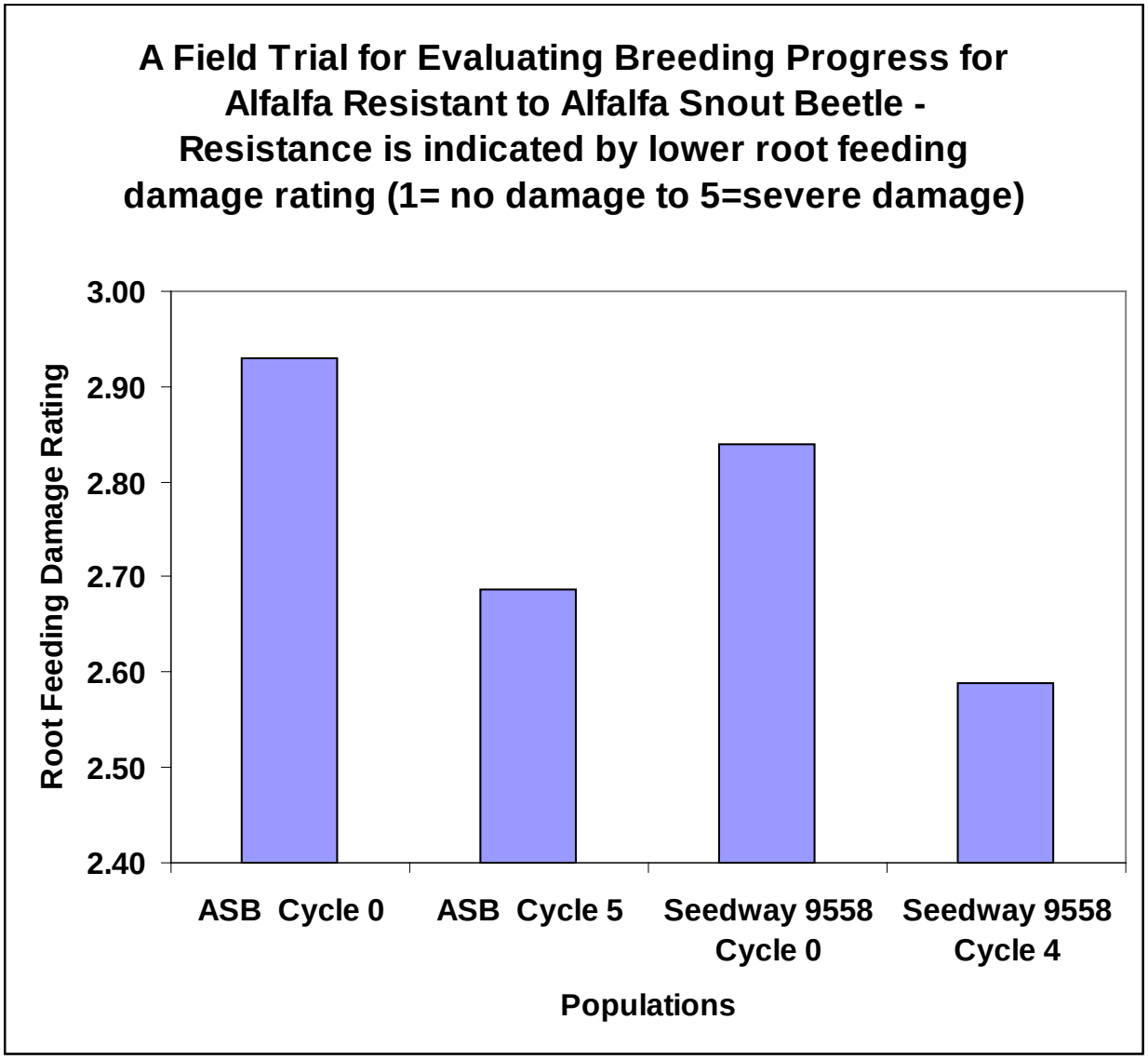

Figure 2: Reduction in root feeding damage on alfalfa from alfalfa snout beetle after 4 or 5 cycles of selection. Field trial was planted in April 2008 and roots were dug and rated in October 2009. Average gain in resistance was 9%.

Photo 1: Seeding alfalfa trial at Doug Shelmidine's farm with experimental alfalfa selected for resistance to alfalfa snout beetle. Photo credit: Jamie Crawford, Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY.

Photo 2: Alfalfa snout beetles collected in Jefferson County. Eggs are collected from the beetles and used to inoculated alfalfa under controlled conditions. Photo credit: Julie Hansen, Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY.