Northern NY Agricultural Development Program
2021 Project Report

Breeding Alfalfa Cultivars with Higher Resistance to Alfalfa Snout Beetle: On-Farm Selection of Surviving Alfalfa Plants

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Background:
Alfalfa snout beetle (snout beetle, ASB), *Otiorhychus ligustica*, is the most destructive insect pest of alfalfa in Northern New York (NNY). This pest causes severe yield and stand losses on alfalfa by larval feeding on alfalfa roots. The Cornell alfalfa breeding program (previously led by D. Viands, now transitioning to Dr. Virginia Moore upon D. Viands retirement), J. Crawford, and J. Hansen, and the Cornell entomology program (led

by Elson J.Shields, Ph.D., and A. Testa, who pioneered the use of native New York nematodes as a biocontrol for ASB) have been cooperating to develop a two-pronged approach to control alfalfa snout beetle on alfalfa:

1) identify and incorporate resistance genes into alfalfa cultivars adapted to northeastern USA (breeding for resistance) and
2) identify and establish biological control organisms for use in NNY.

Both of these insect control strategies are necessary to reduce ASB to sub-economic numbers.

The alfalfa snout beetle feeds on alfalfa, but can also survive on red and white clover, broad-leafed dock, wild carrot, wild strawberries, blackberries, dogwood, other legumes and weeds. In the Czech Republic, alfalfa snout beetles are a serious pest of hop plants.

We have used the greenhouse screening method developed by E. J. Shields and A. Testa, with funding from Hatch and the farmer-driven Northern New York Agricultural Development Program, to identify and select alfalfa plants that appear to be resistant to alfalfa snout beetle. Screening more than 30,000 seedlings annually, we have completed up to 16 cycles of selection in many genetically-unique alfalfa populations.
Other plant breeding strategies for developing alfalfa with resistance to alfalfa snout beetles have been digging surviving plants from trials on alfalfa snout beetle-infested fields in Northern New York to develop new populations, intercrossing resistant populations to capture hybrid vigor, and incorporating alfalfa snout beetle resistance into alfalfa populations with resistance to potato leafhopper.

In previous NNYADP project reports, we showed significant progress developing alfalfa snout beetle-resistant alfalfa in experiments conducted under controlled greenhouse conditions and in field trials on Northern New York farms. From the results of several field experiments, breeding for ASB-resistant alfalfa has resulted in genetic gains. A cultivar developed at Cornell, 'Seedway 9558 SBR', has moderate resistance to alfalfa snout beetle in both greenhouse and field screenings at Sheland Farms near Adams in Northern New York.

We believe that higher levels of resistance are achievable. The goal for this project is to develop one or more alfalfa cultivars with yet-higher resistance to alfalfa snout beetle to help protect the alfalfa crops so valuable to New York's dairy and livestock businesses. Research plans for this project were to select surviving plants from five low-root feeding populations identified at Grace-Way Farm in Lowville, NY. These alfalfa populations are expected to have improved alfalfa snout beetle-resistance and improved yield on alfalfa snout beetle-infested fields.

Methods:

An alfalfa trial at Grace-Way Farm in Lowville was planted in 2017 to evaluate new ASB-resistant populations. Alfalfa plants surviving years of attack by alfalfa snout beetle at Grace-Way Farm have genetic profiles that were important to capture for the alfalfa snout beetle breeding project.

This trial was initially harvested three times in 2018 and three times in 2019. In fall 2019 about 4,000 plants were dug from the field (approximately 60 plants per plot). Results from 2019 indicated partial resistance to alfalfa snout beetle in some alfalfa populations/varieties. Populations did not differ in the percent of plants with little to no alfalfa snout beetle root feeding damage (a score of 1 or 2). Rather, differences were noted in percent of roots with moderate to severe alfalfa snout beetle damage. Alfalfa populations in the Lowville trial were found to be partitioned in to two groups: one group of populations with more plants showing moderate root feeding damage (a score of 3) and another group with more severe root feeding damage (a score of 4 or 5).

Selection of genetically strong alfalfa plants from ASB-infested field in Lowville NY
In 2021, the research trial at Grace-Way Farm was harvested as part of the larger field by the farm in late July. On August 10, research associate Hansen spent the day at the research trial locating the plot outlines and taking notes on plot survival (Table 1). In early August, 2021, the fourth production year, the visual estimate of average-percent-stand-per-plot was 28%. Then, on August 23, four project staff traveled to Lowville and cut 15 to 20 stems from each of 63 plots (3 populations in 9 replicate plots; and 2 populations in 18 replicate plots). The stems from each plot were carefully bundled in moist paper towels with a stake identifying the plot, and placed in a cooler for transport back to Ithaca.

Over two days, the alfalfa stems from each plot were cut into sections (stem cuttings) and planted in greenhouse flats of vermiculite. Plot cuttings were grouped by alfalfa population such that five new alfalfa populations were developed from six of the nine alfalfa populations planted in the trial at Lowville.

Advanced Alfalfa Genetics with Resistance to Alfalfa Snout Beetle
Five new populations were selected at Grace-way Farm in 2021 for field resistance to alfalfa snout beetle. These populations were derived from:

- Seedway 9558 SBR (two seed lots),
- NY 1517 (a cross of Seedway 9558 ASB12 (12 cycles of selection for resistance to alfalfa snout beetle) and leafhopper alfalfa ASB12),
- NY1204 + NY9117 (ASB9 and ASB1 selections from J. Peck Farm, Great Bend, NY,respectively),
- NY1518 (a cross of Guardsman II from Sheland Farms, Adams, NY and Seedway 9558 ASB9 from Lowville, NY), and
- NY1201 (leafhopper alfalfa ASB9).

Table 1: Alfalfa populations in trial planted at Grace-Way Farm in 2017 on a field with high populations of alfalfa snout beetle, percent visual estimate of percent alfalfa stand averaged over 9 replicates, and new alfalfa population number developed from selecting surviving alfalfa plants in August 2021. Breeding Alfalfa Cultivars with Higher Resistance to Alfalfa Snout Beetle, NNYADP project, 2021.

Alfalfa Population Name/Number	% Stand	New Alfalfa Population Number
Seedway 9558 SBR	36	NY2201
NY1517	18	NY2202
NY1204+NY9117	30	NY2203
NY1518	32	NY2204
NY1201	10	NY2205
Seedway 9558	30	*
Guardsman II	42	*
SW 315LH	13	*
Trial Mean	28	
LSD(0.05)	7	
CV(%)	27.1	

*Plants were not selected from these populations.

After two months of growing in the greenhouse, the cuttings were put in individual greenhouse pots and grown to flowering stage. The number of plants in each population averaged around 175 plants. Early in December 2021, a hive of bumblebees was placed in a special room for alfalfa pollination. Then, the five new alfalfa populations were added to this room, one population at a time, so that the plants were interpollinated by the bees. Between pollination groups, the bees were given 24 hours to remove pollen before pollinating the next group. By the end of December, all five populations had been through the bee pollination room twice. At the time of this report, the seed pods that formed on the plants were maturing and the pods waiting to be harvested and processed over the next month or two.

The seed harvested in winter 2022 will be used for a second cycle of seed production such that enough seed will be produced for use in future trials and plant breeding efforts.

Conclusions/Next Steps:

From the results of several field and greenhouse experiments, progress is being made in selection of alfalfa with resistance to alfalfa snout beetle. Future research projects are to

test the most advanced selections of the alfalfa snout beetle-resistant alfalfa in greenhouse evaluations and field trials.

As noted earlier, alfalfa plants surviving years of attack by alfalfa snout beetle at Grace-Way Farm have genetic profiles that were important to capture for the alfalfa snout beetle breeding project. For this successive research, we selected and propagated surviving alfalfa plants from the low-root feeding populations at Grace-Way Farm yield trial in summer 2021. From these plant propagules, five alfalfa populations have been developed for evaluation and further plant breeding. One of these populations or a hybrid cross of these populations may replace Seedway 9558 SBR as an alfalfa snout beetle-resistant cultivar with enhanced resistance and adaptation.

Outreach:

The progress in developing alfalfa snout beetle-resistant alfalfa was shared at:

- July 1, 2021 Seedsmen's Field Day, Ithaca, NY, 60 participants.
- November 15, 2021 Cornell Cooperative Extension In-Service Meeting, Ithaca, NY, about 20 participants*.
- Winter 2022, Annual Meeting with Seedway and Allied Seed LLC, Ithaca, NY.

Summer 2021: Three Cornell University undergraduates participated in this research to develop new alfalfa populations with resistance to alfalfa snout beetle and learn about plant breeding methods.

* Outreach to extension educators extends the value of the research as the extension specialists educate the farmers in their areas. This research also provides extension educators working in areas not yet impacted by ASB with information about how to recognize the impact of ASB feeding which can appear to be winterkill.

Next Steps:

A seed increase of the five alfalfa populations with increased ASB-resistance developed from this project will need to be completed, followed by evaluation in trials in multiple locations.

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

- Yields of Seedway 9558 SBR and other alfalfa populations on fields that are not infested with alfalfa snout beetle: New York Forage Legume and Grass Cultivar Yield Trials Summary for 2021 – Season Totals. J. Hansen, V. Moore, J. Chavez, J. Crawford, J. Schiller, R. Crawford, College of Agriculture and Life Sciences, School of Integrative Plant Science, Plant Breeding and Genetics Section, Cornell University, Ithaca, NY 14853; https://blogs.cornell.edu/varietytrials/forage/

- Northern New York Agricultural Development Program Press Release January 25, 2022. NNY Farms Assist NNYADP Alfalfa Pest Management Research. https://nnyagdev.org/index.php/category/press-releases/

For More Information:

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- Marc Laribee, Grace-way Farm, 9627 State Route 26, Lowville, NY 13367-2939

Photos:

Left: Photo 1: Two plots of alfalfa at Grace-Way Farm, Lowville NY on field with high population of alfalfa snout beetle on August 10, 2022. Surviving alfalfa plants were selected from this trial. Breeding Alfalfa Cultivars with Higher Resistance to Alfalfa Snout Beetle, NNYADP project, 2021.

Right: Photo 2: Example of an alfalfa plant dug from a field with high population of alfalfa snout beetle. In fourth production year, the plant had severe root feeding damage. Breeding Alfalfa Cultivars with Higher Resistance to Alfalfa Snout Beetle, NNYADP project, 2021.

Left: Photo 3: Dr. V. Moore, J. Chavez, and J. Gertin selecting surviving alfalfa plants from a field trial on a field with a high population of alfalfa snout beetle, Lowville, NY. Breeding Alfalfa Cultivars with Higher Resistance to Alfalfa Snout Beetle, NNYADP project, 2021.

Right: Photo 4: Stems of surviving alfalfa plants from a field trial on a field with a high population of alfalfa snout beetle in Lowville, NY. Breeding Alfalfa Cultivars with Higher Resistance to Alfalfa Snout Beetle, NNYADP project, 2021.

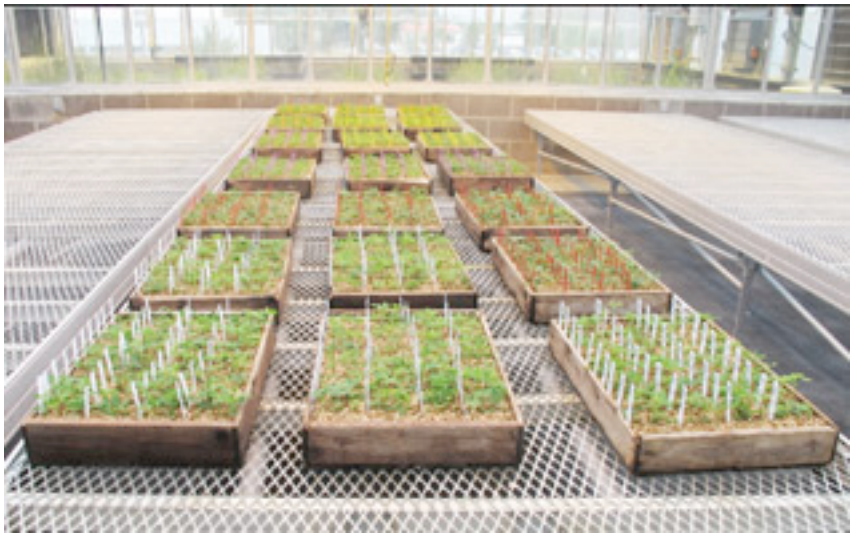

Photo 5: Alfalfa cuttings in the greenhouse from surviving alfalfa plants from a field trial on a field with a high population of alfalfa snout beetle in Lowville, NY. Breeding Alfalfa Cultivars with Higher Resistance to Alfalfa Snout Beetle, NNYADP project, 2021.