Northern New York Agricultural Development Program
2018 Project Final Report

Breeding Alfalfa Varieties with Resistance to Alfalfa Snout Beetle

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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 (led by D.Viands, J. Hansen, and J. Crawford) and the Cornell entomology program (led by 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 into alfalfa varieties adapted to northeastern USA (breeding for resistance and the focus of this grant),
2) and 2) identify and establish in NNY biological control nematodes (Shields and Testa). Both of these insect control strategiesare 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 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.

Other breeding strategies for developing alfalfa with resistance to alfalfa snout beetles have been digging surviving plants from trials on alfalfa snout beetle infested fields 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.

Impacts from past research:
In previous project reports, we showed significant progress developing alfalfa snout beetle-resistant alfalfa in experiments conducted under controlled greenhouse conditions and in field trials. A greenhouse evaluation of alfalfa snout beetle-resistant populations compared to populations not selected for resistance was completed in 2017. The population that is a cross of two populations selected for 12 cycles of resistance to alfalfa snout beetle had the highest percent resistance (44%).

Methods:

Alfalfa Snout Beetle Capture in NNY for Storage in Coolers at Cornell:
On and around May 9, 2018, several thousand alfalfa snout beetles (about 5,500) were collected by Tony Testa and others from the Cornell Forage Breeding Project in the Lowville, NY, area. The beetles were taken back to Ithaca, put in clean petri dishes sealed with parafilm for storage in a cooler, and were put in clean petri dishes every month until the beetles were used for egg production. The beetles were sorted and stored in coolers until needed later in the summer for alfalfa inoculations with alfalfa snout beetle eggs.

Alfalfa Selection for Resistance to Alfalfa Snout Beetles:
Four alfalfa populations for screening for resistance to snout beetle were planted in greenhouses at Cornell the week of May 14, 2018. The seedlings were inoculated with alfalfa snout beetle eggs the week of July 2. After inoculating with eggs, the tubs of alfalfa were left to grow in the greenhouse for another 6 to 8 weeks. In late August, each alfalfa seedling was dug up and roots were washed to evaluate the level of root scarring and feeding 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 pollinating to produce seed, thereby completing another cycle of selection.

Alfalfa Population Evaluation Trial in NNY Field:
In 2018, the alfalfa trial at Lowville (Grace-Way Farm) was harvested for forage yield three times (June 21, July 30, September 13). This trial has nine replicate plots for each of nine trial entries. The yield data collected at each harvest were converted from pounds per plot fresh weight to tons per acre dry matter. For the data from each harvest, the yields were ranked from 1 to 9 where 1 is lowest yield and 9 is highest yield. The ranks over the three harvests for each trial entry were averaged.

The Shields group applied nematodes to three of the nine alfalfa trial replicates on July 11, 2018. Using the multispecies approach, two native NY strains of bio-control entomopathogenic nematodes, (*Steinernema carpocapsae* and *Steinernema feltiae*) were reared, then applied to the replicate numbers 1, 2, and 3. Replicates 4 through 9 remained untreated. Plots were not assayed prior to nematode establishment on July 11, 2018, but experience has shown a low probability of a resident population. Soil cores were collected from the treated plots only to verify a successful inoculation on 60 days post-nematode application. Each sample was removed from the soil and split into upper 2" and 3-6" portions to bioassay for nematode presence. Soil cores were returned to the laboratory and bio-assayed using a standard technique with wax moth larvae as an indicator for the presence of biocontrol nematodes in the sample.

On October 24, plants from three trial entries were dug from all nine replicates to assess alfalfa snout beetle root feeding damage at the end of the first production year of the trial. From the end of each of 27 plots, an area was dug with a shovel so that about 5 to 10 plants were collected. We initially tried to use golf cup cutters to collect the plants, soil and larvae, but this method did not work well because it was difficult to get the cutter into the soil. Plants were dug from the end of the plot that is trimmed off prior to each harvest, so as not to disturb the area of the plot that will be harvested in 2019. Four hundred plants were dug from the 27 plots (3 alfalfa populations x 9 replicates, for about 14 plants per plot). Each plant was rated for root feeding damage in the field. A rating of 1 was given to plants with no root feeding damage, 2 to plants with very minor feeding scars, 3 to plants with moderate damage and deep feeding scars, 4 to plants with severe damage where the taproot was severed and there were many deep feeding scars, and 5 to plants with very severe feeding damage. The number of larvae that were discovered during plant sampling was counted.

Results:

Alfalfa Snout Beetle Capture and Egg Production: Of the more than 5,000 alfalfa snout beetles that were collected in the spring of 2018 in NNY, some were taken out of storage in mid-June and fed alfalfa stem tips. During this eating phase, the beetles started laying eggs. This year, the quantity of eggs collected was adequate for use in greenhouse inoculations. In 2016 and 2017 there were difficulties getting enough eggs for inoculations.

Alfalfa Selection for Resistance to Alfalfa Snout Beetles: From the greenhouse selection protocol in 2018, four alfalfa populations were advanced one more generation

this year. Seed has been produced for these new alfalfa populations for further breeding and testing. Three of the populations are new ones with resistances to both alfalfa snout beetle and potato leafhopper; the other population was produced from surviving plants on farms at Lowville and Adams after being selected for alfalfa snout beetle resistance for four cycles in the greenhouse.

Alfalfa Population Evaluation Trial in NNY Field:
Entomopathogenic nematode application: As shown in Table 1, around 40% of the soil cores taken from the area inoculated with entomopathogenic nematodes tested positive for presence of nematodes. These results are setting the stage for comparison of yield and root damage from field plots with entomopathogenic nematodes and to field plots without nematodes in 2019.

Table 1: Results from nematode application at alfalfa trial sown in 2017 at Grace-Way Farm, Lowville, Lewis County, NY. The alfalfa in replicates 1, 2, and 3 was inoculated with entomopathogenic nematodes on July 11, 2018. Sixty days after inoculation soil cores from replicates 1 and 2 were tested for presence of nematodes.

	% of Soil Cores with Nematodes	
	Average	Standard Error
Replicate #1	43.3	9.2
Replicate #2	40.0	9.1

Yield (Tables 3 and 4, Appendix): Typically differences among alfalfa populations in a trial harvested for the first production year are not statistically significant. For the first production-year alfalfa snout beetle trial at Grace-Way Farm, differences amoung the alfalfa populations were statistically significant at first harvest, but not at the other harvests, or for total over harvests.

The alfalfa populations in the top 50% for high yield included two advance breeding lines NY1517 and NY1518. The areas of the field that had nematodes applied yielded 4.0 tons per acre and the areas that were not inoculated with nematodes yielded 3.7 tons per acre (yields for harvests post-nematode inoculation).

Harvesting this trial in 2019 will be needed for statistically identifying the top yielding group of alfalfa populations and for comparing forage yield of field areas with and without established nematode populations.

Root Feeding Damage and Larvae Counts: For the 385 plants that were dug from the trial in late fall 2018, 4% had no root feeding damage from alfalfa snout beetle, 31% had minor root feeding, 50% had moderately severe root feeding, 15% had severe root feeding and 0% had very severe root feeding.

In past experiments, root feeding damage has been evaluated at the end of the trial (usually end of second production year). For the trial at Grace-Way Farm, the roots were scored at the end of the first production year, so there are no similar results from other

trials for comparison. However, with about one-half of the plants that were rated in the moderately severe root feeding category, this indicates that the alfalfa snout beetle damage will likely impact trial yield in 2019 due to poor plant health and compromised root systems.

In the soil associated with plant samples, six alfalfa snout beetle larvae were discovered. One larvae was in a Seedway 9558 plot, 3 larvae were in three Seedway 9558 SBR plots and two larvae were in two NY1518 plots. The number of larvae discovered in October 2018 was too small to be useful for analyses. It is expected that the number of larvae will be much higher in 2019.

The average number of plants per root feeding score per replicate was computed for the areas treated with nematodes and the untreated areas (Table 2). There were no major differences in the average number of plants in each root feeding score for areas treated with nematode compared to untreated areas. In 2019, the root scoring and analyses will be repeated, once the nematodes have more time to establish and impact the alfalfa snout beetle larvae.

Table 2: Results from nematode application at alfalfa trial sown in 2017 at Grace-Way Farm, Lowville, Lewis County, NY. Alfalfa plants were dug and each plant scored for root feeding damage from none to very severe. The average number of plants per replicate for the field areas treated with nematodes was compared to the field areas not treated with nematodes for each root feeding score class from none to very severe.

	Avg. number of plants per replicate with ASB root feeding damage score from none to very severe				
	None	Minor	Moderate	Severe	Very Severe
Nematode applied	0	11	23	8	0
No Nematodes	2	14	21	6	0

Alfalfa snout beetle root feeding damage was scored on 119 plants from NY1518 (an advanced selection for resistance), on 133 plants from Seedway 9558 SBR (moderately resistant), and on 133 plants from Seedway 9558 (low resistance). Plant roots were scored with visual rating scale of 1: no feeding damage, 2: minor feeding damage, 3: moderate feeding damage, 4: deep feeding wounds or severe damage, and 5: very severe feeding damage. Data from replicate 1 was not included in the analysis because very few plants were rated (used golf hole cutter on replicate 1). Data from replicate 9 was not included in the analysis because there was less root feeding damage at this replicate location compared to the other seven replicates. The percent of plant roots with a score of 1 was averaged with the percent of plants with a score of 2 to compute average percent resistance for each of three trial entries.

The advanced population NY1518 had 38% resistance to alfalfa snout beetle, Seedway 9558 SBR had 32% resistance and Seedway 9558 had 33% resistance. Further root samplings and analyses are needed to compare to these initial results.

Conclusions/Outcomes/Impacts:

From the results of several field and greenhouse experiments, progress is being made in selection of alfalfa with resistance to alfalfa snout beetle. 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, in conventional yield trials and in greenhouse evaluations.

New alfalfa populations that have improved resistance to alfalfa snout beetles are also being tested for other traits of importance to producers such as fall dormancy and disease resistance.

We believe that higher levels of resistance are achievable and would provide more effective control. These research methods and testing procedures should produce one or more new alfalfa varieties with enhanced alfalfa snout beetle resistance. Local New York seed companies are interested in producing and selling seed of a new alfalfa snout beetle-resistant variety for NNY, which indicates the value of alfalfa crops to the regional dairy industry and to agribusiness.

Outreach:

The progress in developing alfalfa snout beetle resistant alfalfa was or will be shared at:

- July 3, 2018 Seedsmen's Field Day, Ithaca, NY, 69 participants.
- July 9, 2018 Dairy Sustainability Interns, Ithaca, NY, 9 participants.
- November 14, 2018 Cornell Cooperative Extension In-Service Meeting, Ithaca, NY, about 15 participants.
- February 20, 2019 Annual Meeting with Seedway and Allied Seed LLC, Ithaca, NY, about 6 participants.
- Summer 2018: Six Cornell University undergraduates participated in the alfalfa snout beetle breeding program and learned about the insect and plant breeding methods.

Outreach is often to extension educators who then can educate the farmers that they work with. An updated factsheet for posting to the web and widely sharing will be published.

Next Steps:

Although significant resistance 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, followed by evaluation, needs to continue to develop alfalfa with lower root damage and higher level of resistance. The alfalfa snout beetle field trial at Grace-Way Farm is set up for second production year data collection on yield, root feeding damage, larval populations, progress from breeding, and nematode application impacts. Also of interest is to investigate the impact of feeding potato leafhopper-resistant alfalfa forage to alfalfa snout beetle adults on egg production and to investigate the egg concentration needed for inoculation of advanced breeding lines as the advanced breeding lines may need higher egg (increased insect pressure) concentrations for selection experiments. Additionally, Seedway 9558 SBR is being tested for resistance to clover root curculio, another serious root feeding insect on alfalfa.

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

- Yields of Seedway 9558 SBR, NY1517 and NY1518 on fields that are not infested with alfalfa snout beetle — New York Forage Legume and Grass Cultivar Yield Trials Summary for 2018 – 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

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Appendix:

Table 3: **Pedigrees of alfalfa populations in 2017 alfalfa trial at Grace-Way Farm, Lowville, NY.**

Alfalfa Populations/Variety	Breeding for Alfalfa Snout Beetle (ASB)	% Resistance to ASB: 2017 Evaluation
NY1518	Guardman II selected 1 cycle crossed to Seedway 9558 selected 9 cycles (average 5 cycles of selection)	36
NY9117	Developed from plants from a 1990 alfalfa snout beetle field near Carthage	26
NY1517	Seedway 9558 selected 12 cycles crossed to leafhopper resistant selected 12 cycles	44
Guardsman II	Not selected for ASB resistance	29
NY1204	NY9117 selected 9 cycles for ASB resistance	37
SW 315LH	Alfalfa cultivar with resistance to potato leafhoppers.	NA
Seedway 9558 SBR	Seedway 9558 selected 7 cycles for ASB resistance	42
Seedway 9558	Not selected for ASB resistance	38
NY1201	Leafhopper resistant selected 9 cycles for ASB resistance	29

% Resistance was evaluated in 2017 Greenhouse trial; NA is not available.

Table 4: Summary of ASB yield trial at Grace-Way Farm, Lowville, NY: yield at each harvest, total season yield, and rank from 9 (highest yield) to 1 (lowest yield).

	2018 Yield							
	21-Jun	21-Jun	30-Jul	30-Jul	Sept. 13	Sept. 13	Total Yield	Average
Trial Entry	T/A	Rank	T/A	Rank	T/A	Rank	T/A	Rank
NY1518 (cross of cycle 1 x cycle 9)	3.22	4	2.06	9	1.91	9	7.19	7.3
NY9117	3.29	5	2.05	8	1.86	5	7.20	6.0
NY1517	3.37	7	1.92	4	1.87	6	7.17	5.7
Guardsman II	3.32	6	2.00	7	1.85	4	7.18	5.7
NY1204	3.18	2	1.93	5	1.88	8	6.99	5.0
SW 315LH	3.52	9	1.92	3	1.82	2	7.26	4.7
Seedway 9558 SBR	3.21	3	1.94	6	1.83	3	6.98	4.0
Seedway 9558	3.16	1	1.88	2	1.88	7	6.93	3.3
NY1201	3.40	8	1.85	1	1.73	1	6.99	3.3

ASB - Alfalfa snout beetle ; Rank: 9 is highest yield and 1 is lowest yield; NA - not available; T/A tons per acre dry matter

Photos

Photo 1, left: Alfalfa snout beetle larvae. These larvae developed from alfalfa snout beetle eggs that inoculated the greenhouse-grown alfalfa plants for the alfalfa snout beetle resistance breeding program.

Photo 2, right: Four experimental alfalfa genetic lines from one greenhouse container in the alfalfa snout beetle breeding program. The four populations show variability in plant size and root feeding damage from alfalfa snout beetle larvae in the greenhouse selection procedure for resistance to alfalfa snout beetle. Photos by J. Hansen

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Photo 3, left: Alfalfa trial at Grace-Way Farm, Lowville, Lewis County, NY. Three of the nine replicate trial ranges had entomopathogenic nematodes applied on July 11, 2018. The nematodes have been shown to attack and kill the alfalfa snout beetle larvae. Photo by E. Shields.

Photo 4, right: Examples of alfalfa roots and root feeding damage by alfalfa snout beetle from an alfalfa trial at Lowville, NY. Left to right: '2's: a plant with minor root feeding damage, '3's: plants with moderately severe root feeding damage, '4's: plants with severe root feeding damage. Photo by J. Hansen

Photo 5, left: A plot of NY 1518, an alfalfa experimental population developed for resistance to alfalfa snout beetle.

Photo 6, right: a sample of plants and roots of NY 1518 from a field with alfalfa snout beetle larvae feeding on the roots (plants on left have no root feeding damage to minor root feeding damage, plants on right have moderately severe to severe root feeding damage. Photos by J. Hansen