NCERA-125 2006 annual report
North Dakota

1) Project title: Control of Yellow toadflax (*Linaria dalmatica*) with *Mecinus janthinus* in North Dakota.

Investigators: Travis Almquist and Rodney G. Lym, Dept. Plant Science, North Dakota State University.

Contact: Rodney G. Lym (Rod.Lym@ndsu.edu)

Project description: An experiment to evaluate the potential of the biological control agent *Mecinus janthinus* to control yellow toadflax (*Linaria dalmatica*) was imitated in Barnes County, North Dakota in June 2005. Approximately 200 adults were released at three locations within a 32 ha area with a dense stand of yellow toadflax. Yellow toadflax density was determined prior to and 12 months following the release of *M. janthinus*. Yellow toadflax density was unchanged and no *M. janthinus* adults were observed one year after release.

2) Project title: Mitochondrial DNA diversity and *Wolbachia* infection in the flea beetle *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) an introduced biocontrol agent for leafy spurge.

Investigators: Richard L. Roehrdanz, Sheila Sears, AB Cortilet, Red River Valley Agricultural Research Center Biosciences Research Laboratory, USDA-ARS; Denise Olson, Gerald Fauske, Dept. of Entomology, North Dakota State University; Rob Bourchier, Environmental Health Program Agriculture and Agri-Food Canada Research Centre.

Contact: Richard L. Roehrdanz (roehrdar@fargo.ars.usda.gov)

Project description: *Aphthona nigriscutis* is one of several species of *Aphthona* flea beetles that have been introduced into North America in an effort to control the weed, leafy spurge (*Euphorbia esula*). It has been a very effective biological control agent at some locations but not at others. Overall genetic diversity is one parameter that could have an effect on *Aphthona* establishment at specific locations. We have examined the genetic diversity of mitochondrial DNA in populations of *A. nigriscutis* from several North American collection sites. The results indicate that the insects are divided into two mtDNA clades. About 78% of the individuals comprise a clade (A) that has little or no mtDNA diversity. The remaining insects in the other clade (B) display extensive diversity with 15 haplotypes observed. The two subpopulations co-exist at most locations. The bacterial endosymbiont *Wolbachia* has been discovered in some individuals. About 86% of the individuals from mtDNA clade A tested positive for *Wolbachia*. Portions of the *Wolbachia* ftsZ and wspA genes were sequenced and the sequences have been shown to fall within the *Wolbachia* Supergroup A. None of the insects from clade B appear to be infected. The association of *Wolbachia* with one, but not both, mtDNA clades of *A. nigriscutis* may play a role in limiting genetic diversity within beetle populations.

3) Project title: Molecular phylogeny and species diversity of *Aphthona* flea beetles introduced to North America.

Investigators: Richard L. Roehrdanz, Sheila Sears, AB Cortilet, Red River Valley Agricultural Research Center Biosciences Research Laboratory, USDA-ARS; Denise Olson, Gerald Fauske, Dept. of Entomology, North Dakota State University; Rob Bourchier, Environmental Health Program Agriculture and Agri-Food Canada Research Centre.

Contact: Richard L. Roehrdanz (roehrdar@fargo.ars.usda.gov)

Project description: Flea beetles of the genus *Aphthona* (Coleoptera: Chrysomelidae) have been imported from Europe and released in the USA and Canada as a biological control agent for the weed, leafy spurge. Long PCR-RFLP (8000+ bp) and nucleotide sequencing (617 bp) of mtDNA were employed to examine both the phylogeny and the intraspecific variation of the North American introductions. A narrow genetic base was observed in some species. A *Wolbachia*-infected mtDNA lineage has been identified in one species, *A. nigriscutis*, and evidence suggestive of cryptic species has been found for another, *A. lacertosa*.

4) Project Title: The importance of groundcover to overwintering survival of *Aphthona* flea beetles (Coleoptera: Chrysomelidae)

Investigators: Ankush Joshi and Denise Olson

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Project Description: *Aphthona* flea beetles have not established or adequately controlled leafy spurge (*Euphorbia esula* L.) in all areas where they have been released in the Northern Great Plains. Effect of groundcover (snow, debris, and snow plus debris), soil temperature, and winter period were evaluated on overwintering *Aphthona* flea beetles during 2001 to 2004 in southeast North Dakota. Winter mortality of the flea beetles was determined by comparing populations before and after each winter by soil samples. Soil temperatures were recorded from first November to first May to correlate winter soil temperature and winter period to spring flea beetle emergence. Spring flea beetle emergence was 52 to 90% lower than the mean population entering the winter. The effect of groundcover on flea beetle emergence was significant during two of these experimental years. When the soil temperature was as low as -4.4˚C, 90% of the *Aphthona* flea beetles emergence were from snow-covered plots. The ranges of soil temperatures and winter periods for overwintering *Aphthona* flea beetles were determined as -5.0 to 4.5˚C and 56 to 132 d, respectively, using linear regression models. A linear relationship between soil temperature and winter period allowed building a model that may help predict the minimum required overwintering period at a given winter soil temperature. These models help to understand the results across the study years. This study provided a better understanding to the overwintering conditions that may limit *Aphthona* spp. establishment or population development in the Northern Great Plains states.

5) Project Title: Post-establishment monitoring and habitat occurrence of *Aphthona* flea beetles and leafy spurge (*Euphorbia esula* L.)

Investigators: Ankush Joshi and Denise Olson

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Project Description: The rate of *Aphthona* spp. establishment and their impact on leafy spurge is variable across habitat types. The population dynamics of *Aphthona* spp. and extent of leafy spurge (*Euphorbia esula* L.) infestations 16 years after initial release of these biological control agents were evaluated at the Katie Olson Wildlife Management Area and a range pasture in southeast North Dakota during 2002 to 2004. High-prairie, mid-prairie, treed, wetland, and thicket habitats were sampled at the sites. *Aphthona* spp. populations in different habitats were determined by soil cores taken in spring of each study year and weekly sweep counts from mid-June to mid-August. Leafy spurge stand densities in habitats were measured in spring of each experimental year. During the three-year study period at the Wildlife Management Area, the *Aphthona* spp. population was almost non-existent in the thicket, treed, and wetland habitats, and <1 beetle/soil core in high- and mid-prairie habitats. Similarly, for leafy spurge <1 stem/0.5 m2 was observed at the wildlife management area. The flea beetle species populations and leafy spurge infestations were of similar levels in the range pasture. *Aphthona lacertosa* constituted >90% of the flea beetle populations across the study habitats at both study sites.

6) Project Title: An integrated approach to sustainable management of leafy spurge (*Euphorbia esula* L.)

Investigators: Ankush Joshi and Denise Olson

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Project Description: Most management tactics used against leafy spurge (*Euphorbia esula* L.) are not economical, practical, or efficacious. Combinations of tactics may, however, provide sustainable and economical control of leafy spurge. Combinations of the biological control agents, *Aphthona* flea beetles, the herbicide imazapic, and native grass species were evaluated against leafy spurge at Sheyenne National Grassland and Albert Ekre Grassland Preserve in southeast North Dakota during 2001 to 2005. Treatment effects on leafy spurge stem counts were measured in the spring, and on root measurements in the fall of each experimental year. Treatment effects on establishment of the flea beetles were determined by taking soil cores in the spring and conducting weekly sweep count in the summer of each study year. At the Sheyenne site, during the five year study period, *Aphthona* spp. population increased to 3.2 beetles/m2 and leafy spurge stem count decreased by 72%. A 90% reduction in leafy spurge stem density was observed one year after herbicide application. One year after this reduction, the

effect of insect was significant on leafy spurge in herbicide plots. This effect continued three years after the herbicide application. Seeding native grass spp. in the leafy spurge infestation did not affect the flea beetle population. At the Ekre site, herbicide application reduced leafy spurge by 98%. However, *Aphthona* spp. establishment was very low (<2 beetle/m2), and it may take one or two additional years to make a conclusion about interseeding native grass species. This study may serve as a model to implement leafy spurge integrated management.

7) Project Title: Comparative Evaluation and Economic Potential of Ecorational versus Chemical Insecticides for Crucifer Flea Beetle (Coleoptera: Chrysomelidae) Management in Canola
Investigators: Frank Antwi and Denise Olson

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Project Description: During 2001 to 2004, field studies were conducted to evaluate the effect of the ecorational insecticides SpinTor® (spinosad), BotaniGard® (*Beauveria bassiana*), Neemix® (azadirachtin) and Surround® (kaolin) against crucifer flea beetle on *Brassica napus* at the cotyledon stage. The ecorational treatments were compared to a standard foliar chemical insecticide, Capture® (bifenthrin), and the chemical seed treatment insecticide, Helix XTra® (thiamethoxam). Flea beetle feeding injury and yields were compared among the treatments. This study indicates that flea beetle injury was significantly lower for Helix XTra, Capture and the ecorational insecticide SpinTor. SpinTor was less effective when flea beetle populations were relatively high (200-300 per trap week). Yields for chemical insecticide treatments were always greater than SpinTor, with differences being the smallest (68-374 kg/ha) at low levels of flea beetle feeding injury. Differences were greatest when canola seedling injury was high (775 to 1364 kg/ha). Yield differences between the conventional insecticides and BotaniGard, Neemix and Surround were 119 to 439 kg/ha and 61 to 2248 kg/ha at low and high flea beetle feeding injury, respectively. Although yield differences between SpinTor and chemical insecticides were relatively small at lower levels of flea beetle injury, net losses ranged from $47 to $151/ha when SpinTor was used as an alternative to a standard chemical seed treatment, Helix XTra. This suggests that SpinTor would not be a viable alternative to the chemical insecticide. Net losses ranged from $30 to $266/ha when BotaniGard, Neemix and Surround were used as alternatives to the seed treatment.

8) Project Title: Spirit Lake Tribe Nation - Leafy Spurge Best Management Practices

Investigators: Denise Olson, Kevin Sedivic, Ankush Joshi

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Project Description: The Spirit Lake Nation Tribe Reservation has encumbered a reduction of productivity and value of their rangelands and natural ecosystems since the

spread of leafy spurge into the reservation during the recent 15 years. The Spirit Lake Nation Tribe has relied on herbicides to control their spurge infestations with minimal reductions and limited economic returns. Best leafy spurge management practices are necessary for the Spirit lake Nation Tribe to preserve the ecological quality of their land, and to increase the productivity and economic returns from their land. The effect of the leafy spurge natural enemies *Aphthona* spp., herbicides, a competitive native grass mixture, and combinations of these treatments were evaluated at three sites of differing habitats during 2004 to 2006 for long term leafy spurge control on the Spirit Lake Nation Tribe lands in northeastern North Dakota.

Treatments plots with insects received a total of 250 adults of a mixed population of flea beetles, *Aphthona lacertosa* and *A. nigricutis*, during late June of the first experimental year. A systemic insecticide, imidacloprid (239 ml/ha) was applied to the non-insect plots to prevent larvae from establishing in the leafy spurge roots. The herbicides 2,4-D or Plateau were applied in mid-October 2004 and 2005 to reduced leafy spurge to 10% or less of its original stand. This percentage is the minimal acceptable leafy spurge density that cattle will graze (Lym 1991; Economic impact, classification, distribution, and ecology of leafy spurge, pp. 169-181. *In* L. F. James, J. O. Evans, M. H. Ralphs, and R. D. Child (eds.), Noxious Range Weeds. Westview Press, Boulder, CO.). The herbicide treatments were applied during the fall to provide maximum leafy spurge control and flea beetle population increase (Nelson et al. 1996; Integration of the flea beetle, *Aphthona nigriscutis*, and herbicide for control of leafy spurge, *Euphorbia esula* L., p. 8. *In* Proceedings, Symposium: Leafy spurge, 13-15 August, Western Soc. of Weed Sci., Brandon, MB, Canada.). A warm season native grass spp. mixture was seeded during early June 2005.

During each study year, beginning with the initial year, we measured the effect of herbicide combined with competitive grass species on *Aphthona* spp. population development, and the effect of an IPM approach, that combines *Aphthona* spp. with herbicide plus native grass species, on the leafy spurge growth pattern. We also measured the impact of the individual treatments on the grasses and other forbs present in the study habitats.

BIOLOGICAL ASSESSMENT

Aphthona flea beetle spp. established in all three habitats and increases in population densities were recorded after beetle releases were made in each habitat. Differences in flea beetle populations were noted among the experimental treatments except for at site one, where insect populations averaged only 0.4 to 2.2 adults/m^2 during 2005 and 2006. Mean seasonal flea beetle counts across sites two and three averaged 15.4 and 7.0 adults/m^2 for the insect and grass only treatments, respectively, and 1.6 and 12.1 adults/m^2 for treatments with Plateau or 2,4-D respectively. The very low flea beetle populations in the Plateau treatments may not be a direct effect of the herbicide, but rather an indirect effect due to unavailability of leafy spurge for flea beetles. At sites two and three, leafy spurge was reduced to less than 15% of the original stand density in the Plateau treatments. Combining flea beetles with Plateau provided little or no additional reduction in leafy spurge compared to herbicide alone. The leafy spurge density increased or only slightly decreased in treatments of grass or insects alone, or in treatments with 2,4-D at site two only. At site three however, these treatments had a substantial reductive effect on leafy spurge. *Aphthona* flea beetles alone reduced leafy spurge to 24% of its original density. Interseeding native grass reduced leafy spurge to

8% of its original density. The herbicide 2,4-5, with or with out grass, provided an 84% to 90% reduction in leafy spurge. When flea beetles were combined with herbicide plus grass, there was a 99% reduction in spurge.

This data indicate that integrated pest management (IPM) using *Aphthona* flea beetles may result in sustainable reduction in leafy spurge, at least in some habitats. More detailed investigation of the data needs to be conducted to ascertain the level of IPM necessary for individual habitat situations. This information will be important to enhancing productivity and economic returns on the Spirit lake Dakota Nation lands.

9) Project Title: Efficacy of ecorational versus chemical insecticides against crucifer flea beetle (Coleoptera: Chrysomelidae) on canola.

Investigators: Frank Antwi and Denise Olson

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Project Description: Studies were done comparing the ecorational insecticides SpinTor®(spinosad), BotaniGard® (*Beauveria bassiana*), Neemix® (azadirachtin) and Surround® (kaolin, a clay) with the chemical insecticides Capture® (bifenthrin) and Helix XTra® (thiamethoxam) all applied at 7 rates in a 7 d assay to assess their impact on adult crucifer flea beetle, *Phyllotreta cruciferae* (Goeze). LT_{25}, LT_{50}, LT_{75} and pairwise comparison test for the insecticide concentration groupings (0, 1X-1.5X and 2X-4X) were estimated for each treatment using survival functions. Flea beetle mortality increased in response to increasing concentrations, especially for Helix XTra, Capture and SpinTor. After 2 d of exposure in trial 1, mortality ranged from 54 to 78% for Helix XTra, 60 to 94% for Capture and 66 to 92% for SpinTor across concentrations. In trial 2, flea beetle mortality ranged from 37 to 60%, 29 to 63% and 23 to 52% on exposure to Helix XTra, Capture and SpinTor, respectively, for 2 d. The LT_{50} values of the insecticides tested showed their efficacy to be in the order of Helix XTra, Capture and SpinTor > BotaniGard, Neemix and Surround. In trial 1, LT_{50}s were 1 d and 2 d for Capture and SpinTor, 2 d for BotaniGard and 3 d for Neemix and Surround. In trial 2, lethal time for 50% mortality occurred between 2 and 4 d following exposure to Helix XTra, Capture and SpinTor, with LT_{50}s of 5 to 7 d on exposure to BotaniGard, Neemix and Surround. SpinTor appears to be a suitable ecorational insecticide in canola for crucifer flea beetle management.

10) Project Title: Integrated Control of Sugarbeet Root Maggot by using Entomopathogens and Cultural Tools.

Investigators: Ayanava Majumdar, Mark A. Boetel, Dept. of Entomology, North Dakota State University

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Project Description: Sugarbeet root maggot is the most damaging insect pest of sugarbeet in the Red River Valley (RRV). Sugarbeet growers rely heavily on a few

chemical insecticides that are available to control this insect pest. The objective of this program is to provide an environmentally friendly and sustainable alternative to chemical control methods by integrating an insect-pathogenic fungus, *Metarhizium anisopliae* (application rate = 3.23×10^{12} viable conidia/acre) with oat or rye cover crops. In field trials at multiple locations and insect infestation levels, the fungus as stand-alone treatment did not provide consistent SBRM control, most likely due to poor post-application persistence. Cover crops seemed to reduce inconsistencies in root protection provided by the insect pathogen Under heavy SBRM infestation levels, rye plus *M. anisopliae*, applied either as planting-time granules or postemergence spray improved root protection (root injury = 3.2 on a 0 to 9 scale) compared to any of the stand-alone control measures (root injury = 5.02). The improvement in recoverable sucrose yield was 1,082 kg/ha by integrating rye and insect pathogen compared to a stand-alone rye treatment. A new pathogen (*Fusarium solani*) was discovered as infecting SBRM pupae. The fungus has been tested for virulence to SBRM, and a pure culture has been deposited to the U.S. Department of Agriculture-Agricultural Research Service Collection of Entomopathogenic Fungal Culture (ARSEF, Ithaca, NY) as isolate ARSEF 7382. A dose of 1.8×10^6 conidia/ml of ARSEF 7382 is sufficient to cause 50% mortality of SBRM pupae. Further research is aimed at continued evaluation of cover crop-*Metarhizium* integrations and virulence testing of ARSEF 7382. Findings have been published in “Sugarbeet Research and Extension Reports”, an NDSU Extension Service publication. Also, one refereed journal article has been submitted.

North Dakota 2005-06 Publications

Antwi, F.B., D.L. Olson, and J.J. Knodel. In Press. Field evaluation of Ecorational versus chemical insecticides for crucifer flea beetle (Coleoptera: Chrysomelidae) in canola. Journal of Economic Entomology

Majumdar, A., M. A. Boetel, S. T. Jaronski, R. J. Dregseth, and A. J. Schroeder. 2006. Cereal cover crops and a microbial insecticide for integrated control of the sugarbeet root maggot (Diptera: Ulidiidae). Sugarbeet Research and Extension Reports. North Dakota State University Coop. Ext. Serv. 36: 222-227.

Majumdar, A., M. A. Boetel, and R. J. Dregseth. 2006. Survey, isolation, and screening for native pathogens of sugarbeet root maggot (Diptera: Ulidiidae) in the Red River Valley. Sugarbeet Research and Extension Reports. North Dakota State University Coop. Ext. Serv. 36: 228-231.

Olson, D. and R. Hanson. 2006. Biological of leafy spurge biological control agents. *In* Biology and biological control of leafy spurge. Forest Health Technology Enterprise Team. USDA Forest Servide.

Roehrdanz, R., D. Olson, R. Bourchier, S. Sears, A. Cortilet, and G. Fauske. 2006. Mitochondrial DNA diversity and *Wolbachia* infection in the flea beetle *Aphthona nigriscutis* (Coleoptera: Chrysomelidae) an introduced biocontrol agent for leafy spurge. Biological Control 37:1-8.