June 30, 2006

LCMR Final Work Program Report

I. PROJECT TITLE: Biological Control of European Buckthorn and Spotted Knapweed.

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Total Biennial LCMR Project Budget: **LCMR Appropriation:** **$ 89,000**
Total Spent: **$ 89,000**
*****Refer to budget on Attachment A.

Legal Citation: ML 2003, Chap. 128, Art. 1, Sec. 09, Subd. 05i 2.

Appropriation Language:
5 (i) Biological Control of European Buckthorn and Spotted Knapweed
$99,000 the first year and $99,000 the second year are from the trust fund. Of this amount, $54,000 the first year and $55,000 the second year are to the commissioner of natural resources for research to evaluate potential insects for biological control of invasive European buckthorn species. $45,000 the first year and $44,000 the second year are to the commissioner of agriculture to assess the effectiveness of spotted knapweed biological control agents. This appropriation is available until June 30, 2006, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.

II. & III. FINAL PROJECT SUMMARY

Spotted knapweed is an exotic-invasive terrestrial plant that threatens the state's roadside, rangeland, agricultural, and grassland/prairie ecosystems. Minnesota land owners/managers are searching for less expensive and more environmentally compatible alternatives to herbicide use for management and control of this invasive weed. The United States Department of Agriculture (USDA) released eleven biocontrol agent species in the state from 1989 through 2000 to manage this weed. Eventually biological control releases became the responsibility of the Minnesota Department of Agriculture (MDA) in 2000. Prior to the MDA inheriting the program from the USDA, few attempts had been made to assess the establishment and success of agents throughout the state to determine if biological control was a viable pest management strategy for spotted knapweed. This research grant was developed in an attempt to increase our knowledge of spotted knapweed biological control in Minnesota and to evaluate the impacts that bioagents have on this highly invasive weed.

Through this research, it was determined that six of the eleven bioagents released in Minnesota, *Urophora affinis*, *Urophora quadrifasciata*, *Larinus minutus*, *Larinus obtusus*, *Cyphocleonus achates*, and *Agapeta zoegana*, are established, have impacted the growth and spread of spotted knapweed on several sites, and are collectable for redistribution to new infestations in the state. Rigorous sampling of selected biological control sites has also provided the MDA with important information pertaining to the extent of spotted knapweed infestations, composition of other vegetation on infested sites, and various landscape, soil, and geographical parameters related to sites in Minnesota.

This research has showed us that biological control can be an important tool for spotted knapweed management in Minnesota. It's not the only tool, but it has the potential to have long-term and sustaining impacts on large infestations where herbicides and other IPM tactics are not practical, expensive, or ecologically unsound. Through this LCMR grant, the MDA has dramatically increased its knowledge of spotted knapweed in the state and the possibilities for extensive biological control management in the future.

IV. OUTLINE OF PROJECT RESULTS:

Result 1: Characterization of spotted knapweed biological control sites and assessment of the establishment and distribution of released knapweed agents in the state.

Description:

Activity 1) All data pertaining to APHIS field insectary sites for spotted knapweed biological control in Minnesota was acquired from the Twin Cities USDA-APHIS, PPQ office. Data was entered into the MDA's Weed Biological Control Geodatabase and each site location was mapped and given a site ID number. Detailed maps were generated for each site using ESRI® ArcMap® and DeLorme X Map®. These maps allowed us to locate biological control field insectary sites to complete the research outlined in this work plan.

Activity 2) Field insectary sites were visited during the 2004 growing season and specific information regarding biological, ecological, and physical characteristics was recorded. The maps created in Activity 1 were used as a computerized data collection system and were programmed on an iPAQ Pocket PC with GPS capabilities. At each study location, the site was characterized according to topographical information, elevation, etc., and information such as ownership was recorded. The knapweed infestation at each site was delineated and acreage recorded. Following site characterization, insect sweep sampling and seedhead collections were conducted to determine the establishment and density of biological control agents known to be released at each site.

Results from these activities allowed us to characterize sites based on ecological and biological parameters. These results also allowed us to select sites with adequate biological control agents to complete the more rigorous sampling activities planned for Result 2.

Summary Budget Information for Result 1: **LCMR Budget: $ <u>41,365</u>**
Total Spent: $ <u>41,365</u>

***Refer to budget on Attachment A.

Final Report Summary: June 30, 2006

<u>Result 1, Activity 1</u>
In early 2004, all of the Minnesota spotted knapweed biological control data was obtained from the Twin Cities USDA-APHIS, PPQ office. The data included records of releases and monitoring of field insectary sites throughout Minnesota before 2000. The data was combined with MDA's spotted knapweed data from 2000 to 2004 and entered in the MDA's Weed Biological Control Geodatabase (WBCG). Release points were mapped using ArcView® software (Attachments B1 & B2). The maps created were used to locate each site and select sites for further research. All spotted knapweed biological control data can now be obtained through the internet via an ArcIMS site developed by the MDA and is synchronized with the WBCG. Data can be viewed online at: http://www.mda.state.mn.us/weedcontrol/mapping.htm

Between 1991 and 2004, USDA and MDA data showed that there were 322 insect biological control agent releases made on a total of 127 sites infested with spotted knapweed in Minnesota. Release records showed that over 790,000 individual bioagents have been released in the state since 1991. These biological control agents consisted primarily of root-feeding, stem-feeding, and seed-feeding insects (Table 1).

Table 1. Bioagent Species and Releases in Minnesota, 1991-2004

Biocontrol Agent	Order	Family	Method of Damage to SK	Released
Agapeta zoegana	Lepidoptera	Cochylidae	Root Borer (L)	1,717
Bangasternus fausti	Coleoptera	Curculionidae	Leaves/Rosettes (A), Seedhead (A/L)	2,975
Chaetorellia acrolophi	Diptera	Tephritidae	Seedhead (A/L)	1,340
Cyphocleonus achates	Coleoptera	Curculionidae	Leaves/Rosettes (A), Root Borer (L)	3,745
Larinus minutus	Coleoptera	Curculionidae	Leaves/Rosettes (A), Seedhead (A/L)	61,646
Larinus obtusus	Coleoptera	Curculionidae	Leaves/Rosettes (A), Seedhead (A/L)	3,519
Metzneria paucipunctella	Lepidoptera	Gelechiidae	Seedhead (A/L)	5,470
Sphenoptera jugoslavica	Coleoptera	Buprestidae	Leaves/Rosettes (A), Root Borer (L)	100
Terellia virens	Diptera	Tephritidae	Seedhead (A/L)	600
Urophora affinis	Diptera	Tephritidae	Seedhead (A/L)	108,550
Urophora quadrifasciata	Diptera	Tephritidae	Seedhead (A/L)	41,400
*Urophora spp. mix**	Diptera	Tephritidae	Seedhead (A/L)	565,663
			(A)=Adult Stage	
			(L)=Larval Stage	
			Totals:	796,725

*Release consisting of mixtures of *U. affinis* and *U. quadrifasciata*.

Result 1, Activity 2

Mobile Data Collection/Mapping System

Following data entry and map creation of the USDA spotted knapweed release sites, a unique computerized data collection system was created and programmed by the MDA Weed Biological Control Program on an iPAQ Pocket PC with wireless GPS capabilities. The computerized system consists of a touch screen data form, GPS unit, and GIS capabilities all in one handheld unit. The Hewlett Packard iPAQ Pocket PC uses Bluetooth wireless technology to communicate with a HOLUX GR-230 Bluetooth GPS receiver. ArcPad 6.0 (the mobile component to ArcView) was used on the iPAQ to visually display the field inspector's location on a map and associate a GPS point location with a data collection form. USGS Digital Orthophoto Quads (DOQ) of each release point were downloaded to the iPAQ for use with the ArcPad site data at each sampling point. This allowed the inspector to not only see the site attributes, but also have an aerial view of the site where data was being collected. Other appropriate layers, county boundaries, roads, and lakes, were added to ArcPad to view while navigating in the field.

This paperless method of data collection eliminated several steps of entering data from the field into a computer database. It also saved hours of time and was very accurate. Furthermore, georeferenced data collected in the field could be immediately synchronized with the MDA's WBCG. The sampling of all biological control release sites began at the end of June and was completed in late August of 2004. When a biological control release site was located in the field using the iPAQ, the field inspector activated the GPS unit and created a waypoint which initiated the opening of data collection forms to complete. The final number of biological control release sites identified was 127. Of these, 103 sites were sampled using the form described above and 24 sites were not sampled due to mowing, unknown location, lack of access, or lack of permission.

Biological Control Agent Recovery and Site Classification

In order to better understand the enormous amount of data that was collected at 103 sites, the data was broken down and analyzed by species of agents recovered. Some challenges existed with analyzing the data because of the huge variability among sites. Some species were found at sites where they were not originally released, meaning the released agents expanded their range or were released by the USDA and not recorded. Table 2 depicts the number of known release sites for each biocontrol agent in Minnesota and also includes the number of sites where recoveries of released agents were obtained at these sites through sampling in 2004. Additionally, the number of sites where agents were not recorded by the USDA to have been released, but were recovered during this activity are also shown. Figure 1, shows a geographical representation of recovered spotted knapweed biological control agent locations throughout Minnesota.

Table 2. Summary of release sites and recoveries for spotted knapweed biological control agents in Minnesota.

Spotted Knapweed Biocontrol Agent	# Release Sites (Pre 2004)	# Recovery sites (2004)	Percent Recovery	# New Recovery sites (2004)
Urophora quadrifasciata (seedhead fly)	33	25	76%	32
Urophora affinis (seedhead fly)	32	18	56%	38
Larinus minutus (seedhead weevil)	66	49	74%	12
Larinus obtusus (seedhead weevil)	7	4	57%	6
Cyphocleonus achates (root-boring weevil)	26	7	27%	1
Agapeta zoegana (root-boring moth)	10	1	10%	0
Metzneria paucipunctella (seedhead moth)	3	0	0%	0
Terellia virens (seedhead fly)	2	0	0%	0
Chaetorellia acrolophi (seedhead fly)	5	0	0%	0
Bangasternus fausti (seedhead weevil)	5	0	0%	0
Sphenoptera jugoslavica (root-feeding beetle)	1	0	0%	0

Overall, six of the eleven bioagent species released in Minnesota were recovered during this activity (Table 2, Figure 1). Approximately 17 of the 103 bioagent release sites sampled (16.5%) were found to have no biological control agents existing on them. Furthermore, five of the bioagents released, *Metzneria paucipunctella, Terellia virens*, *Chaetorellia acrolophi*, *Bangasternus fausti*, and *Sphenoptera jugoslavica,* were not recovered on any of the 103 release sites. We have no clear explanation for the apparent failure of these agents to establish in Minnesota. One possibility to consider would be that some of these agents were established at sites in the state, but exist at such low numbers that our sampling methodology was not able to detect them. We used sweep netting procedures as a sampling method to recover insect biological control agents at all sites. This sampling methodology for insects is widely used in entomological research. Because of the large geographical area that was sampled for this activity and the timing of insect emergence versus timing of sampling, some insect species may actually exist on sites that were sampled but were not collected in our sweeps. However, our sampling methodology was consistent among the 103 release sites and we feel that even if our sampling excluded some agents at particular sites, our data is a good overall representation of agent establishment per site throughout Minnesota.

Two seedhead fly species, *Urophora affinis* and *Urophora quadrifaciata*, and two seedhead weevil species, *Larinus minutus* and *Larinus obtusus*, had the highest percent recoveries of all biocontrol agents released in Minnesota (Table 2). These species were also recovered more frequently at new sites where they were never recorded to have been released, showing a strong tendency and ability to travel from their initial release site to new spotted knapweed infestations. This is considered to be a desirable trait for a biocontrol agent. In fact, both seedhead fly species were found at as many new sites or more than the number of initial sites they were released onto by the USDA (Table 2). Seedhead weevils were found on new sites but not as frequently as the seedhead flies. This may be attributed to the design of both *Larinus* species being so similar that they may have been misidentified in original releases and recorded as such. Flies, in general, can move greater distances than weevils due to the nature of their wings. Both seedhead weevil species have the ability to perform short “burst-like” flying, but usually only exhibit this behavior when disturbed. Seedhead flies in contrast, are able to travel longer distances due to the fact that they fly between destinations. Weevils primarily walk or crawl.

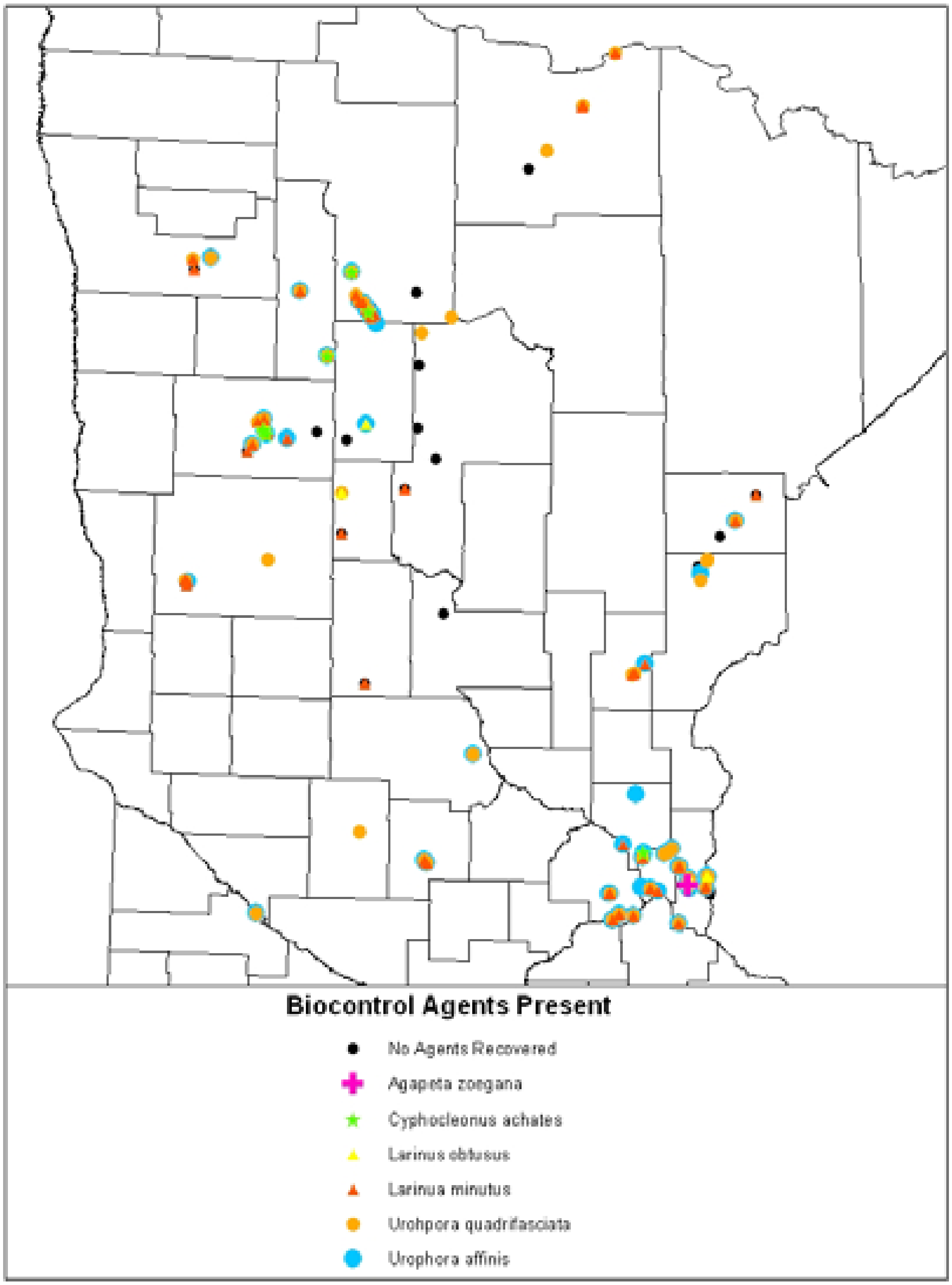

Figure 1. Locations of recovered biocontrol agents in Minnesota at the 103 sites sampled.

Two root feeding agents, *Cyphocleonus achates* and *Agapeta zoegana*, were released on a smaller number of sites statewide than both seedhead flies and weevils and also had a smaller percentage recoveries (Table 2). *Cyphocleonus* was also found at one new site where it was not released, showing some ability to travel between spotted knapweed infestations. Lower numbers of root feeding weevils and moths were released per site than all other spotted knapweed agents. This was partially a result of the difficulty in obtaining these agents from

USDA field insectaries in Montana and Wyoming where most of Minnesota's populations originated from and could also be the reason for their low percentage recoveries at release sites in the state. However, we are not able to determine this with absolute certainty from our data.

Analysis of variance procedures (Statistica Stat Software, 1999) were used to compare the means of agents recovered in our samples against specific variables collected at each release site: 1) topography, 2) land use, 3) elevation, 4) the area of knapweed infestation, and 5) general soil descriptions. These comparisons were performed to attempt to classify biological control sites for future use in the MDA Spotted Knapweed Biological Control Program. In each analysis, variability among sites was enormous due to the uniqueness of each individual site and the parameters being analyzed. Therefore, large significant differences were found among sites and data could only be analyzed within sites. Because of this, sites had to be classified individually and few common trends were found among the 103 sites sampled to discuss in this report. However, the site classification data obtained through this research will be used in the future by the MDA Spotted Knapweed Biological Control Program for determining potential sites to target for annual biological control agent collections and redistributions throughout the state.

Result 2: Research and analysis of the effects that individual biological control agents are having on spotted knapweed growth and spread.

Description:

Ten currently undisturbed sites with high recoveries of biocontrol agents in 2004 were chosen for research in 2005 and were mapped using ArcView software. The digitized maps were used to create template maps for our mobile, computerized data collection system programmed onto an iPAQ Pocket PC with GPS capabilities. The iPAQ was used in the field to locate each site's delineation from Result 1 activities and to find pre-determined random sampling point coordinates at each site. The mobile system was also programmed with data collection forms to efficiently suit the needs of this project.

Random sampling points generated at each site represented starting points for sampling transects. Transects included a vegetation sample before and after a sweep net sampling to collect biocontrol insects. The number of sampling points per site was relative to the scale of the knapweed-infested area. The sites were sampled three times throughout the growing season (late-June and early-July, late-July and early August, late-August and early-September). Vegetation and biocontrol agent sampling at these sites in 2005 determined insect abundance, knapweed density, and composition of other ground cover at the sites. Statistical analysis was performed on all collected data in order to determine the establishment of bioagents and to look for trends among sites pertaining to spotted knapweed density and the existing plant community. Data from both Results 1 and 2 are currently being used to define future management decisions and best management practices for spotted knapweed in Minnesota.

Summary Budget Information for Result 2: **LCMR Budget: $ 47,635**
Total Spent: $ 47,635
***Refer to budget on Attachment A.**

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RESULT 2:

The following summary highlights several trends that we were able to describe among all 10 study sites (Refer to site maps in Attachments C1 & C2).

Spotted Knapweed Bioagent Recoveries

2005 sampling led to the recovery of the same six species of bioagents that were recovered in the 2004/Result 1 field season (Table 1). Two seedhead fly species, *Urophora affinis* and *Urophora quadrifaciata*, and two seedhead weevil species, *Larinus minutus* and *Larinus obtusus*, had the highest recoveries in 2005. By far, the most abundant species recovered was *U. affinis*. Only one *Agapeta zoegana* and a total of 36 *Cyphocleonus achates* were recovered in the 2005 sweep samples (Table 3). In general, seedhead weevils and flies tended to be relatively abundant at sites and were easy to sample with sweep nets. *Agapeta zoegana* and *C. achates* tend to be more difficult to collect using sweep nets, which could be a cause for lower sample recoveries for these two bioagents.

Table 3. 2005 Bioagent Totals Recovered Per Site at 10 Spotted Knapweed Biological Control Sites in Minnesota.

Site Name	Acres	%SK	Uro qua	Uro aff	Lar min	Lar obt	Cyp ach	Aga zoe
AHATS	6.29	19.8	104	957	0	0	0	0
County Pit	9.64	21.8	36	337	91	0	16	0
Gruss	10.47	30.4	8	109	351	0	2	0
Hubble Pond WMA	15.79	21.2	144	198	0	280	0	0
Knutson	11.93	18.4	57	172	86	94	2	0
Lake Elmo Maintenance	9.62	18.2	85	366	63	211	0	0
Lake Elmo P	0.42	13.5	26	74	81	46	0	0
MN Valley NWR-Old Cedar	0.46	17.7	2	0	116	0	0	0
St. Croix SNA	28.96	9.3	31	135	10	3	0	0
Tamarac NWR-Job Corps	13.89	17.6	64	24	303	0	16	1
		Totals	557	2372	1101	634	36	1

Regardless of numbers collected per sample, having recovered all six of the above spotted knapweed bioagent species two years in a row, in addition to recoveries that have been made by USDA and MDA in routine monitoring and collections in prior years which MDA now has on record, establishment of these species in Minnesota can now be assumed. This was one of the greatest results obtained from this research grant. Prior to developing this study, the MDA Spotted Knapweed Biological Control Program had little knowledge of the extent or locations of spotted knapweed bioagent releases in Minnesota. Therefore, there was little basis to promote spotted knapweed biological control in Minnesota other than what results had been witnessed in the western Great Plains states.

Spotted Knapweed Bioagent Peak Emergence Times

In general, the total number of bioagents recovered in 2005 was greatest early in the summer (6/21/05-7/6/05) and fell off as the sampling season ended (8/24/05-9/12/05). Our data shows that sampling at different times over the field season results in differing recovery numbers for certain agents. For example, *Urophora affinis* was the most abundant agent recovered in the first sample round (Table 4). Following the first sample round, collected numbers of *U. affinis* decrease dramatically. *Urophora quadrifaciata* was most abundant during the second sampling period, but overall was collected at much lower numbers in all sampling periods than *U. affinis*. Both *Larinus* species had fairly steady recoveries throughout the season, with the best numbers being recovered in the first sample round. Although our data shows that both *Larinus* species can be collected throughout the entire season successfully, sampling in late June or early July appears to be the prime time to collect (Table 4). *Cyphocleonus achates* and *A. zoegana* were only recovered in the second sample period (7/25-8/10), and appear to be more susceptible to collecting in late July or early August (Table 4).

Table 4. 2005 Biocontrol Agents Recovered at Spotted Knapweed Sites in Minnesota During Three Sample Periods

2005 Totals	Uro qua	Uro aff	Lar min	Lar obt	Cyp ach	Aga zoe	TOTAL
1st Sample Period (6/21/05-7/6/05)	191	2327	422	292	0	0	3232
2nd Sample Period (7/25/05-8/10/05)	356	34	333	173	35	1	932
3rd Sample Period (8/24/05-9/12/05)	10	11	346	172	1	0	540
All Sample Periods Combined	557	2372	1101	637	36	1	4704

Timing of agent recovery is important for any biological control implementation program. Through our data analysis, we have constructed a peak emergence graph that highlights when a species is most active and when it will be most collectable for redistribution to other spotted knapweed infestations in the state (Figure 2). This information will be extremely beneficial for the MDA Spotted Knapweed Biological Control Program, in terms of coordinating bioagent collections with the vast number of cooperators participating in this statewide program. Prior to this LCMR grant, we had little knowledge of when species were the most collectable at a site. Now that we have this information, we can use our database to plan specific biological control events well in advance of each field season.

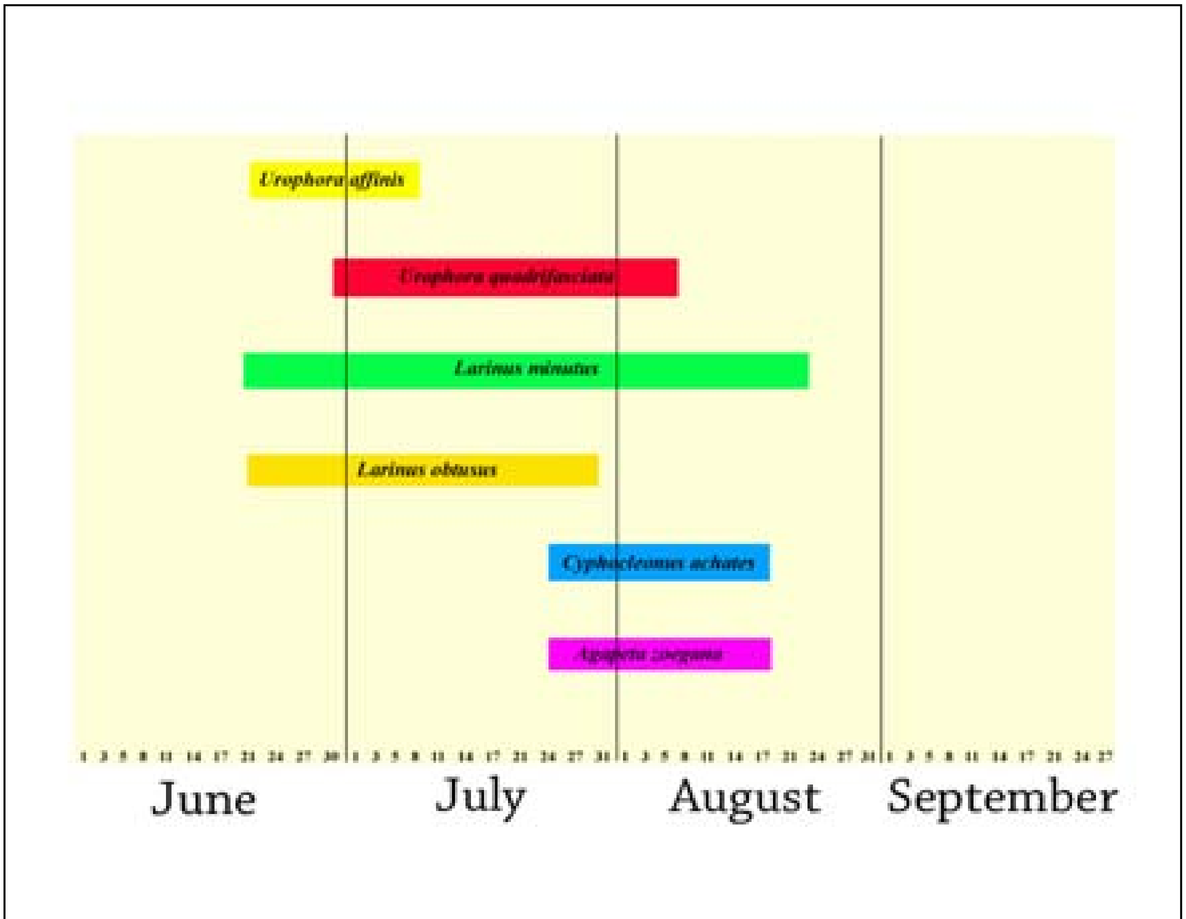


Figure 2. Primary collection times for six spotted knapweed agents in Minnesota.

<u>**New Bioagent Harvest Sites Identified**</u>

Another huge benefit derived through this study was the number of new bioagent collection sites that were discovered and previously not known to the MDA. After sampling all 103 sites in 2004 (Result 1), five sites were found to have extremely large populations of seedhead and root-feeding weevils existing on them. All five of these sites were chosen for Result 2/2005 sampling and included the Beltrami County Pit, Gruss, Tamarack Job Corps, and Lake Elmo Maintenance sites (Table 3). Both seedhead and root-feeding weevils are the preferred mixture of bioagents sought after by the MDA for new spotted knapweed biological control releases because together they have been shown to have the quickest and longest lasting impacts on spotted knapweed plants in northwestern Minnesota and the western United States (Figure 3). Prior to having the knowledge of these five new sites, the MDA had knowledge of only two field collection sites in the state where agents could be obtained for new biological control releases. This increase in the number of field collection sites has allowed the MDA to more than double its redistribution of seedhead and root-feeding weevils to new spotted knapweed infestations statewide. Without this opportunity to gather all of the site release data from the USDA and complete the work in this LCMR grant, we may have never known about these sites.

BEFORE: Tamarac National Wildlife Refuge 2000. Over 100 acres infested with dense spotted knapweed.

AFTER: Tamarac National Wildlife Refuge 2003. Bioagent releases controlled over 100 acres of spotted knapweed.

Figure 3. Before and after biological control pictures of seedhead and root-feeding weevils working at Tamarac National Wildlife Refuge near Detroit Lakes, MN.

<u>Spotted Knapweed Biological Control Success in Minnesota.</u>

Many of the 103 sites sampled in 2004 showed us that biological control of spotted knapweed is aiding in lessening the density of spotted knapweed on biological control sites in the state. One of the most notable examples of this success was witnessed at the Tamarac National Wildlife Refuge in northwestern Minnesota. The refuge had approximately 100 acres of upland that was completely dominated by spotted knapweed. The intention of the U.S. Fish and Wildlife Service was to manage this land for the benefit of native plant and wildlife species. Spotted knapweed began to outcompete native plants leaving only a monoculture of knapweed that hindered the use of this area for wildlife. The USFWS began treating the area with herbicides, but due to product and application costs, its large size, and the ecological sensitivity of being close to several lakes and bogs, herbicide treatments were abandoned. In the mid 1990s several species of knapweed bioagents were released. By 1999, the seedhead weevil *Larinus minutus* and two seedhead flies, *Urophora quadrifasciata* and *U. affinis*, were established and highly visible. In 1999, the first *Cyphocleonus achates* were recovered from the site. Five years later the site was almost void of any spotted knapweed and estimated to be below 90% of the knapweed density in 2000 (Figure 3). Samples made by the MDA between 2002 and 2003 showed that a large increase and distribution of *C. achates* and *L. minutus* throughout the site correlated with the large decrease of spotted knapweed during the same time. The suppression of spotted knapweed by these bioagents was so great that the USFWS was able to go back to this site in 2004 and replant it with native ecotype plants to begin meeting their management goals for wildlife species on that part of the refuge. They were never able to accomplish this through herbicide applications alone. Similar results have been observed and recorded by the MDA at the Beltrami County Pit, Gruss Site, Lake Elmo Maintenance site, and Hubble Pond WMA (Table 3).

<u>Ground Cover Composition and Spotted Knapweed Plant Data at 2005 Sites:</u>

Due to the large amount of statistical variability, only simple trends could be found among sites for the various vegetation parameters that were analyzed. Our data showed strong correlations between rosette numbers on a site and the composition of other knapweed parameters. In general, sites with the highest number of spotted knapweed rosettes tended to also have the highest number of stems, seedheads, percentage spotted knapweed cover and percentage bloom. The reverse was true when low numbers of rosettes were counted on a site. For example, St. Croix SNA had the lowest average rosette, stem, and seedhead counts, the lowest percentage bloom, and it also had the shortest spotted knapweed plants of all 10 study sites (Table 5).

Table 5: 2005 Field Season Means of Rosettes, Stems, Stem Length, % Bloom, Seedheads & %SK Per Sub sample at 10 MN SK Biological Control Sites

Site Name	Acres	%SK	#Rosette	#Stem	StemLength	%Bloom	#Seedhds
AHATS	6.29	19.8	49.2	28.8	49.3	63	119.9
County Pit	9.64	21.8	58.9	32.5	38.2	72.9	168.4
Gruss	10.47	30.4	86.8	29	46.2	19.9	155.7
Hubble Pond WMA	15.79	21.2	40	23.6	50	61.1	122.1
Knutson	11.93	18.4	31.8	29	41.1	16.6	136.5
Lake Elmo Maintenance	9.62	18.2	43.8	21.4	51.6	62.7	137.9
Lake Elmo P	0.42	13.5	26.7	18	41.9	17	81.5
MN Valley NWR-Old Cedar	0.46	17.7	18.8	18.2	62	29.5	109
St. Croix SNA	28.96	9.3	6.1	10.6	23.9	9.2	76
Tamarack NWR-Job Corps	13.89	17.6	45	22	36.3	87.3	110.1

Table 6: 2005 Field Season Mean % Ground Cover Per Sub sample at 10 MN SK Biological Control Sites

Site Name	Acres	%SK	%Grass	%Forb	%Crypto	%SHRTRE	%Bare	%Rock	%Litter
AHATS	6.29	19.8	30.1	9.8	1.3	0.7	12.7	6.8	14
County Pit	9.64	21.8	9.9	16.2	6	1.1	16.1	21.1	8
Gruss	10.47	30.4	41.6	4.6	0.2	0.2	7.1	0.3	15.6
Hubble Pond WMA	15.79	21.2	33.8	30	0.7	2.8	2.3	0.1	9.1
Knutson	11.93	18.4	20.1	15.6	7.9	4.8	7	4.6	21.7
Lake Elmo Maintenance	9.62	18.2	44.5	17.1	1.8	0	7.9	1	7.8
Lake Elmo P	0.42	13.5	31.5	26.8	4.5	2.2	5.7	0.1	16
MN Valley NWR-Old Cedar	0.46	17.7	12.5	41.4	1.9	7.2	8.8	3.5	7
St. Croix SNA	28.96	9.3	33.4	31.4	1.5	0.1	9.1	1.4	13.9
Tamarac NWR-Job Corps	13.89	17.6	38.8	5.3	3.8	1.4	15.6	3.2	14.5

*Percentages are based on averages of three sample periods between 6/21/2005 and 9/12/2005.

In general, the average rosette counts at each study site increased as the season went on, whereas stem counts stayed fairly consistent across the entire season. This would make sense since rosettes are a result of germinating seeds, and measurable stems are produced from bolted/mature plants. Spotted knapweed is a long-lived biennial/short-lived perennial and germinates from seed becoming a rosette until year two when it “bolts” producing the mature plant stage. Germination occurs throughout the growing season, so new rosettes continue to appear on a site throughout the summer. However, spotted knapweed plants tend to bolt all at the same time in the early spring of the second year (and or subsequent years of the plants life cycle). Over the past few years, we have determined that rosette establishment and development is one key to spotted knapweed’s invasiveness and potential management on a site.

Most of the 10 study sites had very few shrubs and/or trees that were sampled. Tree/shrub coverage never exceeded a percentage cover of more than 7% at any of the ten sites, and the average percentage cover of all ten sites was only 3%. Grasses and forbs generally dominated the ground cover composition at sites. Sites with the lowest grass cover averaged 14.2%, and sites with the highest grass cover averaged 41.6% grass cover. Sites with the lowest forb cover averaged 6.5%, and highs averaged 34.3% cover (Table 6). Grasses were usually more common within sites than forbs. Spotted knapweed generally thrives in arid environments such as sandy gravel pits or roadsides that are highly disturbed and have very little competition from other plants, such as grasses or forbs.

A balanced variety of plants creates increased biodiversity and is considered to be a component of a healthy ecosystem. This is also correlated with soil organic composition. In many cases, organic composition of soils is determined by the plants growing within a particular ecosystem. Many of our native plant systems depend on soil composition for their success and species richness. Our research showed that sites consisting of significant amounts of grasses and other forbs had much higher soil litter content than sites dominated with large densities of spotted knapweed alone. Spotted knapweed doesn't produce much litter when compared with other forbs because it primarily consists of stems, small stem-like leaves, and seedheads. It also has allelopathic properties that allow it to release a chemical into the soil via its root system that prevents other plants from growing in the immediate area. This results in patches of bare ground where spotted knapweed seeds can germinate and eliminates competition from other plants. As this invasive process continues, lower amounts and diversity of decomposing biomass on infested sites eventually leads to changing soil organic composition and soil chemistries that favor the growth and development of knapweed plants instead of other types of more desirable vegetation. Eventually, a site becomes dominated by spotted knapweed through this process. We found that the presence of grasses and forbs on an infested site increased the amount of soil litter biomass. One way to increase the amount of grasses and forbs would be to decrease the competitive ability of spotted knapweed.

It has become apparent that some sites delineated during this research project were classified as more robust than others. In this case the term robust refers to sites where it appears that bioagents are stressing spotted knapweed plants enough to allow native plants to begin growing on these sites and establishing themselves in greater numbers. This level of robustness varies between all spotted knapweed biological control sites in the state and was especially evident at all 10 of the 2005 study sites, thus helping to explain the large amount of statistical variability among them. Without knowing the vegetative composition of the sites prior to spotted knapweed invasion, or even when the knapweed started to encroach, it is unknown whether certain sites have a better competitive edge against knapweed, whether or not bioagents are positively impacting the site, or if these sites are still in the earlier stages of invasion. However, the MDA has seen strong correlations throughout the state between increasing numbers of bioagents (primarily both *Larinus* spp. and *C. achates*) and decreasing spotted knapweed densities. Pre-release data certainly would have made these correlations much stronger.

To gain a better understanding of this phenomenon, the MDA plans to continue monitoring sites throughout Minnesota over the next decade to track changes in site vegetation characteristics. Our hope is to see less knapweed and more native plant types at all of our biological control sites. This two year grant gave us an opportunity to discover all of the spotted knapweed sites in Minnesota, delineate them, and research a small percentage of them. Now that we have visited and delineated each site, we will be able to track vegetative changes on sites and correlate them better with bioagent population levels. This will help us to gain a better understanding of the role bioagents are having at sites and their impacts not only on spotted knapweed but also on the resultant vegetation.

Conclusion

When we first developed the work plan for this grant, we had envisioned being able to visit all known spotted knapweed sites in Minnesota and provide some type of consistent explanation on the status of biological control agents and their impacts on spotted knapweed infestations. However, with most large-scale field research projects, unforeseen variables can quickly change the intended goals of any research project. That said, we were able to accomplish many things through the completion of this grant that may never have been studied without the aid of LCMR. Overall, the results and observations obtained through this research grant have benefited the MDA's statewide implementation program for spotted knapweed immensely. Due to several findings as a result of this grant, the MDA will be better prepared to plan for future biological control activities pertaining to spotted knapweed.

The largest benefit obtained from our work was through the collection and organization all of the biological control agent release information from USDA and various counties in Minnesota. We were able to enter all known release data, dating back to the late 1980s, into a geographical database that will allow the MDA and future land managers to have easy access to all spotted knapweed biological control site information for the entire state at the push of a button. The second biggest outcome of this grant was that we were able to successfully visit all existing spotted knapweed sites (103 total sites) in the state, delineate their boundaries for georeferencing, determine their basic ecological make-up, and determine through sampling what biological agents were established on them. Finally, we were able to take a full growing season to conduct research on 10 of the 103 sites in Minnesota to see if we could observe what impacts spotted knapweed bioagents were having on the plant and existing vegetation at each site. From our data we were able to identify that six bioagents have established at sites in Minnesota and that each bioagent is active at a specific time of the year. Prior to the work completed in this grant, none of the previously mentioned outcomes were known.

Looking back there are several areas for improvement. Our original intention was to be able to describe what was happening at knapweed biological control sites in Minnesota, many years following bioagent releases where little or no pre-release site data existed. This became a huge problem in our data analysis. Huge variability existed among research sites for almost all of the vegetation parameters we analyzed. Therefore, we were only able to look at our data on a site by site basis. Furthermore, without any prerelease data and limited site data with the original bioagent release information obtained from USDA (not to mention that a lot of the information was 10 – 15 years old and most of those sites had not been revisited until our work began in 2004) it was difficult for us to determine if biological control was the result of low knapweed counts on a site or the presence of more natives in sub samples, etc. Therefore, with the lack of statistical trends among sites due to enormous site variability, we focused on biologically significant trends that did exist among sites. We were able to see that the presence or absence of the rosette stage of knapweed alone can be a sign of what that habitat will look like in a few years if untreated or could help explain bioagent activity on a site. We did the best we could with what time we had and what information we were given. If we had to do this again, we would probably focus on sites without any biological control activity and set them up in such a way that controlled releases of agents were made and then studied over a three year period. That way we would know what we started with before bioagents were released and could monitor the sites each year showing how the site may or may not change.

Given the fact that this project had its obstacles, we feel confident that our knowledge base pertaining to spotted knapweed ecology and biology, its bioagent complex, and its distribution in Minnesota has increased dramatically. Throughout the course of this grant, we compiled a diverse library of peer reviewed literature pertaining to spotted knapweed research, biological control, and general ecology/biology. Being within a large state agency that is responsible for many terrestrial biological control programs other than spotted knapweed, we have been able to adopt several of the aspects of this grant into our other biological control programs. For example, the mobile mapping system that was created separately by several MDA staff to aid in the data collection and site delineation portions of this research, has been incorporated into the MDA's statewide nuisance weed mapping program and its other biological control programs for both insects and weeds. This system of data collection is so efficient and useful that it has changed the way many land managers in the state collect data today. Visit our website to see an article and video about this project: http://www.mda.state.mn.us/ipm/thicket/volume4no1/kwdatacollection.htm. We have also become strong proponents of having pre-release/pre-treatment site data for any biological control or weed IPM project so that implications of management/treatments can be correctly documented. It is surprising the number of biological control projects nationwide that lack pre-release information.

Overall, the completion of this grant has resulted in a number of accomplishments. Already, we have been able to use the data from this research to strategize more efficiently for the 2006 spotted knapweed biological control field season. We've also seen some tremendous results attributed to the release of bioagents on spotted knapweed in Minnesota (see Figure 3 – before and after pictures from Tamarac National Wildlife Refuge), and have been getting positive feedback from land owners and managers who have participated in the program. Over the past few years we have learned that spotted knapweed biological control can be an important tool in managing this invasive weed. It's not the only tool, but it has the potential to have long-term and sustaining impacts on large infestations where herbicides and other IPM tactics are not practical, are expensive, or ecologically unsound. This grant has allowed us to increase our knowledge of spotted knapweed and the possibilities for extensive biological control management in Minnesota. The MDA plans to continue the efforts of this research over the next several years so that we can gain a better understanding of spotted knapweed infestation and spread and how biological control agents may impact that process.

V. TOTAL LCMR PROJECT BUDGET:

All Results: Personnel: $ 85,807 (Research Scientist 1)
All Results: Equipment: $ 1,470 (Lab and Field Equipment)
All Results: Development: $ 0
All Results: Acquisition: $ 0
All Results: Other: $ 1,723 (Travel – In State)

TOTAL LCMR PROJECT BUDGET: $ 89,000.00; See Attachment A.

Explanation of Capital Expenditures Greater Than $3,500: Not Applicable for this project.

VI. PAST, PRESENT AND FUTURE SPENDING:

A. Past Spending: Approximately $10,000 dollars has already been spent by the MDA General Fund (2002) to locate sites in Minnesota with biological control agents released on them and to begin a small amount of data transfer of past releases from the USDA-APHIS office in Minneapolis, MN. MDA funds (approximately $8,000) were used to purchase research and laboratory equipment, a laptop computer, and office supplies for the incumbent who is funded by this grant.

B. Current Spending: MDA funds (approximately $1,400) purchased the current mobile computer/GPS system which includes an hp iPAQ Pocket PC, HOLUX Bluetooth GPS unit, and other items to complete this field data collection system. Approximately $1,500.00 of MDA 2004 and 2005 funds were used to rent a car for field work. Another $5,000 of MDA funds was used to pay for the remainder of Ms. Northrop's time analyzing data and working on the preparation of this report in 2006. MDA funds ($9,000 – 12,000) were encumbered for this project, in addition to LCMR funding, for field and laboratory supplies, insect identifications, and monies for a student intern to assist with field and lab work in 2004 and 2005.

C. Required Match (if applicable): No Match Required.

D. Future Spending: MDA plans to continue providing general fund dollars to further research and exploration into several aspects of work investigated in this LCMR grant.

VII. PROJECT PARTNERS:

A. Partners Receiving LCMR Funds: This was only used to fund a full-time, temporary unclassified position within the MDA for Jill Babski (2004) and Natasha Northrop (2005 -2006) to conduct the research.

B. Project Cooperators: This project will fund a position at the MDA to complete Results 1 and 2. Although weed biological control cooperators throughout the research study area may help with collection of data and field locations, they will not be funded in any part by this grant.

VIII. DISSEMINATION: Through collection of data over the course of this project, a geospatial database was created that will be used to show trends in spotted knapweed biological control with respect to available landscape, soil, temperature, precipitation, and land-use databases throughout the state. The results for objectives 1 and 2 were presented in 5 semi-annual status reports to LCMR throughout the course of this study and in a detailed final project report presented in July, 2006. Additionally, we hope to publish at least one manuscript from this study in a peer reviewed journal such as Weed Science or Weed Technology and plan to present the final results at the 2006 Weed Science Society of America Annual Meeting (time and place to be announced). Ultimately, we are hoping that the data generated from this research will allow us to develop an efficient and informative protocol that will benefit local communities as we strive to reach Phase 3 with spotted knapweed biological control.

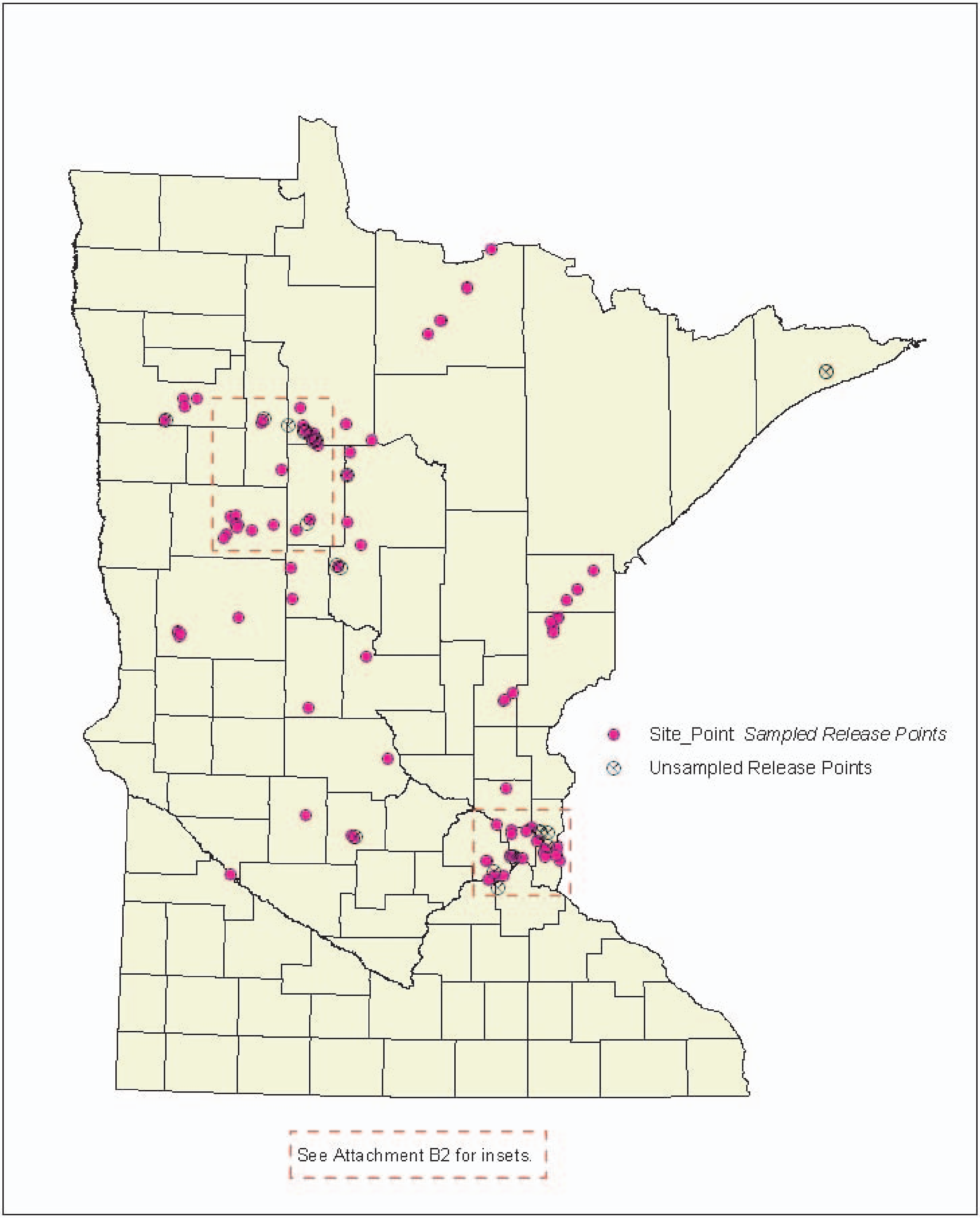
IX. LOCATION: During the 2004 field work, 127 spotted knapweed biological control sites were identified. Of these, 103 sites were sampled using the form described above and 24 sites were not sampled due to mowing, unknown location, lack of access, or lack of permission.

Ten sites were chosen for summer 2005 based on 2004 sampling data. These ten sites are located in Becker, Beltrami, Clearwater, Hennepin, Ramsey, and Washington counties of Minnesota. Random points for sampling transects will be generated for each site, and will be located in the field using an iPAQ mobile GPS. See attachment B.

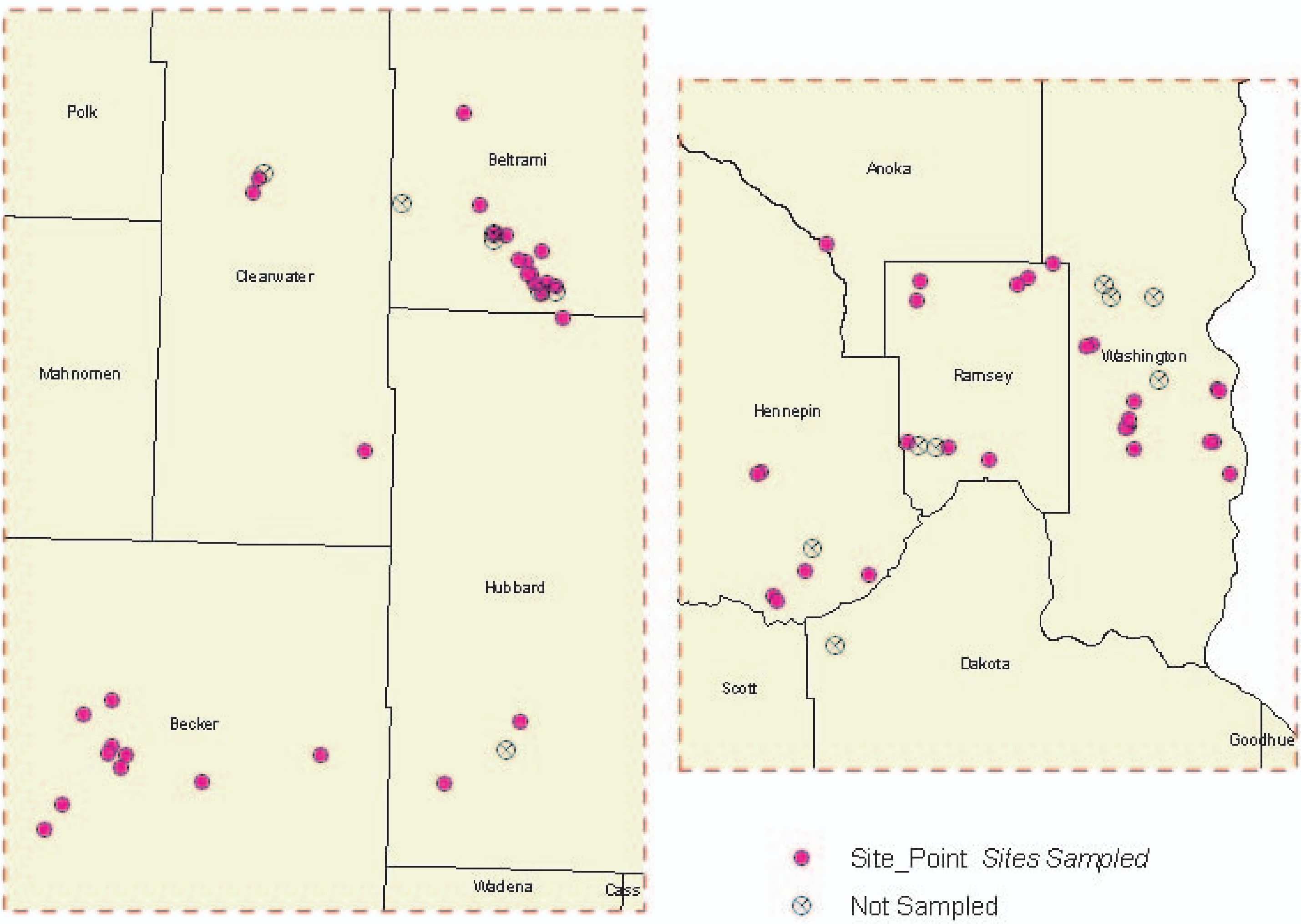
X. REPORTING REQUIREMENTS: Periodic work program progress reports will be submitted not later than 1) February 20, 2004, 2) September 17, 2004, 3) March 18, 2005, 4) Sept 16, 2005, 5) February 17, 2006. A final work program report and associated products will be submitted by July 31, 2006.

XI. RESEARCH PROJECTS: Research Addendum – will be part of final report.

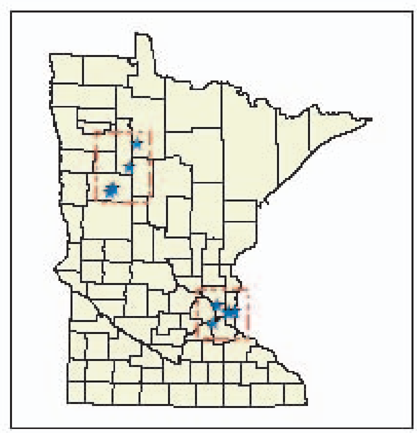
Attachment B1: Spotted Knapweed Bioagent Release Locations Sampled and Unsampled, September 2004

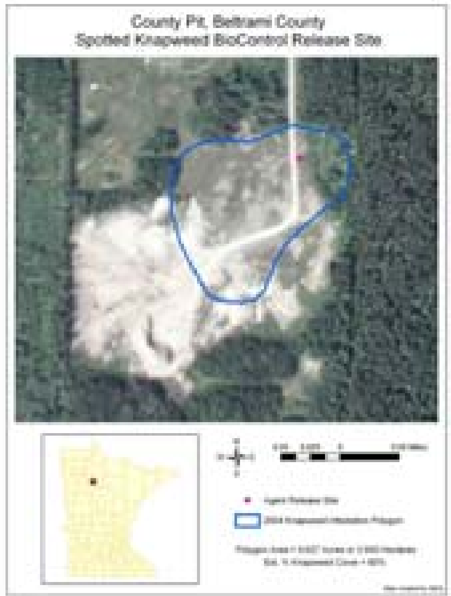


Attachment B2: Inset of Spotted Knapweed Release Point Locations, September 2004



Attachment C1: ★ 2005 Spotted Knapweed Research Sites





Attachment C2 : 2005 Spotted Knapweed Biocontrol Site Maps (Lake Elmo Maintenance and “P” are on the same map)