FINAL REPORT

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Document 1 – Work Program Final Report

Date of Report: July 1, 2002

I. PROJECT TITLE: ***Evaluate establishment, impact of leafy spurge biocontrol agents.***

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Total Biennial Project Budget:

LCMR:	$ 140,000.00
LCMR Amount Spent:	$ 140,000.00
LCMR Balance:	$ 0.00

A. Legal Citation: ML 1999, Chap. 231 Sec.16 Subd. 16(b) Exotic Species Language:
Evaluate Establishment, Impact of Leafy Spurge Biocontrol Agents $70,000 the first year and $70,000 the second year are from the trust fund to the commissioner of Agriculture to study flea beetles introduced to control leafy spurge by site characterization and assessment for biological control. This appropriation is available until June 30, 2002, at which time the project must be completed and final products delivered, unless an earlier date is specified in the work program.
B. Status of Match Requirement: (None)

II. and III. FINAL PROJECT SUMMARY.

Research was conducted to assess the establishment and control success of *Aphthona* flea beetles introduced to control leafy spurge, *Euphorbia esula* L. Since 1989, five species of flea beetles, *Aphthona* spp., were released in Minnesota to control leafy spurge.

The results suggest that *Aphthona lacertosa* is the most effective species in controlling leafy spurge in Minnesota. *Aphthona lacertosa* has established at 100% of the release sites and significantly reduced leafy spurge by 63% across all sites studied. *Aphthona nigriscutis* established at 73% of the study sites, but at significantly lower densities. *Aphthona nigriscutis* most likely contributed to the control success at sites where both species occurred. Other introduced *Aphthona* species are difficult to locate in Minnesota and contributed little to the overall control success. Correlations between biotic/abiotic factors and flea beetle density were not clearly evident. *Aphthona nigriscutis* was observed at highest densities in dry sites with either sand or sandy loam soils.

Interspecific competition between *A. lacertosa* and *A. nigriscutis* was not affecting flea beetle populations. Small release quantities (<500 beetles) may have contributed to lack of establishment on early releases made in Minnesota. Although the beetles became established, all treatment populations were small one year after release. This suggests that the current practice of releasing >4,000 flea beetles per site will increase establishment, reproduction and eventual redistribution.

Phenology models were developed for predicting peak emergence. The model results can be used in two ways. First, the lower developmental threshold (LDT) and accumulated degree-days (ADD) to peak emergence can be used to calculate current ADD with local weather station temperature data. This allows resource managers to track degree-day accumulations and plan collection events on or near the predicted ADD for each species. The second method is to use maps developed on 30-year temperature data with estimated peak abundance dates in the field.

IV. OUTLINE OF PROJECT RESULTS:

Summary of Results 1, 2 and 3. (Please refer to Document 3 – Detailed Research Addendum for additional information).

BUDGET UPDATE:

Result 1:	**LCMR Budget**	**$ 56,523**
	Balance 6/30/01	$ 19,044
	Balance12/31/01	$ 10,040
	Balance 7/01/02	$ -0-
Result 2:	**LCMR Budget**	**$ 58,089**
	Balance 6/30/01	$ 31,186
	Balance 12/31/01	$ 14,885
	Balance 7/01/02	$ -0-
Result 3:	**LCMR Budget**	**$ 25,388**
	Balance 6/30/01	$ 20,188
	Balance 12/31/01	$ 16,593
	Balance 7/01/02	$ -0-

Objectives of this research include:

1) Assess the relationships between establishment of *Aphthona* spp. and biotic/abiotic factors.
2) Test for interspecific competition between *Aphthona lacertosa* and *Aphthona nigriscutis.*
3) Test the effect of release quantity on establishment and control by *Aphthona lacertosa* and *A. nigriscutis.*
4) Develop phenological models for *Aphthona* spp using accumulated degree-days.

Objective 1. Assess relationship between establishment of *Aphthona* flea beetles and biotic and abiotic factors.

Results:

To effectively utilize the variety of available biological control agents against leafy spurge, it is important to understand which biotic and abiotic factors influence the success of establishment and integration within spurge communities. Although there is some information in this area, a comprehensive look at current insect releases, establishment rates and environmental factors (both density dependent and density independent) will provide valuable information towards control efforts. Armed with this knowledge, biocontrol agents can be applied into situations where they have the greatest chance to succeed.

Table 1. Number of *Aphthona* spp. released for control of leafy spurge in Minnesota.

(Data summarized from MN Department of Agriculture leafy spurge database)

YEAR	No. of Releases	No. Insects Released
1989	8	4,500
1990	5	3,000
1991	5	4,000
1992	4	1,700
1993	3	2,000
1994	2	1,550
1995	13	14,250
1996	67	91,000
1997	158	1,511,153
1998	362	1,584,350
1999	439	4,650,322
2000	436	7,162,400
2001	539	6,797,870

Twenty-six sites were selected in three counties with 16 sites in Clay, 8 sites in Otter Tail, and 2 sites in Becker counties.

Table 2. Site characteristic information collected from each release site sampled.

Characteristics	**Categories**			
Site Type	open field/prairie	shrub-prairie mix	wet prairie	woodland/meadow
Shade	none	slight (5-30%)	moderate (31-60%)	heavy (>60%)
Water Drainage	well drained	moderately drained	poorly drained	
Topography	flat	valley or swale	hillside	hilltop
Slope	level	slight slope	steep slope	
Slope Direction	north	south	east	west
Soil Texture	multiple categories			

The most successful *Aphthona* species in moist, loamy sites has been the combination of *A. czwalinae* and *A. lacertosa*. It is known that *A. lacertosa* is the dominant species where it is released. Their populations tend to build up quickly, potentially affecting establishment of the other introduced *Aphthona* species, where mixed releases occur. In many of the release sites, it is unknown how well the other *Aphthona* species would establish in the absence of *A. lacertosa*.

Despite a range of microclimates, we were not able to associate *Aphthona* spp. abundance with biotic or abiotic factors with one exception. It was reported that soil texture influences *Aphthona* spp. abundance (Rees et al. 1996, Lym 1998 and Nowierski et al. 2002). Our study shows that *Aphthona lacertosa* abundance may be negatively affected by soils with high sand content. Nowierski et al. (2002) suggested that *A. lacertosa* in Europe are associated with sites containing higher levels of silt and clay. We found no relationship between *A. nigriscutis* and soil texture. This is in contrast to previous research that shows an apparent relationship of *A. nigriscutis* to sandy soils and xeric conditions (Rees et al. 1996, Lym 1998, and Nowierski et al. 2002).

The lack of association with biotic and abiotic factors may suggest that *A. lacertosa* may be able to establish on a wide variety of site types. This seems to be the case in our study where *A. lacertosa* established in 100% of the sites with a wide range of population densities. This is supported by previous research that suggested *A. lacertosa* can establish in hydric, mesic, and moderately dry sites (Fornasari 1996, Gassmann 1996, Rees et al. 1996, and Nowierski et al. 2002). For *A. nigriscutis*, we cannot make this same assumption. *Aphthona nigriscutis* established in 73% of the sites but the numbers were very small and are only a fraction of *A. lacertosa* densities. The extreme low densities of all but one of the *A. nigriscutis* populations most likely affected the regression analysis. It is our impression from field observations however, that *A. nigriscutis* prefers the drier

sandy sites. Rees et al. (1996) and Nowierski et al. (2002) both suggest this pattern of *A. nigriscutis* preferring drier sites with sandier soils.

The sex ratio for *A. lacertosa* and *A. nigriscutis* was approximately 50/50. This is of particular interest for *A. nigriscutis* because sex ratios for this species in western states were found to strongly favor females. It is thought that a bacteria, *Wolbachia* spp., is lethal to male *A. nigriscutis* and thus may be a barrier to establishment (D. Kazmer pers.comm.). It would be of interest to know if *A. nigriscutis* populations in Minnesota are infected with *Wolbachia* spp.

Aphthona lacertosa and/or *A. nigriscutis* have shown that they can reduce leafy spurge densities. *Aphthona lacertosa* density appears to follow a classical biological control model where the population is small shortly after release, increases exponentially, peaks then decreases as the food source (spurge) decreases. *Aphthona lacertosa* populations tended to increase then peak shortly after spurge density began to decrease (Fig. 1). For many sites, the maximum *A. lacertosa* density was reached in 2000 then dropped in 2001. There were insufficient numbers of *A. nigriscutis* to discern general trends.

Figure 1. Mean spurge density for sites by number of years after *A. lacertosa* release compared to the relative *A. lacertosa* density by number of years after release. The relative *A. lacertosa* density is a ratio of the mean number of *A. lacertosa* per sweep by year.

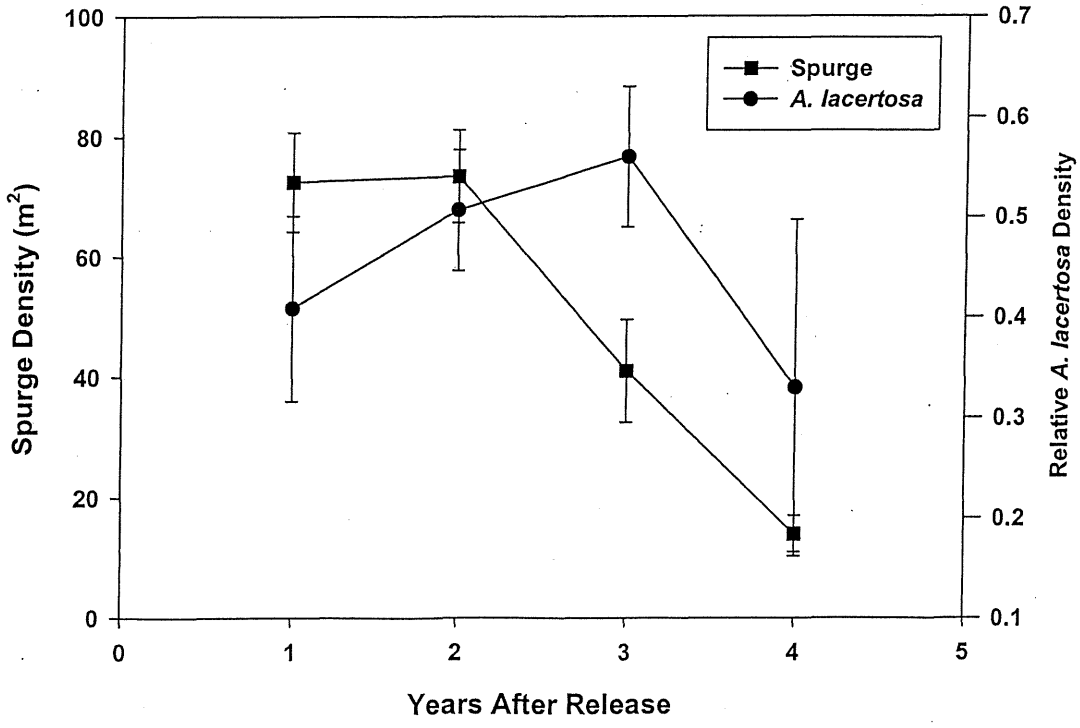
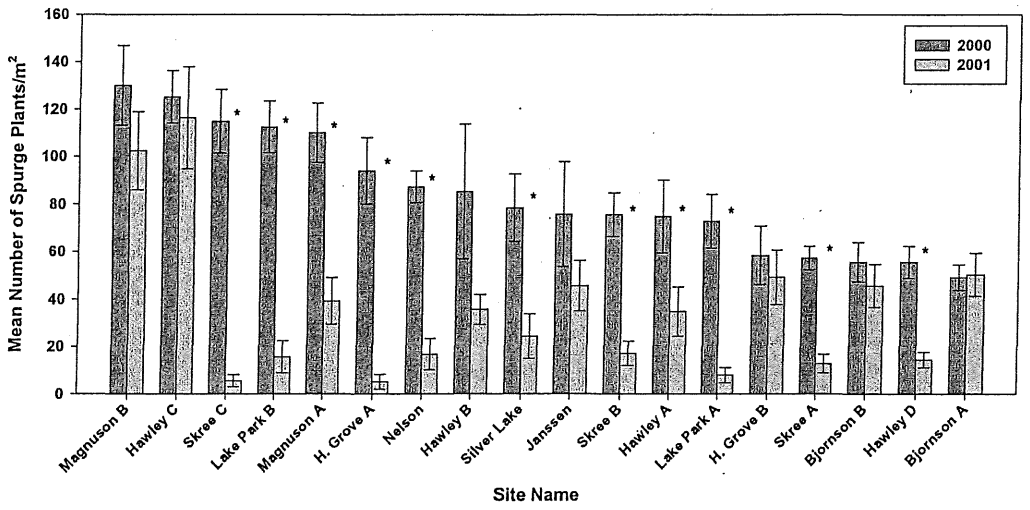


Fig.2. Mean with standard error for combined totals of flowering and non-flowering spurge plants per square meter for sites in Clay and Becker Counties.*denotes significant differences (p=0.05).



Leafy spurge density (total number, flowering and non-flowering) and percent cover were all significantly reduced in 73% of the sites (Fig.2, Fig.3). This reduction on average took three years. This is exceptionally fast for biological control to be successful. The root-feeding larvae are most likely the key, stressing or killing the plants by destroying the carbohydrate reserves in the roots (Gassman et al. 1996). Biocontrol agents that only defoliate plants tend to take longer in controlling perennial plants due to reserves in the roots, which may take two or more years of defoliation to kill the plant (Katovich et al. 1999) We would expect a greater percentage of significant decrease in spurge density in the summer 2002 than 2001.

Many of the sites were former pasture comprised of smooth brome grass. Since this grass was the predominant species prior to spurge infestation, it is not surprising that we found this species extensively in our study. As spurge density decreased, the resulting area was filled with plant species already present at the site which in this study was primarily smooth brome.

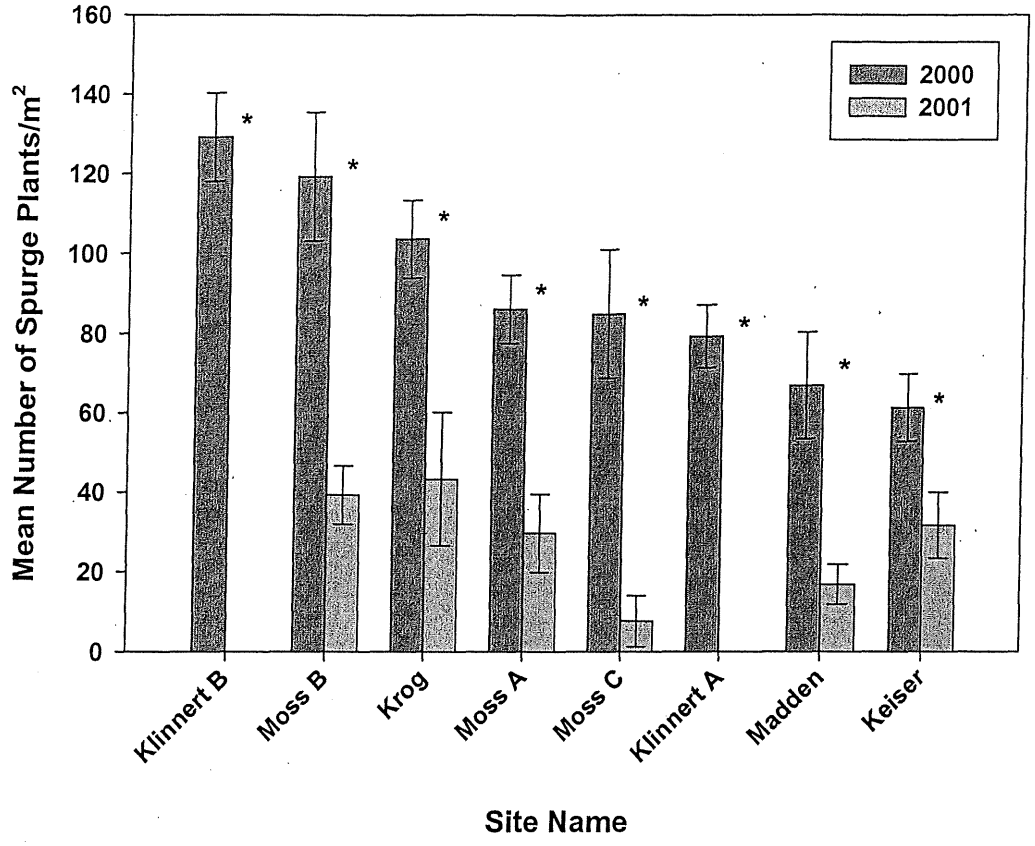


Fig. 3. Mean with standard error for combined totals of flowering and non-flowering spurge plants per square meter for sites in Otter Tail County. * denotes significant differences (p= 0.05).

Objective 2. Test for interspecific competition between *Aphthona lacertosa* and *Aphthona nigriscutis.*

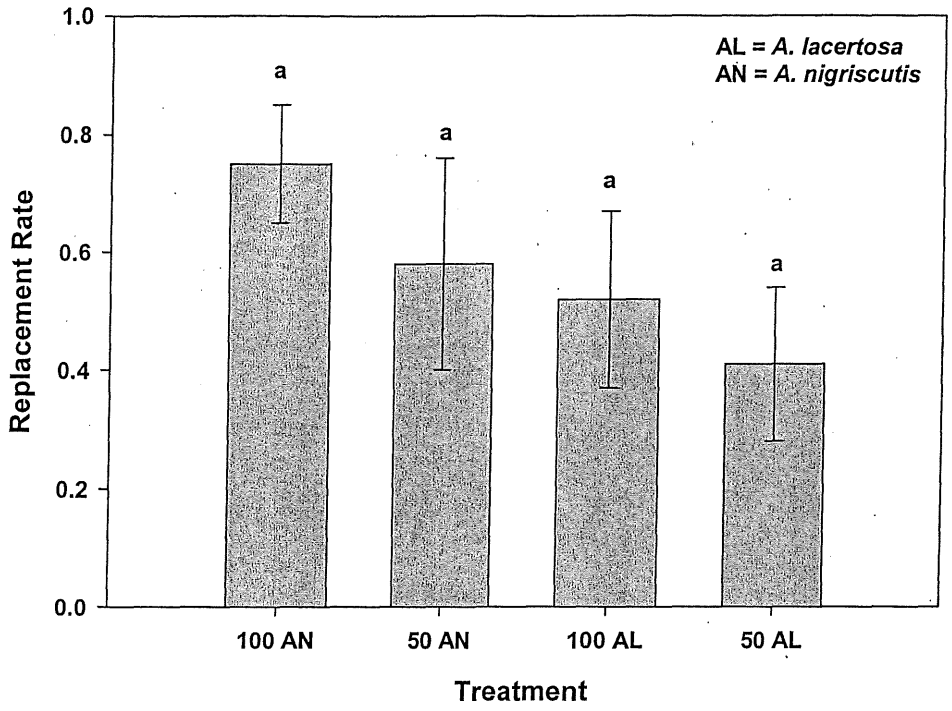
Results:

One year post treatment, there were no significant differences between treatment replacement rates. (Figure 4). The mean replacement rate for all *Aphthona* species and treatments was 0.56. Replacement rates across all treatments ranged from 0.07 to 0.92.

Leafy spurge stem densities significantly decreased one-year post treatment for all treatments. Prior to treatment the mean leafy spurge stem density per plot was 239.6. Leafy spurge densities per plot ranged from 178 to 301 stems. Leafy spurge stem densities, however, significantly decreased one-year post treatment (Figure 4). The mean

stem density per plot across all treatments was reduced to 86.4 stems, and stem density ranged from 6 to 123.

Figure 4. Replacement rates of *Aphthona* one year post treatment. There were no significant differences by treatment as indicated by letter 'a'



Objective 3. Test effect of release quantity on establishment and control by *Aphthona lacertosa* and *A. nigriscutis*.
Results.

Within treatments, *A. lacertosa* established at all plots and *A. nigriscutis* was recovered from all but one plot. At peak emergence, the total number of *A. lacertosa* per plot ranged from 9 to 188 compared to *A. nigriscutis* ranging from 0 to 69 per 50 sweeps. Neither species affected the establishment of the other in the combined 500 *A. lacertosa*/500 *A. nigriscutis* treatment.

The 1,000 *A. lacertosa* treatment produced the highest insect density that was significantly higher than all treatment except the combined 500 *A. lacertosa*/500 *A. nigriscutis* release rate ($P = 0.05$, Fig. 14). In contrast, there were no differences among the release rates of *A. nigriscutis*.

All plots initially contained spurge with a mean number of total (flowering and non-flowering stems) stems ranging from 42 to 169 stems per m^2 (Fig. 13). Density of flowering, non-flowering, or total number of spurge stems in general was not significantly different for most treatments ($P = 0.05$, Table 13). In 2001, mean number of spurge stems across all treatments ranged from 26 to 110 and 1 to 89 stems per m^2 for flowering and non-flowering spurge respectively. Significant reductions in the total number of spurge stems were observed in the 1,000 *A. nigriscutis*, 250 *A. lacertosa*, and the combined 500 *A. lacertosa*/500 *A. nigriscutis* treatments. Spurge height ranged from 15 to 97 cm with a mean of 65.9 cm which was not different from stem height prior to release and one treatment, 250 *A. lacertosa,* showed a significant increase in stem height. Mean percent spurge cover for all plots ranged from 6-25% to 76-100%. The only significant change was an increase in spurge cover with the 1,000 *A. nigriscutis* treatment. The mean number of plant species per plot other than spurge was 1.6 species. Perennial grasses were the predominant vegetation type found in the plots followed by perennial forbs, annual or biennial forbs, and woody perennials respectively (Table 12).

Table 12. Categories of plant species other than leafy spurge found during vegetation surveys.

Category	**2000**	**2001**
annual or biennial forb	8.65%	6.85%
perennial grass	62.63%	83.06%
perennial forb	25.95%	9.68%
woody perennial	2.77%	0.40%

Objective 4. Develop a degree-day emergence model for *Aphthona* adults.
Results.

Developmental Rates and Lower Developmnental Threshold Determination:
A total of 3,355 *A. lacertosa* individuals were collected from the growth chambers while only 277 *A. nigriscutis* individuals were collected. Developmental times decreased with increasing temperatures. Average days to emergence ranged from 68.6 days at 15°C to 25.6 days at 26°C for *A. lacertosa* and 84.3 days at 15°C to 26.1 days at 26°C for *A. nigriscutis*. The development rates (1/d) for *A. lacertosa* and *A. nigriscutis* were linear with temperature as shown in Figures 15 and 16 respectively. Based on the regression analysis, the lower developmental threshold estimate for *A. lacertosa* and *A. nigriscutis* are 8.3 °C and 10.1 °C respectively. The degree-days required for adult emergence are 448 for *A. lacertosa* and 425 for *A. nigriscutis* based on the reciprocal of the slope of the linear regression.

Phenology Model
Pooled data resulted in four "location years" as described by Legg et al. (2002). Model equations and R^2 value for non-linear models are provided. The estimated accumulated degree-days to peak abundance of *A. lacertosa* is 513 (based on a lower developmental threshold of 8.3 °C). The estimated accumulated degree-days to peak abundance of *A. nigriscutis* is 610 (based on a lower developmental threshold of 10.1 °C). Calculations,

estimates and standard errors for predicting the number of accumulated degree-days at peak abundance are listed.

Displaying Models Spatially
Maps displaying estimated average dates to peak abundance for *A. lacertosa* and *A. nigriscutis* are displayed in Figure 21. Average date to peak abundance for *A. lacertosa* ranged from June 17th to July 22nd depending on location in the state. Average date to peak abundance for *A. nigriscutis* ranged from June 27th to August 8th, approximately 10 days later statewide than *A. lacertosa* in similar geographic locations. Peak emergence occurred earlier in the year in the southern part of the state for both species and became progressively later as you move north and east in the state.

V. DISSEMINATION

Data will be shared with each of the Counties as well as provide statewide maps. Presentations will be made at the County Agricultural Inspectors' Workshop and at district weed meetings.
Findings will be shared with other State and Federal Agencies.

VI. CONTEXT:

A. Significance:

The five species of *Aphthona* flea beetles have been released since 1989 and all of them are established in Minnesota. However, some of them have had delayed establishment taking 3-4 years to reveal damage symptoms. This variability has prompted this investigation to analyze flea beetle establishment and habitat characteristics.

Special conditions such as unusual weather events and records of treatment history, including information on treatment applications (where, how, cost, and successes) will allow evaluation and fine-tuning of treatments. Biological control does not aim to eradicate weeds, but to keep them at low, manageable levels. The five flea beetle species have taken 5-10 years to establish and increase to effective numbers. This study is expected to provide information on action thresholds and prioritizing and balancing treatments with resources. County Weed Inspectors will be able to use this biological control tool to manage weeds in their counties in addition to or as an alternative to current control practices.

B. Time: The proposed project will exceed two fiscal years because of its seasonal field work.

C. Context: MDA enforces the Noxious Weed Law which entails surveying for noxious weeds in each county by the county Ag.Inspectors who are paid by the counties but supervised by MDA. MDA's Plant Pest Survey and Biological Control Program also interacts with the county ag.inspectors by providing biocontrol agents and assisting in their establishment and monitoring. USDA, APHIS cooperates in the redistribution of biocontrol agents from their established sites.

LCMR Project Budget Breakdown (revised)

BUDGET: (2000-2002)

Personnel: Dr. Dharma Sreenivasam, MDA. 5% in-kind contribution.
Dr. David Ragsdale, Professor, Dept. of Entomology, University of Minnesota. 5% in-kind contribution.

	Budget	Expenses
Research Associate (100%) MDA	$ 71,736	71,736.00
Research Assistant (50%) U of M	$ 50,264	50,264.00
~~**Space Rental Office and Lab.**~~	~~$ 3,000~~	
Communications, Telephone, Mail	$ 600	600.00
Contracts: Professional & Technical	$ 5,000	5,000.00
	$ 3,000	3,000.00
In-State Travel	~~$ 5,000~~	
	$ 6,000	6,000.00
Out-of-State Travel	~~$ 2,000~~	
	$ 1,000	1,000.00
Office Equipment, Supplies	$ 2,400	2,400.00
Totals	**$140,000**	**140,000.00**

VII. COOPERATION: As shown in the budget.

VIII. LOCATION: As shown in the report.

Recommendations:

We recommend that *A. lacertosa* be used as the primary agent for control of leafy spurge in Minnesota. We also recommend that *A. nigriscutis* continue to be redistributed statewide, particularly to sites where *A. lacertosa* may not do well. Mixed colonies are preferred for sites that are very dry with sandy soils. Efforts to collect and redistribute *A. cyparissae* and *A. flava* should be considered low priority, unless field populations become highly abundant.

The current practice of releasing >4,000 beetles per leafy spurge infestation, should be continued. If flea beetle abundance becomes low, smaller release quantities can be released with some success.

We recommend that release sites be visited starting two years post release. Our results show that flea beetle populations increase dramatically in the second or third year. Leafy spurge reduction, on average, also begins to decline rapidly three years post release, but may occur as early as two years post release. Importance of monitoring two years post release are two-fold, first to monitor success of the biocontrol release and secondly to determine if flea beetle populations are large enough that a collection can be made for redistribution

Document 2 – Final Report Abstract

Title: *Evaluate establishment, impact of leafy spurge biocontrol agents.*

Research was conducted to assess the establishment and control success of *Aphthona* flea beetles introduced to control leafy spurge, *Euphorbia esula* L. Leafy spurge is a Eurasian perennial plant that seriously impacts native plants, wildlife, and grazing land for cattle and horses. Since 1989, five species of flea beetles, *Aphthona* spp., were released in Minnesota to control leafy spurge. Some of the species, however, have had difficulty establishing and have not contributed to control success. Factors that may affect insect establishment include soil type, soil moisture, leafy spurge density, leafy spurge biotype, vegetation type, litter cover, release quantity, and interspecific competition.

The results suggest that *A. lacertosa* is the most effective species in controlling leafy spurge in Minnesota. *Aphthona lacertosa* established at 100% of the release sites and significantly reduced leafy spurge by 63% across all sites studied. *Aphthona nigriscutis* established at 73% of the study sites, but at significantly lower densities than *A. lacertosa*. *Aphthona nigriscutis* most likely contributed to the control success at sites where both species occurred. Other introduced *Aphthona* species are difficult to locate in Minnesota and contributed little to the overall control success occurring statewide. Correlations between biotic/abiotic factors and flea beetle density were not clearly evident. Only soil texture seemed to affect *A. lacertosa* densities, which may not have biological significance. Early indications showed that interspecific competition between *A. lacertosa* and *A. nigriscutis* was not affecting flea beetles populations. Small release quantities (<500 beetles) may have contributed to lack of establishment on early releases made in Minnesota. Currently it is recommended that >1,000 beetles should be released at new leafy spurge infestations. Phenology models predicting peak emergence of *A. lacertosa* and *A. nigriscutis* were developed to provide information to resource managers on when to collect beetles for redistribution.