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STATE HIGHWAY ADMINISTRATION

RESEARCH REPORT

DEVELOPMENT OF BENEFICIAL BIOLOGICAL AGENTS FOR INVASIVE SPECIES CONTROL

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MARYLAND DEPARTMENT OF AGRICULTURE**

**SP009B4T
FINAL REPORT**

May 2013

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16. Abstract Noxious and invasive weeds readily colonize disturbed areas and outcompete and displace native and other desirable vegetation. This can result in a loss of pollinators (i.e. animals such as birds, bees, and other insects that move pollen between plants making them very important to plant reproduction), wildlife food and nesting resources, and decrease biodiversity in general. In cases where invasive plants establish monocultures (i.e. the growth of only one plant species), or where remedial management of noxious or invasive plants must be employed, winter cover can be lost and soils destabilized, the filtering quality of wetlands and riparian areas are diminished, and siltation is increased. The Maryland State Highway Administration (SHA) spends over \$300,000 per year purchasing herbicides to control exotic weeds. This figure does not include administrative and labor costs to apply herbicides. Current practices of pesticide use and mowing are costly and often high impact, causing even further disturbance and thus perpetuating the cycle of colonization by invasive plants. Labor, material, equipment, and fuel are other costs incurred by SHA. The objective of this study was to develop methods for the use of biological control that would provide SHA with a sustainable, cost effective, and beneficial method for dealing with noxious and invasive weeds on highway rights-of-way.					
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I. Executive Summary

The objective of this study was to develop methods for the use of biological control that would provide the Maryland State Highway Administration (SHA) with a sustainable, cost effective, and beneficial method for dealing with noxious and invasive weeds on SHA rights-of-way (ROW). As a result, the research team at the Maryland Department of Agriculture (MDA) propagated and released two beneficial biological agents, *Galerucella* leaf beetles and *Rhinoncomimus latipes* (a weevil or small beetle), for the control of two invasive plant species (purple loosestrife and mile-a-minute weed) at strategic locations. The research team then monitored their effects on weed populations over the course of two growing seasons. Other biological agents were also monitored for their potential to aid in the management of Canada thistle, *Cirsium arvense*.

Rearing protocols developed by the New Jersey Department of Agriculture (NJDA) Beneficial Insect Laboratory were adapted for use at the MDA Insect Rearing Facility. Refinements were made over the two season timeframe and the numbers of insects reared significantly increased during the course of the study. The result of this work is a year-round weevil-rearing process where colony numbers can be increased quickly. MDA also developed a method for holding weevils in cold storage (similar to hibernation) for several days prior to release to ensure that sufficient numbers are collected.

During the study period, high levels of activity of the Canada thistle bud weevil, *Larinus planus*, were found in Canada thistle populations in Maryland. However, the impact of this weevil could not be determined. Due to the widespread distribution and abundance of this herbivore, it is likely that the impact on seed development is significant, although not enough to eliminate populations of Canada thistle. It may, however, reduce colonization of new sites due to the reduction in spread of viable seed.

The numbers of the leaf beetle, *Cassida rubiginosa*, while high in certain areas, remained relatively low overall. The research team speculated that the impact of the leaf beetle on Canada thistle is low, as many plants still are able to flower and set seed after early season leaf beetle

herbivory. However, coupled with other biocontrol agents, additional stressors, including the leaf beetle, may have a long term impact on Canada thistle populations.

Based on site visits, the disease causing apical chlorosis in Canada thistle continued to spread in Maryland. The disease is of interest to the research team because it appears to delay and/or reduce flowering and viable seed production. The team hypothesized that mechanical means of transmission and spread of the disease, including feeding by certain insects and human activities (e.g. mowing), may help spread the disease.

II. Introduction and Background

Noxious and invasive weeds¹ colonize disturbed areas and outcompete and displace native and other desirable vegetation. This can result in a loss of pollinators, wildlife food and nesting resources, and decrease biodiversity. In cases where invasive plants establish monocultures², or where remedial management of noxious or invasive plants must be employed, winter cover can be lost, soils destabilized, and the filtering quality of wetlands and riparian areas may be diminished and siltation increased.

Wetlands, particularly those connected to storm water treatment systems, are critical at enhancing the water quality that enters the Chesapeake Bay Watershed. Invasive plants such as purple loosestrife and mile-a-minute weed kill off plants species that support water filtration, decreasing the effectiveness of the wetland or even destroying it. Mile-a-minute weed is a rapidly growing vine with triangular leaves and thorns of Asian origin. It grows on shrubs, trees and other native plant species blocking necessary sunlight, thus ultimately killing the host plants. Because the weed is widely propagated (i.e. reproduced), it is often difficult to control. The mile-a-minute vine is an annual plant that dies in the fall and winter, but its seeds last up to seven years and are spread by wildlife such as deer and birds. Purple loosestrife is an attractive European perennial plant with vibrant colors and thought to have medicinal value. However, its thick stands in wetlands crowd native plants, limiting biodiversity and potentially altering naturally occurring water flow, which can change the wetland hydrology. A mature purple loosestrife plant can produce up to two million seeds a year making it highly prolific.

In addition to the environmental problems invasive and noxious plants cause, they have a financial impact as well. The SHA spends over \$300,000 annually in purchasing herbicides to control exotic weeds. That figure is much larger when factoring in administrative and labor costs. Current practices of pesticide use and mowing are costly and often high impact, causing even further disturbance and thus perpetuating the cycle of colonization by invasive plants. Material, equipment, and fuel are other costs incurred by SHA.

¹ Noxious and invasive weeds are plants that have an adverse effect on other plants/crops, ecosystems, humans and/or animals.

² The presence and/or growth of only one plant species.

The use of biological controls (i.e. using a natural predator such as an insect) to stop invasive plants, could result in the reduction of costs related to chemical and mechanical control measures and lead to more sustainable landscape management practices. Additional benefits include an increase in the richness and diversity of more ecologically beneficial species, improvement in water quality by reduction of runoff, and soil stabilization from year-round vegetation cover, with negligible impacts on food webs and nesting sites.

The Maryland Department of Agriculture (MDA) has an established Integrated Pest Management (IPM) program for weed management in Maryland. In the late 1980's, MDA weed research plots were established at several locations around the State to evaluate IPM of state-regulated *Cirsium* and *Cardus* thistles. More recent research has concentrated on the evaluation of organisms for potential biocontrol, herbicide formulation efficacy, and the use of competitive vegetation (including native grasses and forbes) in an effort to provide environmentally sound and cost-effective methods for suppression of noxious thistle species in Maryland.

Other efforts have demonstrated the benefits of insects in reducing populations of musk and plumeless thistle. Such an approach may be possible for a reduction in Canada thistle. A Native Grass and Forbe Nursery as well as a Germplasm Repository at the MDA Cheltenham facility, serve as a seed source for native plants used in the experiments described above and for right-of-way (ROW) re-vegetation. It also serves as a germplasm bank for many ecotypes of native grass species, including several local Maryland ecotypes. The Weed IPM program has expanded beyond the listed noxious species over the years as invasive weed introductions have increased. Biological control efforts have expanded as this valuable tool has been deployed against purple loosestrife and mile-a-minute weed.

The objective of this study was to develop methods for the use of biological control that would provide SHA with a sustainable, cost effective, and beneficial method for dealing with noxious and invasive weeds on SHA ROW. The MDA propagated and released two biological control agents, *Galerucella* leaf beetles and *Rhinoncomimus latipes* (a weevil or small beetle), at strategic locations and monitored their effects on targeted weed populations. Other biological agents were monitored for their potential to aid in the management of Canada thistle, *Cirsium arvense*.

III. Research Methodology

This study was conducted from April 2010 through December 2011, and included the tasks listed below. The rest of the chapter describes in more detail, the work that was completed by quarter.

Research Tasks:

1. Develop and refine a rearing protocol for *Galerucella* leaf beetles at the MDA greenhouse and quarantine facility and develop and test release strategies for the biological control of purple loosestrife on SHA ROW and associated sites.
2. Develop a strategy to control mile a minute weed, *Persicaria perfoliata*, using the biocontrol agent, *Rhinoncomimus latipes*, a weevil (i.e. small beetle). Develop a greenhouse and laboratory rearing program for *R. latipes* and field test the efficacy of the weevil at sites where mile-a-minute weed is most problematic on SHA ROW.
3. Determine the efficacy of the Canada thistle bud weevil, *Larinus planus*, and the leaf beetle, *Cassida rubiginosa*, at suppression of Canada thistle by monitoring study sites during the two year project period.
4. Document the occurrence and evaluate the spread and impact of the apical chlorosis disease of Canada thistle likely caused by *Pseudomonas syringae* p.v. *tagetes* on SHA ROW.
5. Prepare a final report including the results of these tasks and applications of information obtained relative to SHA weed management strategies.

April 7, 2010 through June 30, 2010:

Work for this project began when overwintered³ adult *Galerucella spp.* beetles were removed from refrigeration at MDA's insect rearing facility and placed in rearing cages. Three cages were started with the overwintered adult beetles. Overwintering mortality ranged from 25-50% of the adult beetles placed in refrigeration.

³ Overwintering describes how insects, plants, and animals survive over the winter. Hibernation is a common method.

Purple Loosestrife or *Lythrum salicaria* plants, were overwintered in coldframes outside of the MDA greenhouse and then brought inside and propagated to increase plants for beetle feeding during the rearing operation. Adult beetles began egg laying on April 12th. By April 29th, two additional adult rearing cages were started, and by May 21st, the first new generation (F1) adults began emerging.

On June 23rd, the first beetle release on SHA ROW occurred at a purple loosestrife infestation in a drainage ditch at SHA's Hanover facility. Over 600 adult beetles were released. The photo to the right was taken during the release.



Information learned during a visit to the New Jersey Department of Agriculture (NJDA) Beneficial Insect Lab in Trenton, in March, was taken into consideration when equipment and supplies were purchased and the MDA rearing facilities in Annapolis were modified. Rearing cages for both weevils and for mile-a-minute plants were constructed. Mile-a-minute plants were grown from seeds at the greenhouse and were also grown from field-collected plants as they began to germinate in the field. Plants were propagated to increase numbers for the weevil rearing program. The first rearing cages were stocked with adult beetles from NJDA on May 27th. The first *Rhinoncomimus* adults (F1) reared at MDA began to emerge on June 21st in low numbers. Additional cages were stocked with adult weevils from NJDA to help quickly increase numbers. Surveys were conducted on SHA ROW sites and two fitting the necessary parameters for release, were identified.

Early season monitoring determined high levels of activity of the Canada thistle bud weevil, *Larinus planus*, but numbers of the leaf beetle, *Cassida rubiginosa* were lower than in 2009. The occurrence of the disease causing apical chlorosis of Canada thistle, likely caused by *Pseudomonas syringae* p.v. *tagetes*, was documented and monitored. Emphasis was placed on identifying new locations where the disease spread, and monitoring the spread of the disease within thistle populations where the disease had been previously identified.

July 1, 2010 through September 30, 2010:

Adult *Galerucella spp.* beetles were reared at the MDA facility. A new rearing cage design was developed and prototypes were built and tested side-by-side with the older type of rearing cage. Purple loosestrife (*Lythrum salicaria*) plant propagation also continued, and an approximate total of 600, one-gallon container plants were grown for feeding the adult and larval beetles at the facility. New releases were conducted at SHA ROW locations in Prince George's County: at I-95 and US 1 in College Park in July, and near the MD 450/202 crossing of the Anacostia River in Bladensburg in June and again in August. These were locations where purple loosestrife infestations were spreading and expanding in size. A total of 717 beetles were released at the College Park location, and 3,488 beetles were released at Bladensburg. Additional releases were also made along county roads in purple loosestrife infestations that had the possibility to impact or spread to SHA ROW sites. During this time, 8,914 adult *Galerucella spp.* beetles were reared and 8,367 beetles were released.

Mile-a-minute weevil, *Rhinoncomimus latipe*, rearing protocol continued to be refined specifically for the MDA rearing facility, and plants were propagated to increase numbers for the weevil rearing program. The first filial generation (F1) *Rhinoncomimus* adults reared at MDA began to emerge on June 21st in low numbers from the rearing cages originally stocked with adult beetles from the NJDA Beneficial Insect Laboratory. The first MDA-reared weevils available in sufficient numbers for field use were released in a small controlled study plot in Greenbelt on August 2nd so they could be closely monitored.

Weevils supplied by the NJDA were used for the first two release sites on SHA ROW and MDA-reared weevils were used for the third site. On July 14th, 500 adult weevils were released at a site in northern Prince Georges County on MD 201 in Beltsville. This site was especially overrun with mile-a-minute weed, which had spread along large expanses of the ROW and into adjacent riparian areas along Indian Creek. These locations are sensitive areas and other methods of control, especially chemical, are limited.

On July 28th, 1,000 adult weevils were released on an infestation of mile-a-minute on the eastbound ramp from MD 32 to I-70 in Howard County. On August 25th, the first MDA-reared weevils were released on SHA ROW; a total of 300 were released on MD 550 in Frederick County between Thurmont and Sabillasville in August and September. All mile-a-minute weevil release sites were then monitored seasonally to determine weevil population establishment and growth and to try and quantify the impact on the mile-a-minute plant populations.

Early season monitoring determined high levels of activity of the Canada thistle bud weevil, *Larinus planus*. However the impact of the beetle activity was not able to be determined due to extreme heat and early senescence of the flower buds at sites where monitoring was conducted. Numbers of the leaf beetle, *Cassida rubiginosa*, were lower than in 2009 and sites were monitored for both herbivore impact as well as thistle and herbivore phenology.

The occurrence of the disease causing apical chlorosis of Canada thistle, likely caused by *Pseudomonas syringae* p.v. *tagetes*, was documented and monitored. New sites where this disease appeared were noted in Garrett County on US 40 near Keyser's Ridge, and along county roads in the southern part of the County.

October 1, 2010 through December 31, 2010:

The final releases of adult *Galerucella* spp. leaf beetles were performed in late September. Active rearing of the adult and larval *Galerucella* spp. leaf beetles was curtailed in mid to late October and over 1,000 adult beetles were put into hibernation in the MDA laboratory for the winter. In 2010, over 15,000 beetles were reared and of those, over 8,600 adult beetles were released as biocontrol agents.

A new cage design was developed and tested side by side with the previous rearing cage model. The new cage proved to be designed for optimal rearing of the beetles and a marked improvement over the older design in terms of efficiency of use. Purple loosestrife (*Lythrum salicaria*) plant propagation was curtailed in mid autumn and a smaller number of healthy plants were prepared to be held over the winter for use as stock plants for propagation in the spring.

Further refinements were made to the mile-a-minute weevil, *Rhinoncomimus latipes* rearing protocol at the MDA rearing facility and mile-a-minute plants continued to be propagated at the MDA greenhouse. In addition to the plants propagated by rooting vegetative cuttings, MDA experimented with propagation of mile-a-minute plants by tissue culture. This method successfully produced viable plants, and the plants appeared to be more robustly branched as compared to conventionally propagated mile-a-minute plants.

The benefits and effects on mile-a-minute weevil rearing continued to be evaluated, however the plants with an increased number of branches were likely to be improved hosts due to the increase in numbers of stems per plant to serve as a developmental resource for larval mile-a-minute weevils. While the total number of released adult mile-a-minute weevils was not large in 2010, rearing was expected to increase in 2011. Rearing equipment repairs were made and refinements and modifications to the rearing protocol continued. Scouting for appropriate release sites on SHA ROW continued until the mile-a-minute plants senesced. Site visits and evaluations of weevil impact in mile-a-minute populations were made until mile-a-minute plant senescence and/or frost caused the plants to die back.

As of the end of the season, weevils were active at nearly every release site (one site had been sprayed with an herbicide), had produced a light to moderate impact on mile-a-minute weed plants at most sites, and in several cases, had spread up to two miles off-site to colonize new patches of mile-a-minute weed. Monitoring of all mile-a-minute weevil release sites continued in 2011. Weevils were reared in small numbers through the winter at the MDA laboratory facility.

Early season monitoring determined high levels of activity of the Canada thistle bud weevil, *Larinus planus*, however ultimate impact on Canada thistle directly due to beetle activity was not able to be determined due to extreme heat and early senescence of the flower buds at sites where monitoring was conducted. The number of leaf beetles, *Cassida rubiginosa* were lower than in 2009. The sites continued to be monitored for both herbivore impact and thistle and herbivore phenology during 2011. Monitoring of the occurrence of the disease causing apical chlorosis of Canada thistle, likely caused by *Pseudomonas syringae* p.v. *tagetes* was curtailed for the season but resumed in 2011.

January 1, 2011 through March 31, 2011:

The 1,000 adult *Galerucella* spp. leaf beetles remained in hibernation until April 2011. The cage design developed during the first year of the study was used to construct additional cages for beetle rearing. Purple loosestrife (*Lythrum salicaria*) plants were held over the winter for use as stock plants for propagation in the spring. Trials continued with tissue culture propagating of purple loosestrife plants in the MDA laboratory.

Mile-a-minute weevil, *Rhinoncomimus latipes*, rearing continued at a low level throughout the winter and mile-a-minute plants continued to be propagated. During the previous quarter and into the this one, experimentation was conducted on growing mile-a-minute plants from tissue cultures. While it was thought that these plants would be more desirable to the weevils, it was ultimately determined that the ochrea (modified leaf at each internode of the stem) of the tissue-cultured plants was slow to develop, and this appeared to negatively affect plant suitability for development of *Rhinoncomimus latipes*. Since the weevil colony had been kept at active, low level numbers over the winter (rather than put in hibernation), it was expected that with an early start, both the MDA colony and the release numbers would increase significantly in 2011.

Selected sites continued to be monitored for both herbivore impact and thistle and herbivore phenology during 2011. Canada thistle bud weevil, *Larinus planus*, thistle rosette weevil, *Trichoseracalus horridus*, thistle head weevil, *Rhinocyllus cornicus*, and the leaf beetle, *Cassida rubiginosa*, continued to be monitored on Canada thistle, *Cirsium arvense*.

April 1, 2011 through June 30, 2011:

In April, rearing cages were prepared for vernalization of overwintered adult *Galerucella* leaf beetles. However, the overwintered population suffered from extreme mortality so the colony was supplemented by 100 adult leaf beetles received from the NJDA Beneficial Insect Laboratory. After a slow start, populations of the beetles increased rapidly over the following weeks. Field releases of *Galerucella* adults were made in Anne Arundel County and in Prince George's County. In Anne Arundel County, 434 adult beetles were released at MD 178 in

Crownsville. In Prince George's County, 1,259 adult beetles were released along MD 150 at the Church Road underpass in Bowie. Scouting for purple loosestrife populations continued, and the presence of new populations and expansion of some existing populations, currently uncolonized, by *Galerucella* was documented in central Maryland and the Eastern shore.

Repairs to the "Percival" incubators were made after a significant equipment failure. Rearing and increase of the weevil colony resumed once the repairs were completed. Mile-a-minute plants continued to be propagated at the MDA greenhouse. Follow-up data collection was performed at sites where weevils were previously released and a survey was conducted for future weevil release sites. Surveys of existing and/or expanding mile-a-minute weed populations continued. Significant expansion of weevil populations at release sites and movement of weevils to new locations, up to 15 miles from documented release sites, was noted. Weevils were released at new locations in Prince George's, Anne Arundel, and Washington Counties. The Washington County release was the first in that County. In Prince George's County, 473 MDA-reared weevils were released on Brooke Lane in Upper Marlboro. In Anne Arundel County, 250 weevils were released at a mile-a-minute weed- and kudzu-infested site on MD 214 near Davidsonville, and in Washington County, 500 weevils were released on SHA ROW on I-70 at the Frederick/Washington County line.

Early season monitoring determined high levels of activity of the Canada thistle bud weevil, *Larinus planus*, at sites in Howard County, and a high percentage of flower buds were infested with bud weevil larvae. The numbers of the leaf beetle, *Cassida rubiginosa*, were somewhat higher than in 2010. Sites continued to be monitored for both herbivore impact and thistle and herbivore phenology during the remainder of the 2011 season. It should be noted, however, that most herbivore activity occurs in the early season.

Monitoring of the occurrence of the disease causing apical chlorosis of Canada thistle, likely caused by *Pseudomonas syringae* p.v. *tagetes*, was performed statewide during this period. In 2011, the incidence of the disease was more prevalent and widespread regionally than in the past. It is undetermined if growing conditions (e.g. rainfall and temperature) contributed significantly to the increased presence of this disease.

July 1, 2011 through September 30, 2011:

The colony of beetles reared at MDA increased rapidly over the summer. A process had been developed for holding adult beetles in cold storage for short periods of time prior to release. This procedure proved to be very advantageous, allowing for strategically planned release of significant numbers of beetles. Purple loosestrife plant propagation continued at the MDA greenhouse with 1,000 fully developed purple loosestrife plants as of September for *Galerucella* colony feeding. New field releases of *Galerucella* adults were completed in Anne Arundel, Howard, Prince George's, and Caroline/Talbot Counties during the reporting period. In Howard County, 455 adult beetles were released on MD 32 eastbound just east of the I-95 underpass. In Anne Arundel County, 1,011 adult beetles were released at MD 32 eastbound at the ramp to MD 295 southbound. In Prince George's County, 1,745 adult beetles were released next to the Anacostia river levee along US 1 in Bladensburg, and in Caroline/Talbot Counties, 1,333 adult beetles were released on purple loosestrife growing in the marsh on the Choptank river at the Dover Bridge on MD 331. Additional beetle releases were completed on non-SHA ROW sites to bring the total number released during this reporting period to 6,563. Scouting for purple loosestrife populations continued and the presence of new populations and expanded populations was documented.

Increase of the mile-a-minute weevil colony continued at the MDA-rearing facility and mile-a-minute plants continued to be propagated. During this period, 1,900 mile-a-minute plants were produced. Significant expansion of weevil populations at release sites and movement of weevils to new locations was again documented. Weevils were released at new locations in Baltimore, Carroll, and Wicomico Counties. All of these releases were the first made in those counties. In addition, an established population was documented in Caroline County at Adkins Arboretum, near Ridgely, MD, likely having spread from an earlier release made at the Eastern Neck Wildlife refuge approximately 15 miles away. In Wicomico County, 347 weevils were released on state forest land near Whitehaven with help from personnel from the Maryland Department of Natural Resources (DNR). While this site was not technically on SHA ROW, the research team believed it important to establish a weevil population on the lower eastern shore, and this was the first release made in that region. In Carroll County, 500 weevils were released on MD 84 just north of Meadow Branch creek. In Baltimore County, a release of 250 weevils

was made at a site on I-83 in the Pikesville area, and a second possible site along I-795 was found to have been already colonized, likely earlier in the year, by weevils naturally dispersing. 250 weevils were also released at a site on MD 550 near Sabillasville to supplement the weevil population established there in 2010. An additional 43 weevils were released at the site in Anne Arundel County on MD 214, which was established during the spring of 2011. Surveys for new populations of mile-a-minute weed continued statewide. Due to heavy rainfall during this period, mile-a-minute weed growth was much more rampant late in the season.

IV. Results and Conclusions

Successful rearing and release of biological control agents was concluded in the fall of 2011. In addition, the monitoring of several biological control agents on Canada thistle concluded at the end of the year and the findings indicated widespread establishment of the insect agents and spread of the occurrence of the disease that causes apical chlorosis.

Rearing protocols developed by the NJDA Beneficial Insect Laboratory were adapted for use at the MDA Insect rearing facility. Refinements were made over a two season timeframe, and numbers of insects reared was increased over that period. Overwintering adult *Galerucella spp.* beetles in an artificial environment was somewhat problematic, with good success one year, and very high mortality of beetles the next. Once rearing was initiated, MDA staff were successfully able to increase beetle numbers and the result was rapid colony increase. Over the two year timeframe, MDA greenhouse staff propagated and grew thousands of purple loosestrife plants for feeding the leaf beetles. In 2010, 15,000 adult *Galerucella* leaf beetles were reared at MDA. Of those, 12,800 were released at six sites. In 2011 another 9,834 adult beetles were reared. Of those reared, over 8,600 were released four times at three sites proximal to SHA ROWs.

Rearing protocols developed by the NJDA Beneficial Insect Laboratory were again adapted for use at the MDA Insect rearing facility. Refinements were made over a two season timeframe, and numbers of insects reared was increased over that period. MDA staff developed a year-round weevil-rearing process that allowed for quick build up of colony numbers earlier in the season. Staff also developed methods for holding weevils in cold storage for several days prior to release so that sufficient numbers could be collected for each planned release. In 2010, when MDA first initiated the rearing program for *R. latipes*, the rearing process began slowly. The first MDA-reared weevils were not ready for release until August of 2011. The research team also supplemented 2010 releases with weevils obtained from the NJDA. By the end of 2010, MDA had reared approximately 1,500 weevils, and released weevils at two SHA ROW sites.

There was greater success in 2011. MDA staff reared over 3,000 weevils. Of those reared, 1,500 were released at six SHA ROW sites. Release numbers were supplemented by an additional 2,000 weevils acquired from NJDA. Weevils were released in three counties where no prior releases had been made: Carroll, Wicomico, and Washington. *R. latipes* has now been released by MDA staff in those counties as well as Anne Arundel, Baltimore, Cecil, Frederick, Montgomery, and Prince George's counties. Future emphasis will continue to focus on releases on the Eastern Shore and in southern and western Maryland. As with other biocontrol agent-rearing programs, MDA greenhouse staff propagated and grew thousands of mile-a-minute plants for the effort.

Monitoring over two years determined high levels of activity of the Canada thistle bud weevil, *Larinus planus*, widespread over most of the range of Canada thistle in Maryland. Specific Canada thistle population impact directly due to the beetle activity was not able to be determined. Due to the widespread distribution and abundance of this herbivore, it is likely that impact on seed development is significant, although not high enough to eliminate individual populations of Canada Thistle. It may however, reduce colonization of new sites due to the reduction in spread of viable seed.

During the study period, the numbers of the leaf beetle, *Cassida rubiginosa*, while high in certain areas, remained relatively low overall. It is speculated that the overall impact of the leaf beetle on Canada thistle is relatively low, as many plants still are able to flower and set seed after early season leaf beetle herbivory. However, coupled with other biocontrol agents, additional stressors, including the leaf beetle, may have a long-term impact on Canada thistle populations.

The occurrence of the disease causing apical chlorosis of Canada thistle, likely caused by *Pseudomonas syringae* p.v. *tagetes*, was tracked during the study. The disease continues to spread and new sites were recorded in several counties including Carroll, Garrett, and Washington. The disease is particularly widespread in Frederick, Howard, Montgomery, and parts of Prince George's counties. This disease is of particular interest in that it can apparently delay and/or reduce flowering and viable seed production. It is hypothesized that mechanical means of transmission and spread of the disease including feeding by certain insects and human

activities, such as mowing, may help spread the disease. Earlier cooperative work by MDA and the United States Department of Agriculture, failed to isolate the causal organism; therefore, inoculation trials have not been performed. The “natural” spread of the disease is promising, as more locations in widely scattered areas of Maryland have been documented. The isolation of the causal agent and the development of a type of “bioherbicide” to be used against Canada thistle would be of great benefit. This organism show much promise and the preliminary findings suggest further research is justified.

Appendix - Project Photos



Galerucella leaf beetle



Galerucella release



One of several SHA ROW *Galerucella* release sites



Galerucella rearing



Purple loosestrife plants at MDA greenhouse



Mile-a-minute Weevils mating



Mile-a-minute weevil rearing at MDA quarantine facility



Mile-a-minute weed propagation at MDA greenhouse



MDA & SHA at release site, Baltimore Co.



Mile-a-minute weed effectively competing with Kudzu



Larinus planus , Canada Thistle Bud Weevil



Canada Thistle Bud Weevil larvae in Canada thistle bud



Cassida rubiginosa larva on Canada thistle leaf

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