Project Abstract
For the Project Ending June 30, 2019

PROJECT TITLE: Biological Control of Canada Thistle
PROJECT MANAGER: Roger Becker
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2015, Chp. 76, Sec. 2, Subd. 06c

APPROPRIATION AMOUNT:	**$300,000**
AMOUNT SPENT:	**$300,000**
AMOUNT REMAINING:	**$0**

Overall Project Outcome and Results

Canada thistle is a serious threat to natural and managed ecosystems in Minnesota. In 1998, the Canada thistle biocontrol stem-mining weevil *Hadroplontus litura* was introduced into a limited area in Minnesota with a resulting decline in Canada thistle populations. Although showing a preference for Canada thistle, initial host range testing of *H. litura* revealed that it attacked other native thistles. Before continuing biocontrol efforts with additional *H. litura* releases in Minnesota, we wanted to clarify whether *H. litura* would attack thistles native to Minnesota. The two objectives of our research were: 1.) determine whether *H. litura* could feed, oviposit and complete development on native thistles, and 2.) determine the phenology of native thistles in relation to Canada thistle. In no-choice tests, female *H. litura* accepted all native thistle species for oviposition and was able to complete development to the adult stage on swamp, field, tall, Flodman's and wavy-leaved thistle. In Hill's and the federally threatened Pitcher's thistle, no adults were found in development tests. However, since more than half of Hill's and Pitcher's thistle plants died during the course of the experiment and it is unclear whether the plants died as a result of *H. litura* attack or other causes. Delayed spring emergence on native thistles could temporally escape *H. litura* oviposition and afford some protection from *H. litura*. However, all tested native thistles could be attacked because they have shoots present when *H. litura* eggs are laid in the spring. In conclusion, we recommend that tests should be conducted in open field conditions to document the ecological host range of *H. litura* prior to the continued released of *H. litura* as a biocontrol agent of Canada thistle in Minnesota.

Project Results Use and Dissemination

Becker, R.L. 2017. Update included as part of a broader presentation: Overview of Canada Thistle *(Cirsium arvense)* Management in Minnesota. Iowa Invasive Species Conference. Honey Creek Resort at Rathbun Lake, Moravia, Iowa. March 28 - 29, 2017.

Becker, R. 2018. Update included as part of a broader presentation: Canada thistle in Minnesota Prairies: Now you see it, now you don't. Webinar hosted by MIPN. International audience (Canada and US). 70 participants. Invited talk, of on 4 in a series. Feb 13 2018. Available online https://www.mipn.org/proceedings/restoration-webinar-series/

Katovich, E., R. Becker, M. Marek-Spartz. 2018. Host Specificity of *Hadroplontus litura* on native *Cirsium* Species. Proc. North Central Weed Science Soc. Hyatt Regency, Milwaukee, WI. Dec. 3–6, 2018. Poster (35)

Katovich ES, RL Becker, M Marek-Spartz, M Chandler, L Van Riper. 2016. Biological Control of Canada Thistle. UMISC. LaCrosse WI Oct 17-19. Poster.

Marek-Spartz, M., E. Katovich, R. Becker, M. Chandler, and L. Van Riper. 2018. Biological Control of Canada Thistle: Host Range of *Hadroplontus Litura* on Native *Cirsium spp.* Presentation at the Upper Midwest Invasive Species Conference. Rochester Convention Center, Rochester MN. Oct. 15-18, 2018.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2015 Work Plan Final Report

Date of Status Update: 08-18/2019

Final Report

Date of Work Plan Approval: 06-11/2015

Project Completion Date: 06-30/2019

PROJECT TITLE: Biological Control of Canada Thistle

Project Manager: Roger Becker

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Location: Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$300,000**
	Amount Spent:	**$300,000**
	Balance:	**$0**

Legal Citation: M.L. 2015, Chp. 76, Sec. 2, Subd. 06c, as extended M.L. 2018, Chp. 214, Art. 4, Sec. 2, Subd. 20

Appropriation Language:
$300,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to develop a biological control for Canada thistle, an invasive plant species in Minnesota. This appropriation is available until June 30, 2018, by which time the project must be completed and final products delivered.

Carryforward; Extension (a) The availability of the appropriations for the following projects are extended to June 30, 2019: (5) Laws 2015, chapter 76, section 2, subdivision 6, paragraph (c), Biological Control of Canada Thistle.

I. PROJECT TITLE: Biological Control of Canada Thistle

II. PROJECT STATEMENT: Canada thistle (*Cirsium arvense*) is native to Eurasia and has been introduced worldwide. It is considered as one of the worst weeds of agricultural and natural systems. In North American, Canada thistle is has been introduced into 42 states, 12 Canadian provinces and has a noxious weed status in 31 states. It is the most prevalent invasive plant in Minnesota and, with a prohibited noxious weed designation, control can be required by law. This results in considerable time and expense to control this weed on state lands. Absent biological control, currently available control options include herbicides, mowing, or tillage. These control methods can harm desirable plants and interfere with or alter wildlife management practices.

Canada thistle is a herbaceous perennial plant, with aboveground shoots dying back over the winter and underground roots surviving from year to year. Plants reproduce through seed dispersal and vegetatively via spreading underground lateral roots. Canada thistle plants are dioecious, with male and female flowers produced on separate plants. Flowers are pollinated by honeybees and other native pollinators. Seeds are attached to a plumose achene that can aid in dispersal.

In North America, the biological control agent and stem-mining weevil, *Ceutorhynchus litura*, was first introduced into Canada in 1965. It was subsequently introduced into the United States in 1972, with the first released in Montana. *C. litura* has since been established in Idaho, Montana, Nebraska, North Dakota, Oregon, Utah, Virginia, Washington and Wyoming. In 1998, *C. litura* was introduced into a limited area in Minnesota, with a resulting decline in Canada thistle populations long-term.

Ceutorhynchus litura adults overwinter in leaf litter, and begin to feed on Canada thistle leaf and stem tissue in early spring (April and May). Females oviposit in the mid-vein on the underside of leaves on rosette shoots. Larvae mine leaves, stems and crowns of Canada thistle plants throughout the spring and summer. Third instar larvae emerge from Canada thistle plants in late summer, pupate in the soil, and emerge as adults from July to October, depending on location. There is one generation per year.

There are conflicting reports about the efficacy of *C. litura* as a biocontrol agent against Canada thistle. Some have reported that *C. litura* did not control thistle stands, but could contribute to a decline in thistle populations when combined with other plant stressors, such as other insects or pathogens. It also has been reported that *C. litura* infestations did not reduce thistle stem counts, flowering or overwinter survival in Canada thistle stands on two South Dakota wildlife refuges over four years of study.

In contrast, others have found 75 to 92% of Canada thistle stems infested with *C. litura* larvae at four sites 15 years after release. Underground roots suffered higher winter mortality rates as a consequence of *C. litura* larval mining. Adults dispersed 9 km over 15 years. Significant declines in Canada thistle abundance were also documented after ten years when *C. litura* was released in combination with the gall forming fly, *Urophoa cardui*, and the seed-head weevil, *Larinus planus*. Total non-structural carbohydrates were 1.5 times lower on early season sampling in Canada thistle roots after attack by the three biocontrol insects mentioned previously, plus the leaf defoliator, *Cassida rubiginosa*. Similar reduced levels of free sugars and fructans were found in Canada thistle roots after spring larval mining. However, sugar levels recovered later in the summer. Lastly, competition from the native, cool-season, needle and thread grass (*Hesperostipa comata*) in addition to *C. litura*, has been shown to reduce Canada thistle root biomass. The combination of cool-season grass competition with *C. litura* may compliment restoration methods over each agent alone.

The host range of a weed biological agent is defined as the set of plant species attacked by the agent. In North America, *Ceutorhynchus litura* attacks Canada thistle (*Cirsium arvense*), although its host range includes the *Cirsium-Silybum-Carduus* complex of the Asteraceae subtribe Carduinae. In North America, there are no native *Carduus* or *Silybum* species, but there are at least 118 native species of *Cirsium.* Initial host range testing indicated that *C. litura* fed on the natives, *Cirsium brevistylum, Cirsium undulatum* and *Cirsium flodmanii*. Slotta and colleagues found that the host range of Canada thistle biocontrol insects, *Larinus planus* and *Rhinocyllus conicus*, did not follow phylogenetic lines developed for *Cirsium* species derived from native *Cirsium* DNA - sequences. Therefore, they recommend a more comprehensive list of *Cirsium* species should be included in host range testing of Canada thistle biological control insects.

In 1998, the stem-mining weevil, *C. litura,* was introduced into a limited area in Minnesota with a resulting decline in Canada thistle populations, generating interest in supporting a biological control effort with *C. litura*. Before we can support additional release of this biocontrol weevil in Minnesota, we need to determine whether *C. litura* will attack Minnesota's native thistles. If *C. litura* does not develop on our native thistles, a program to augment and support biological control of Canada thistle with *C. litura* can be implemented in Minnesota to provide cost-effective, long-term management of Canada thistle in Minnesota's natural areas. This project will determine the host range of *C. litura* on Minnesota's native *Cirsium* species. The first objective of our research is to determine whether *Cirsium* spp. native to Minnesota are attacked by *C. litura*. Specifically, we will investigate whether *C. litura* can feed, oviposit and complete development on native *Cirsium* spp. This project will help to define whether the host range of *C. litura* includes Minnesota's native *Cirsium* species. The second objective of this research is to determine the phenology of *C. litura* in Minnesota. This information will be invaluable for the implementation of a future Canada thistle biocontrol program.

III. OVERALL PROJECT STATUS UPDATES:

Amendment Request (12/23/2015): Amendment approved by LCCMR 1-8-2016

We request to drop Agassiz National Wildlife Refuge as one of two sites to complete **Activity 3: Determine Phenology of *Hadroplontus litura* in Minnesota.**

This change to the work plan is needed because of our findings in 2015 at Agassiz. We made two trips to the Agassiz National Wildlife Refuge in the spring and summer of 2015. We timed our trips to coincide with adult activity periods of *H litura.* During both trips, we surveyed the known release sites of *H. litura*. Despite several findings of the Canada thistle gall fly, *Urophora cardui*, we were unable to collect any *H. litura* adults at all *H. litura* release sites. Because we were unable to collect any adult *H. litura*, and few Canada thistle plants remain at release sites, we concluded that it would be difficult to determine the phenology of *H. litura* at Agassiz National Wildlife Refuge. In addition, since the original work plan was written, Gramig et al. (2015) reported that collecting third instar larvae and adult *H. litura* from study sites in eastern North Dakota was "extremely challenging". As a result, the authors were unable to determine and predict emergence time of adults. This amendment request will not require any budget changes as the current travel budget and salary will not be impacted by these changes in our work plan. See Activity 3 for changes based on this request.

We will complete the research objectives in Activity 3, but request it be amended to rely on the University of Minnesota St. Paul Campus site for 2016 on for the definitive work on thistle and *H. litura* phenology. We can determine the phenology of *H. litura* at the St. Paul Campus site for the early spring 2016 data collection period, where we have adult weevils overwintering on individually caged Canada thistle plants. In 2016, we will double the number of caged plants with weevils at St. Paul to make up for the loss of data at the Lake Agassiz site.
We were going to rely on salaried personnel at the University of Minnesota Roseau station to do future work at Agassiz, so travel will not change as we will not be conducting fewer trips to Agassiz. The salary amount would have been used weather conducted at Agassiz or at the St. Paul campus.

An aside, *Ceutorhynchus litura* is now reclassified as *Hadroplontus litura*, and will be call *H. litura* in future reports.

Project Status as of January 1, 2016:
We began work *pro bono* in the fall of 2014 to obtain seed sources and establish plants in the greenhouse and in field plots in the spring of 2015. The perennial and biennial biology of these thistles meant considerable lead-time was needed to have plants of various life-cycle stages available during the funded period of study. Absent this preparatory work, performing the funded work beginning July 1, 2015 through June 30, 2018 would not be possible. Thistles of all *Cirsium* species native to Minnesota, the federally and state listed threatened species, dune thistle, and Canada thistle have been established on campus.

Hadroplontus litura (formerly (*Ceutorhynchus litura*) adults were purchased from Biological Control of Weeds Inc., Bozeman, MT in early July, 2015 and a *H. litura* nursery has been established on the St. Paul Campus. In July, 2015, we established a common garden of native and Canada thistle to conduct the comparative phenology studies transplanting seedlings or plants of thistles into a field block on the University of Minnesota St. Paul Campus. In October, 2015, seeds of biennial thistles were planted in the thistle common garden to ensure a continuation of biennial thistles after the summer of 2016. Two trips to the Agassiz National Wildlife Refuge were completed in the spring and summer of 2015. We timed our trips to coincide with adult activity periods of *H litura.* At all release sites, we were unable to collect any *H. litura* adults. In addition, few Canada thistle plants were present. Because we were unable to collect any adult *H. litura*, and because we found few Canada thistle plants at release sites, we concluded that it would be difficult to determine the phenology of *H. litura* at Agassiz National Wildlife Refuge as proposed in the work plan. We conclude that we need to amend the plan of work to expand the proportion of this effort conducted at the St. Paul Campus and reduce the effort at Lake Agassiz NWR to be able to study the phenology of *H. litura* and native *Cirsium*.

Project Status as July 1 2016:
The plan of work amendment request to focus H litura work at the St. Paul Campus and reduce effort at Lake Agassiz National Wildlife Refuge was approved. In spring of 2016 on the St. Paul Campus, sequential no-choice oviposition tests were conducted with *Hadroplontus litura* adults overwintered in caged Canada thistle plants. In March, 2016, we began to collect weekly phenology data from *H. litura* and native thistles planted in a common garden on the University of Minnesota campus.

Project Status as of January 1, 2017: All native thistles have been established on the St. Paul Campus and have been prepped to overwinter, many with a pot-in-pot technique perfected on our garlic mustard biological control efforts, historically funded by LCCMR. Since Hill's thistle is designated a Minnesota Species of Concern, a permit was obtained and live rosettes of Hill's thistle obtained form the Nature Conservancy's Ordway Prairie to transplant into our St. Paul nursery as we were unsuccessful in getting seeds obtained under permit from the same prairie to germinate. We have made progress on sequential no-choice oviposition specificity testing of *H. litura* on all but the Hill's thistle to date. Preliminary results show female *H. litura* oviposited on all native thistles. No-choice larval development tests in 2017 will determine if this oviposition behavior is of concern. We have collected the first year of phenology data on *H. litura* in Minnesota related to phenology of native thistles in our common garden established on the St. Paul campus.

Amendment Request (01/24/2017): Amendment Approved by LCCMR 1/30/2017
I am requesting that $2,200 be shifted to Lab/Medical supplies by taking $200 from Lab/Medical Services, and $2000 from Travel. We have had to purchase more of the biological control agent *Hadroplontus litura* insects than anticipated to conduct the research and establish colonies at the St. Paul campus, and the insects are costing more than initially anticipated. Purchase of insect charges were made to Lab/Medical supplies. We anticipate we may need to order more insects, depending on survival and health of the *H. litura* colony we have established on the St. Paul campus by next spring. The insects are integral to all activities in his grant and the cost shifts occur in all 3 activities. The $200 should be available from Lab/Medical Services based on cost accrued to date for land use, watering, etc. The $2000 in Travel monies are available as I made the decision to go to Agassiz in June 2015 before the grant began as the biology of *H. litura* was progressing such that we could not be certain that observations and collections would still be optimal in July when the grant started. We also went in July 2015, but did not ask for reimbursement for travel to Agassiz on either occasion ($1,581), or two trips to the Ordway Prairie ($298) when our project pickup was used. I will cover travel costs to conduct this research that is not covered by this grant with unrestricted gift monies from my project accrued for activities unrelated to this grant. This budget amendment is for 0.7% of the total budget of $300,000. An aside, we also incurred considerable travel costs to collect seed of native thistles in the fall of 2014 and in 2015 before the grant began in July of 2015 to be able to start the project in the spring of 2015 so the biology still works within the confines of the grant calendar that I am covering internally. Language in the work plan does not need to be changed further as the work will still be done as proposed. The accompanying budget document has been changed to reflect these changes.

Project Status as of July 1, 2017: Life-cycle completion tests were initiated with tall, Flodman's, swamp and field thistle. Single-choice oviposition tests were completed with tall, Flodman's, swamp and field thistle. The second year of phenology data on *H. litura* and thistles is in progress. We are collecting the second year of phenology data on *H. litura* in Minnesota related to phenology of native thistles in our common garden established on the St. Paul campus. Despite successfully establishing the field common garden and protected nursery plants the previous winter, loss of plants in our *Cirsium* collection due to winterkill was widespread in the field common garden, and in the protected nursery planting, we lost all species of all native *Cirsium* due to predation, likely by small mammals. The invasive Canada thistle, of course survived everywhere. Re-establishing the native collections has required continuation of effort in Activity 1.

Amendment Request (12/28/2017): Amendment Approved by LCCMR 1/9/2018
I am requesting to move $800 from salaries to Lab/Medical Services. In August of 2017, we again needed to purchase more of the biological control agent *Hadroplontus litura* insects than anticipated to conduct the research to maintain the colonies at the St. Paul campus ($652.50). Purchase of insect charges were made to Lab/Medical supplies. We anticipated we may need to order more insects, depending on survival and health of the *H. litura* colony we have established on the St. Paul campus in 2017. Salaries, while not yet showing all charges in the system to end 2017 should be on target to allow this shift. This budget amendment is less than 0.3% of the total budget of $300,000. An aside, we continue to incur travel costs to re-collect seed and rosettes of native thistles this year to rebuild the nursery that I am covering internally. Language in the work plan does not need to be changed further as the work will still be done as proposed. The proposed changes are added to the accompanying budget document. We will update with just the amended budget if confirmed as the way to proceed.

Amendment Request (12/28/2017): Amendment Approved: May 30, 2018
We are also requesting an extension to June 30, 2019 to complete the work and final reporting. This is in part due to the loss of plants overwintering from 2016-17. Since the thistles are biennial and perennial and we re-established plants in 2017, more data than originally proposed will be collected in the growing season of 2018. Additionally, several of the surviving native thistles that we have been able to monitor completed the flowering and seed maturation stages considerably later than Canada thistle, extending into September. To follow all species through their complete life-cycles in 2018, we need to extend our trials past the June 30, 2018 timeline.

Project Status as of January 1, 2018. Life-cycle development tests were completed for tall, Flodman's and field thistle and partially completed for swamp thistle. Adult *H. litura* emerged from tall, Flodman's and field thistle, indicating that these species are able to act as hosts for *H. litura*. Single-choice oviposition tests were completed with tall, Flodman's, dune and field thistle. In these tests, *H. litura* females were able to choose whether to lay eggs in Canada thistle or test plant leaves when presented with leaves of the two species simultaneously. Eggs were found in all native thistles tested. These results indicate that *H. litura* females will accept the native thistles as hosts for oviposition, even in the presence of Canada thistle. Effort continues on Activity 1 due to considerable difficulty in successfully over-wintering plants and losses due to predation. Since all of our *Cirsium* species are biennial or perennial, multiple seasons are required to grow plants to stages for testing. All species of native thistles were re-established in 2017 from seed or field-collected over-wintered rosettes. The phenology of native *Cirsium* and Canada thistle was followed for the second year in our native thistle common garden. As in 2016, native thistles initiated spring growth approximately three weeks earlier than Canada thistle.

Project Status as of July 1, 2018: Successfully cultivating all six native *Cirsium* species at one site in a common garden to enable comparative phenology data has proven the biggest challenge in this study. These thistles are biennials or perennials requiring multiple years to grow plants to study. Some are likely adapted to soil and climate very different than that at our St. Paul site. Winter-kill and rodent predation on thistle crowns during the first winter decimated the plant test materials. The noxious weed Canada thistle of course, is doing very well. We were granted a no-cost extension to June 30, 2019 to enable cultivating additional thistles building on tricks we have learned to ensure survival. We had improved survival this past winter so we can continue our research activities. Hill's thistle continues to be one of the most challenging *Cirsium* to cultivate. Despite these setbacks, we are on target to achieving the primary goal of our research, defining the host specificity of *Hadroplontus litura* as a biological control agent for Canada thistle.

Project Status as of January 1, 2019: We successfully finished life-cycle completion tests on all thistle species, with the exception of wavy-leaved thistle. Wavy-leaved thistle plants failed to overwinter. This species does not appear to successfully overwinter in St. Paul, as it did not survive in our common thistle garden either year. Results show that *H. litura* was able to complete development on swamp, Flodman's, field, and tall thistle in no-choice development tests. These *Cirsium* spp. are within the fundamental host range of *H. litura*. In no-choice oviposition tests, female *H. litura* laid eggs on both Hill's and dune thistle, but no adults emerged in development tests. In addition, single-choice oviposition tests were conducted with all native *Cirsium* spp. For these tests, *H. litura* females were able to choose whether to lay eggs in Canada thistle or native *Cirsium* spp. leaves when presented with leaves of the two species simultaneously. These results indicate that *H. litura* females will accept all native thistles tested as hosts for oviposition, even in the presence of Canada thistle. With the exception of single-choice oviposition tests, all trials were no-choice, where *H. litura* adult females were not able to choose their host for oviposition. These trials represent the most conservative type of host-range test. Field tests, where *H. litura* females are able to select their host through normal host acceptance behavior, could provide an additional assessment and help define the ecological host range of *H. litura* on native *Cirsium* spp.

Amendment Request (01/18/2019): Amendment Approved by LCCMR 1/24/2019
I am requesting within Activity 2 to move $400 to Equipment /Tools/ Supplies and $155 to travel, both from Personnel, and within Activity 3 to move $243 to Equipment /Tools /Supplies from Personnel. The overall budget total remains the same and activity totals remain the same. We spent a little more than anticipated in Equip/Tools/Supplies in both Activities 2 and 3 because we keep experiencing thistle mortality so we had to rebuild the colonies which required more wire mesh, pots, etc. Additionally, for Travel in Activity 2, for Hills thistle, the Special Concern species for MN, we had to do an unanticipated trip to the Ordway Prairie TNC site to collect yet another round of Hill's thistle with the generous permitting by TNC to try again to propagate this species in our common garden on the St. Paul Campus.

Overall Project Outcomes and Results: Canada thistle is a serious threat to natural and managed ecosystems in Minnesota. In 1998, the Canada thistle biocontrol stem-mining weevil *Hadroplontus litura* was introduced into a limited area in Minnesota with a resulting decline in Canada thistle populations. Although showing a preference for Canada thistle, initial host range testing of *H. litura* revealed that it attacked other native thistles. Before continuing biocontrol efforts with additional *H. litura* releases in Minnesota, we wanted to clarify whether *H. litura* would attack thistles native to Minnesota. The two objectives of our research were: 1.) determine whether *H. litura* could feed, oviposit and complete development on native thistles, and 2.) determine the phenology of native thistles in relation to Canada thistle. In no-choice tests, female *H. litura* accepted all native thistle species for oviposition and was able to complete development to the adult stage on swamp, field, tall, Flodman's and wavy-leaved thistle. In Hill's and the federally threatened Pitcher's thistle, no adults were found in development tests. However, since more than half of Hill's and Pitcher's thistle plants died during the course of the experiment and it is unclear whether the plants died as a result of *H. litura* attack or other causes. Delayed spring emergence on native thistles could temporally escape *H. litura* oviposition and afford some protection from *H. litura*. However, all tested native thistles could be attacked because they have shoots present when *H. litura* eggs are laid in the spring. In conclusion, we recommend that tests should be conducted in open field conditions to document the ecological host range of *H. litura* prior to the continued released of *H. litura* as a biocontrol agent of Canada thistle in Minnesota.

IV. PROJECT ACTIVITIES AND OUTCOMES:

<u>ACTIVITY 1:</u> Collect and develop the techniques to successfully grow Canada thistle and native thistles in phenological synchrony with each other and with *C. litura* to allow specificity testing.

Description: The University of Minnesota herbarium lists six thistles in the *Cirsium* genus as native to Minnesota (Table 1). A seventh species, *Cirsium x Iowense*, is also listed as native but it is now considered synonymous with *Cirsium altissimum* (tall thistle). We will collaborate with the Minnesota Biological Survey to locate sources for each of Minnesota's native thistles. *Cirsium* plants will need to be established the summer prior to host range testing as *C. litura* adults are active and oviposit in the spring. In spring and summer of 2015, we will collect roots or stems of perennial *Cirsium* species (Table 2). Two years prior to testing, seeds will be collected from biennial *Cirsium* species, planted and rosettes overwintered outside. Seedlings (for biennials) or plant parts (for perennials) will be planted into 3-gallon pots using a standard potting mix and greenhouse soil in a 1:1 ratio. Plants will be fertilized as necessary. Potted thistle plants will be overwintered using the pot-in-pot method to ensure winter survival. This technique is similar to that used by researchers to overwinter potted

Canada thistle plants in Regina, Saskatchewan. Multiple plants of each species will be established so that replicated host-range field trials can be conducted. Scientist and technical staff at the University of Minnesota will conduct this work.

Table 1. Native Thistles (*Cirsium* spp.) of Minnesota

Scientific Name	Common Name	Life Cycle	Status
Cirsium altissimum	tall thistle	biennial	
Cirsium discolor	field thistle	biennial	
Cirsium flodmanii	Flodman's thistle	perennial	
Cirsium x Iowense		perennial	(Considered synonymous with *Cirsium altissimum*)
Cirsium muticum	swamp thistle	biennial	
Cirsium pumilum var. Hillii	Hill's thistle	perennial	Species of Special Concern-MN
Cirsium undulatum	wavy-leaved thistle	perennial	Native to IA, ND, SD, WI,

Table 2. Time table for *Cirsium* species collection and host range testing. St. Paul, MN

Scientific name	Common name	Life-cycle	Seed collected	Roots/stems collected	Planted into pots	Host range testing
Cirsium altissimum	tall thistle	biennial	Fall 2014 (*pro bono*) Fall 2015		Spring 2015 (*pro bono*) Spring 2016	Spring 2016 Spring 2017

Scientific name	**Common name**	**Life-cycle**	**Seed collected**	**Roots/stems collected**	**Planted into pots**	**Host range testing**
Cirsium discolor	field thistle	biennial	Fall 2014 (*pro bono*) Fall 2015		Spring 2015 (*pro bono*) Spring 2016	Spring 2016 Spring 2017
Cirsium muticum	Swamp thistle	biennial	Fall 2014 (*pro bono*) Fall 2015			Spring 2016 Spring 2017
Cirsium flodmanii	Flodman's thistle	perennial		Summer 2015	Summer 2015	Spring 2016
Cirsium pumilum var. *Hillii*	Hill's thistle	perennial		Summer 2015	Summer 2015	Spring 2016
Cirsium undulatum	wavy-leaved thistle	perennial		Summer 2015	Summer 2015	Spring 2016
Cirsium arvense	Canada thistle	perennial		Summer 2015	Summer 2015	Spring 2016

Summary Budget Information for Activity 1:	**ENRTF Budget:**	**$ 60,000**
	Amount Spent:	**$ 60,000**
	Balance:	**$ 0**

Outcome	**Completion Date**
1. Collect thistle plants and/or seeds. Thistle seed or vegetative propagules will need be located and collected at that appropriate time to ensure successful, replicated propagation. Propagation of Canada thistle is understood and collection sites are numerous. The native thistles are not common on the landscape and collection times will vary and will be defined to ensure consistent, replicated propagation under controlled conditions. We will work with Laura Van Riper (Terrestrial Invasive Species Coordinator), Welby Smith (State Botanist), and Dan Wovcha (Plant Ecologist) with the Minnesota Department of Natural Resources to locate potential sites for collection of native thistles. Hill's thistle poses the most potential difficulties to locate and successfully propagate to enable testing at the correct phenological synchrony to conduct valid host specificity testing. We will find suitable populations of Hill's thistle and develop techniques to do valid testing.	November 30, 2015 - Completed
2. Develop techniques to successfully establish and overwinter each of the *Cirsium* species. We will document the growth and development phenology of each species. Despite public perceptions, growing native thistles and Canada thistle under controlled conditions to enable replicated, valid testing is not a simple task. For example, approx. two years of experimenting with overwintering techniques of container grown garlic mustard were required before we could repeatedly overwinter plants of the quality and phenological synchrony required for valid host specificity testing. We anticipate similar challenges with the various *Cirsium* species, and are currently beginning some techniques testing *pro bono* because we do not have two years to develop mass propagation techniques on this grant.	December 31, 2015 - Completed
3. Overwinter thistle plants of each species of suitable quality in preparation for spring host range tests. We will be able to report on the success or challenges that need to be overcome by the first spring of the grant.	March 31, 2016 - Completed (except Hill's thistle)

Activity 1 Status as of January 1, 2016:

Outcome 1. Collect thistle plants and/or seeds. We have completed Outcome 1 on time, with the caveat that the suitability of the methodology to rear and synchronize phenology of Hill's thistle will require the growing season of 2016 to validate that our approach will work. In the fall of 2014, we collected seeds of tall, and field thistle from local sources. Seeds of swamp thistle were purchased from a local native plant source. Wavy-leaf thistle seeds were obtained from USDA-National Genetic Resources Program via Germplasm Resource Information Network (GRIN). Seeds of the federally and state listed threatened species, dune thistle (*Cirsium pitcheri* (Torr. ex Eaton) Torr. & A. Gray) were generously provided by Kay Havens of the Chicago Botanic Gardens. Flodman's thistle plants were purchased from a native plant nursery in west central Minnesota. Canada thistle plants were collected on the University of Minnesota, St. Paul Campus. Additionally, we used native species inventory data obtained from Minnesota Biological Survey to locate sources of native thistle. In summer of 2015, we obtained permission from The Nature Conservancy and collected Hill's thistle seeds, a listed species of concern in Minnesota, from the Ordway Prairie in west central Minnesota. Personnel at the Burnham Wildlife Management Area, near Crookston, Minnesota, collected swamp thistle seeds. Field/tall thistle was collected at the Lac Qui Parle Wildlife Management area, near Appleton, Minnesota. Thistles of all *Cirsium* species native to Minnesota and Canada thistle have been established on campus.

Outcome 2. Develop techniques to successfully establish and overwinter each of the Cirsium *species.* We have completed Outcome 2 on time having established all *Cirsium* species the summer of 2015, to ensure we had perennial or biennial stages of each species available for host range testing in 2016, as *H. litura* adults are active and oviposit in the early spring. Perennial *Cirsium* plants; Canada, Flodman's, and wavy-leaved thistle were established in the summer of 2015. Seeds of the biennial *Cirsium* thistles; swamp, tall, and field, were planted and overwintered in 2014/2015. In spring of 2015, all seedlings or plants were planted into 3 gal pots using a standard potting mix and greenhouse soil in a 1:1 ratio. Plants were fertilized as necessary. In July, 2015, tall and field thistle plants were removed from pots and planted in a field on the University of Minnesota, St. Paul Campus as they grew too large for their pots. Potted thistle plants were overwintered in 2014/2015 using the pot-in-pot method to ensure winter survival (Katovich unpublished data). This technique is similar to that used by Peschken and Derby (1992) to overwinter potted Canada thistle plants in Regina, Saskatchewan. Multiple plants of each species were established so that replicated host-range field trials can be conducted. The caveat here is that this will be the first winter for some, the second for other thistles. Each winter provides a unique environment that may cause winter-kill in perennial or biennial herbaceous plants, so our techniques will be validated each winter of study.

Activity 1 Status as of July 1, 2016:
Outcome 3. Overwinter thistle plants of each species of suitable quality in preparation for spring host range tests. We will be able to report on the success or challenges that need to be overcome by the first spring of the grant.

We successfully overwintered all species except Hill's thistle in-ground or in pots in the nursery - both on the St. Paul campus. We were unsuccessful in getting Hill's thistle seed to germinate and are seeking a permit to remove seedlings from the TNC Ordway Prairie in July to establish living plants on campus to enable specificity and phenology testing.

Activity 1 Status as of January 1, 2017: Only one Hill's thistle plant germinated from seed after undergoing stratification treatment. For this reason, we applied for and obtained a permit to harvest Hill's thistle on the Ordway Prairie from the Nature Conservancy in June 2016. On July 7, 2016, we collected Hill's thistle rosettes from the Ordway Prairie and immediately transplanted them on-site into pots. Rosettes were propagated during the summer and pots were dug into the soil using the pot-in-pot technique and mulched with straw for overwintering. If they survive, we will include Hill's thistle in sequential no-choice oviposition tests and no-choice development tests in spring, 2017.

All other species of thistles were grown in pots during the summer of 2016. In the fall, all native thistles were dug into the ground using the pot-in-pot technique for overwintering. Plants were mulched with straw in November 2016. Flats of all native thistles were planted in November 2016 and placed outside in raised beds and mulched with straw for overwintering. This will ensure that we have a supply of native thistle plants and seedlings for testing spring of 2017.

Activity 1 Final Report Summary: Despite successfully establishing the field common garden and protected nursery plants the previous winter, loss of plants in our *Cirsium* collection due to winterkill was widespread in the field common garden in the winter of 2016-2017. In the protected nursery planting, we lost all species of all native *Cirsium* due to predation, likely by small mammals in the winter of 2016-2017. The invasive Canada thistle, of course survived everywhere. We had stratified seed and seedlings available to re-establish the common garden and collected field-grown rosettes of some species to conduct host range tests. We will use protective wire mesh over pots in the nursery this coming winter, and attempt to have more cover in the field common garden to protect plants in the event of winterkill conditions next winter.

Activity 1 Status as of January 1, 2018. All thistle species, including a limited number of Hill's thistle (four plants), were successfully propagated in pots during the summer of 2017 to re-establish the nursery. Seed was successfully produced and collected from all plants for future work. Plants were dug into the ground for overwintering. To protect against predation that killed all the native thistles that occurred during overwintering from 2016 to 2017, all native thistles were caged with hardware cloth designed to exclude rodents and larger predators. Plants were mulched with straw for overwintering. It continues to be difficult to collect viable seed of

Hill's thistle, and once collected and determined to include viable seed, difficult to germinate. We appreciate the continued support and permits from The Nature Conservancy to acquire seed from the Ordway Prairie.

Activity 1 Status as of July 1, 2018: All native thistle plants successfully overwintered, with the exception of wavy-leaved thistle. The hardware cloth cages installed over the pots last fall prevented animals from eating the rosettes over the winter. We obtained a permit from the Nature Conservancy and in May 2018 and collected additional Hill's thistle rosettes from the Ordway Prairie to complete our host range studies. We would like to thank the Nature Conservancy for their continued support of our project.

Activity 1 Status as of January 1, 2019: All of our thistle species, with the exception of wavy-leaved thistle, successfully overwintered. We obtained a permit from the Nature Conservancy and in May 2018 and collected additional Hill's thistle rosettes from the Ordway Prairie to complete our host range studies. We would like to thank the Nature Conservancy for their continued support of our project. As a result of successful overwintering of these native thistles, we were able to complete our host range testing with the native *Cirsium* spp.

Activity 1 Final Report Summary: Same as **Activity 1 Status as of January 1, 2019 above.**

ACTIVITY 2. Determine whether *Ceutorhynchus litura* attacks thistles native to Minnesota.

Overview: *Ceutorhynchus litura* adults will be purchased from Biological Control of Weeds Inc., Bozeman, MT in the late summer or fall preceding the spring of host range testing. Weevils will be overwintered outside on caged Canada thistle plants. Two separate studies will be conducted with each native thistle species in replicated trials to include; sequential no-choice feeding and oviposition (egg-laying) and life-cycle completion experiments. All tests will also be conducted on Canada thistle as a control plant. Scientist and technical staff at the University of Minnesota will complete this work. The methodology of each test is described below.

Sequential no-choice feeding and oviposition tests. Tests will be conducted in the spring and early summer when *C. litura* females are laying eggs. Procedures are similar to those described by Esther Gerber at CABI, Delémont, Switzerland, for *Ceutorhynchus scrobicollis*. Prior to inclusion in oviposition tests, females will be tested to ensure that they are laying eggs. Only ovipositing females will be used in experiments. An excised Canada thistle or test plant leaf will be inserted into a hydrated piece of florist foam encased in a self-sealing plastic bag. Leaves will be a minimum of 5 cm in length as *C. litura* does not oviposit on leaves shorter than 5 cm. The leaf will be placed into a pint Mason jar and covered with nylon mesh. A mating pair of *C. litura* will be placed into the jar. After 3- to 4-days, leaves and stems will be dissected and checked for eggs. Feeding will be recorded. The test plant leaf will be replaced with a Canada thistle leaf to ensure *C. litura* females are ovipositing, and leaves will dissected after allowing 3- to 4-days for oviposition. A minimum of 10 replications will be completed.

Life-cycle completion tests. Prior to inclusion in these trials, females will be tested to ensure that they are laying eggs. Only ovipositing females will be used. In late March to early April, 5 marked female and 5 male *C. litura* will be placed on each potted thistle plant placed in a screen cage. After a period of two to three weeks, adults will be removed. In late summer, plants will be checked for F-1 adults. Number of adults collected from each plant will be recorded. All plants will then be dissected after adult emergence and checked for larval mining and tunneling. All plants will be grown outside and covered with nylon mesh bags to contain the *C. litura* during testing. Canada thistle plants will be used as a control plant species.

Summary Budget Information for Activity 2:

ENRTF Budget:	**$ 120,000**
Amount Spent:	**$ 120,000**
Balance:	**$ 0**

Outcome	Completion Date
1. Conduct host range studies. *Ceutorhynchus litura* (name changed to *Hadroplontus litura*) adults will be purchased from Biological Control of Weeds Inc., Bozeman, MT and successfully overwintered outside on caged Canada thistle plants. Two separate studies will have been conducted with each thistle species in replicated trials including sequential no-choice feeding and oviposition (egg-laying) and life-cycle completion experiments. Results of all tests on Canada thistle as a control plant will also have been conducted. Scientist and technical staff at the University of Minnesota will complete this work. The methodology of each test is described above.	July 1, 2018

Activity 2 Status as of January 1, 2016:
Progress towards Outcome 1, Conduct host range studies. *Hadroplontus litura* adults were purchased from Biological Control of Weeds Inc., Bozeman, MT in early July 2015. Adult weevils were placed on caged Canada thistle plants and plants were sub-irrigated to prevent the drowning of adults. Adults are overwintering outside on caged Canada thistle plants dug into the ground using the pot-in-pot method. In the spring, two separate studies will be conducted with each thistle species in replicated trials to include; sequential no-choice feeding and oviposition (egg-laying) and life-cycle completion experiments. All tests will also be conducted on Canada thistle as a control plant.

Activity 2 Status as of July 1, 2016:
In spring of 2016 on the St. Paul Campus, sequential no-choice oviposition tests were conducted with *Hadroplontus litura* adults overwintered in caged Canada thistle plants. Ten replications of the oviposition test were conducted for each thistle species, with the exception of Hill's thistle. We collected Hill's thistle seed in August of 2015, so did not have established plants to test in spring 2016. Results of sequential no-choice oviposition tests are as follows, *H. litura* oviposited eggs into shoots of all thistle species. These results indicate that *H. litura* will accept all tested native thistles for oviposition. The next step in *H. litura* host range testing will be concluded next summer when we conduct no-choice larval development tests. These tests will determine whether the eggs laid by *H. litura* will be able to complete development on native thistles. Additional *H. litura* adults will be purchased from Biological Control of Weeds Inc., Bozeman, MT in early July 2016.

Activity 2 Status as of January 1, 2017: Sequential no-choice oviposition tests were completed in spring, 2016 and were reported in our July 1, 2016 Update. All species of native thistle were tested except Hill's thistle. Steps are underway to successfully propagate Hill's thistle for testing. *Hadroplontus litura* eggs were found in shoots of all native thistle species as reported in July of 2016. These results indicate that *H. litura* females accept native thistles for oviposition. However, no-choice development tests will be conducted in spring 2017 to determine whether larvae can complete development in native thistles. These will be the most critical tests because larvae of *H. litura* are the life stage that cause the most damage to thistle plants.

Activity 2 Status as of July 1, 2017: Life-cycle completion tests on field, swamp, tall and Flodman's thistle were set up in late April/early May of 2017 and are still in progress. Tests have also been established on Canada thistle as a control. Single-choice oviposition tests were conducted with tall, Flodman's, field, and swamp thistle. The single-choice oviposition tests are designed to determine oviposition preference of *H. litura* when females are presented a choice between the test thistle and Canada thistle simultaneously. Eggs of *H. litura* were found in all thistle species in single-choice oviposition tests.

Activity 2 Status as of January 1, 2018: Life-cycle completion tests were completed for tall, Flodman's, and field thistle and partially completed (two replications) for swamp thistle. Adult *H. litura* were able to complete their development on native field, tall and Flodman's thistle, as well as in the biological control target, Canada thistle. No adults emerged from the two replications of swamp thistle. During the summer of 2018, life-cycle completion studies will be conducted for swamp (remaining replications), dune, wavy-leaved and Hill's thistle. In addition, single-choice oviposition tests were conducted with field, Flodman's, tall and dune thistle. In these tests, *H. litura* females were able to choose whether to lay eggs in Canada thistle or test plant leaves when presented with leaves of the two species simultaneously. Eggs were found in all native thistles tested: field, Flodman's, tall and dune thistle. These results indicate that *H. litura* females will accept the native thistles as hosts for oviposition, even in the presence of Canada thistle.

Activity 2 Status as of July 1, 2018: Life-cycle completion trials are in progress for Hill's, swamp and dune thistle (*Cirsium pitcher*). In addition, we have completed single-choice oviposition tests for swamp and Hill's thistle.

Activity 2 Status as of January 1, 2019: We finalized life-cycle completion trials with Hill's, swamp and dune (Pitcher's) thistle. Results show that *H. litura* was able to complete development on swamp, Flodman's, field, and tall thistle in no-choice development tests. These *Cirsium* spp. are within the fundamental host range of *H. litura*. In no-choice oviposition tests, female *H. litura* laid eggs on both Hill's and dune thistle, but no adults emerged in development tests. However, due to the difficulty in growing these two thistles in a common garden, more than half of the Hill's and dune thistle plants died during the course of this experiment. Larval tunneling was documented in dune thistle. It is unclear whether Hill's and dune died as a result of *H. litura* attack, or whether mortality was caused by other factors. In addition, single-choice oviposition tests were conducted with Hill's thistle. For this test, *H. litura* females were able to choose whether to lay eggs in Canada thistle or Hills's thistle leaves when presented with leaves of the two species simultaneously. Although eggs were found in Hill's thistle, they were present at lower numbers than in Canada thistle leaves. These and earlier results indicate that *H. litura* females will accept all native thistles tested as hosts for oviposition, even in the presence of Canada thistle.

Activity 2 Final Report Summary: In no-choice tests, female *H. litura* accepted all native thistle species for oviposition. In addition, *H. litura* was able to complete development to the adult stage on swamp (*Cirsium muticum*), field (*Cirsium discolor)*, and tall thistle (*Cirsium altissimum*) and we confirmed initial host range test results of completed development on Flodman's thistle. These *Cirsium* spp. are within the fundamental host range of *H. litura*. In the remaining species tested; Hills (*Cirsium pumilum var. hillii*) and the federally threatened Pitcher's thistle (*Cirsium pitcher*), no adults were found in development tests. However, more than half of Hill's and Pitcher's thistle plants died during the course of the experiment. Larval tunneling was documented in Pitcher's thistle, but not in Hills thistle. It is unclear whether Hill's and Pitcher's thistle died as a result of *H. litura* attack, or whether mortality was caused by other factors. Wavy-leaved thistle may not be adapted to the environment of the St. Paul location as plants failed to overwinter for our host range studies so we were unable to complete larval development tests for this species.

ACTIVITY 3. Determine Phenology of *Ceutorhynchus litura* in Minnesota.

The phenology of *C. litura* will be followed for the three years of the study at a caged site on the University of Minnesota, St. Paul campus. Canada thistle plants will be dissected at regular intervals during each growing season to determine the weevil's life cycle. At each site we will determine when weevils become active in the spring, when females lay eggs and when a new generation of adults emerge in late summer. If our testing shows a host range limited to Canada thistle, this information will be critical to implementing a Canada thistle biological control program in Minnesota. Scientist and technical staff at the University of Minnesota will complete this work.

Summary Budget Information for Activity 3:	**ENRTF Budget:**	**$ 120,000**
	Amount Spent:	**$ 120,000**
	Balance:	**$ 0**

Outcome	**Completion Date**

1. Complete phenology study-year 1, St. Paul, MN. Data on the synchrony of Canada thistle and *C. litura* will have been collected.	December 31,2016 - Completed
2. Complete phenology study-year 2, St. Paul, MN. Second year of data collection on the synchrony of Canada thistle and *C. litura* will have been collected.	December 31, 2017 - Completed
3. Complete phenology study-year effort, St. Paul, MN. Complete data collection on the synchrony of Canada thistle and *C. litura.* Data analyzed, interpreted and reported in context of building a successful biological control program for Minnesota.	July 1, 2018

Activity 3 Status as of January 1, 2016:
Progress towards Outcome 1. Complete phenology study- year 1.

Phenology of *H. litura*. Two trips to the Agassiz National Wildlife Refuge were completed in the spring and summer of 2015 to delineate the phenology of *H. litura* in Minnesota. We timed our trips to coincide with adult activity periods of *H litura.* During both trips, we surveyed the known release sites of *H. litura*. At all release sites, we were unable to collect any *H. litura* adults. In addition, few Canada thistle plants were present. Because we were unable to collect any adult *H. litura*, and found few Canada thistle plants at release sites, we concluded that it would be difficult to determine the phenology of *H. litura* at Agassiz National Wildlife Refuge as proposed in the work plan. We conclude that we need to amend the plan of work to expand the proportion of this effort conducted at the St. Paul Campus and reduce the effort at Lake Agassiz NWR to be able to study the phenology of *H. litura* and native *Cirsium*. We established a nursery of adult *H. litura* purchased from Biological Control of Weeds Inc., Bozeman, MT on caged Canada plants on the University of Minnesota, St. Paul Campus. *H. litura* will be overwinter in pots using the pot-in-pot method for study in 2016 to estimate Canada thistle shoot and *H. litura* emergence time.

Comparative phenology of native thistles. In July 2015, we established a common garden of native *Cirsium* and Canada thistle to determine the phenology of each native thistle species in relation to Canada thistle to help define the ecological host range of *H. litura.* Seedlings or plants of thistles were transplanted into a field on the University of Minnesota St. Paul Campus in a randomized complete block arrangement with 8 replications. In October 2015, seeds of biennial thistles were planted in the thistle common garden to ensure a continuation of biennial thistles after the summer of 2016. These plants will be monitored during the spring and summer of 2016, 2017 and 2018.

Activity 3 Status as of July 1, 2016:
Since March, 2016, we have collected weekly phenology data for the spring of 2016 from native thistles planted in a common garden on the University of Minnesota campus. We have also collected phenology data from *H. litura* overwintering on caged Canada thistle plants on the University of Minnesota campus. In addition, we have continued our efforts to germinate and collect Hill's thistle so that we can test this species next summer. We are also propagating other native thistles so that we have adequate plants to use in phenology and host range tests next year.

Activity 3 Status as of January 1, 2017: The first year of phenology studies were completed on the common garden established during the summer of 2015 at the University of Minnesota, St. Paul campus to determine the relative phenologies of native thistles included in host range tests (with the exception of Dune and Hill's thistle). During 2016, weekly measurements and counts were collected from March 14, 2016 to Sept. 14, 2016. Data collected included time of spring emergence, length of longest shoot, date and number of buds, partial, full and mature flowers.

All species, except Canada thistle, overwintered as rosettes and initiated new shoots in mid-to-late March. These included both biennial and perennial species (Flodman's and Wavy-leaved thistle). Canada thistle shoots were first recorded on April 13, 2016, three to four weeks later than the native thistles.

***Hadroplontus litura* Phenology.** Eggs were found in Canada thistle shoots on April 26, 2016, approximately two weeks after spring shoot emergence. All native thistles included in the common garden experiment had new shoots developing in the overwintered rosettes by the time *H. litura* were actively laying eggs in the spring of

2016. Larvae were detected in Canada thistle shoots on May 19, 2016 and newly emerged adults on July 11, 2016.

Data on thistles and *H. litura* will be compiled with subsequent year's data and all results reported in 2018.

Activity 3 Status as of July 1, 2017: Despite overwintering in 2015-2016, the majority of swamp and tall thistle plants in the common garden failed to successfully overwinter 2016-2017, most likely due to a lack of insulating snow cover. (Widespread winterkill occurred on agronomic crops such as alfalfa this winter as well.) Canada thistle shoots were first recorded on April 10, approximately three weeks later than the native thistle species in the common garden. Length of longest leaf, plant height and dates of bud and flower development will be recorded during 2017. Thistle seedlings of each species have been transplanted into the common garden. These seedlings will flower next summer and emergence and phenology data collected.
***Hadroplontus litura* Phenology.** Eggs and a first-instar larvae were found in Canada thistle shoots on the St. Paul campus on May 8, 2017, approximately four weeks after shoot emergence. Newly emerged adults were recorded on June 19, 2017. Data on thistles and *H. litura* will be compiled across years and all results reported in 2018.

Activity 3 Status as of January 1, 2018: During the summer of 2017, we collected phenology data for the second year in our native thistle common garden. This garden was established in the summer of 2016 to compare the relative phenologies of native thistles included in host range tests. During 2017, weekly measurements and counts were collected from March 20, 2017 to Sept. 11, 2017. Data collected included; time of spring emergence, length of longest shoot, date of first observation of flower buds, number of buds, and date and number of partial, full and mature flowers. Swamp and tall thistle did not survive the winter of 2016-17, so data were not collected on these species. To re-establish the nursery, native thistle plants were transplanted, seeds were planted, and additional measures taken to protect plants so we are hopeful that all species of native thistles will survive to be plants of sufficient quantity and quality for testing during the 2018 season. In the spring of 2017, fescue and other grasses were seeded into the site to provide cover during transplant establishment and overwintering.

Activity 3 Status as of July 1, 2018: We are collecting phenology data for the third year in our native thistle common garden. This garden was established in the summer of 2016 to compare the relative phenologies of native thistles included in host range tests. All seedling plants and rosettes overwintered successfully, with the exception of wavy-leaved thistle. We continue to collect weekly measurements on; time of spring emergence, plant height, length of longest shoot, date of first observation of flower buds, number of buds, and date and number of partial, full and mature flowers. Stratified seeds were planted in late May 2018 to ensure *Cirsium* spp. rosettes for spring 2019 measurements. In addition to phenology data on the thistles, we continue to collect data on *H. litura* phenology. We take weekly plant and insect-stage measurements. Canada thistle shoots were first recorded on May 2, 2018. Eggs were first detected in Canada thistle stems May 10, 2018 and early instar larvae were first detected on May 24, 2018. To date, F1 adults have not emerged.

Activity 3 Status as of January 1, 2019: We collected phenology data for the third and final year in our native thistle common garden. This garden was established in the summer of 2016 to compare the relative phenologies of native thistles included in host range tests. All seedling plants and rosettes overwintered successfully, with the exception of wavy-leaved thistle. We collected weekly measurements on: time of spring emergence, plant height, length of longest shoot, date of first observation of flower buds, number of buds, and date and number of partial, full and mature flowers. Stratified seeds were planted in late May 2018 to ensure we will have *Cirsium* spp. rosettes for next spring 2019. At this time, we will collect final data on Canada thistle spring shoot emergence and onset of *Cirsium* spp. rosette growth. In addition to phenology data on the thistles, we continued to collect data on *H. litura* phenology. We took weekly plant and insect-stage measurements. Canada thistle shoots were first recorded on May 2, 2018. Eggs from *H. litura* were first detected in Canada thistle stems May 10, 2018 and early instar larvae were first detected on May 24, 2018. F1 adults were first recorded in caged plant on June 14, 2018.

Activity 3 Final Report Summary: We determined the relative shoot emergence or shoot initiation among *Cirsium* species in relation to Canada shoot emergence in the spring. Since *H. litura* attacks and lays eggs in thistle shoots, native *Cirsium* species that had delayed spring emergence could temporally escape *H. litura* oviposition. Shoots of the perennials, wavy-leaved, Pitcher's and Flodman's thistles emerged prior to Canada thistle shoots. Biennial rosettes of field, tall and swamp thistle initiated new leaf growth prior to Canada thistle shoot emergence from the soil as well. These results reveal that all tested native thistles likely are not temporally protected since they have shoots available for *H. litura* attack in the spring.

V. DISSEMINATION:

Description: The results of these studies will be presented at professional meetings, in University of Extension education efforts, and published in the appropriate scientific journal(s).

Project Status as of January 1, 2016:
This status report to the LCCMR will be sent to cooperators and suppliers of *Cirsium* species to keep them informed. Dissemination will be most impactful in the final period of reporting in 2018.

Project Status as July 1 2016:
This status report to the LCCMR will be sent to cooperators and suppliers of *Cirsium* species to keep them informed.

Project Status as of January 1, 2017: This status report to the LCCMR will be sent to cooperators and suppliers of *Cirsium* species to keep them informed.

Project Status as of July 1, 2017: This status report to the LCCMR will be sent to cooperators and suppliers of *Cirsium* species to keep them informed. A poster was presented at the 2016 Upper Midwest Invasive Species Conference (UMISC) at LaCrosse WI Oct 17-19, 2016.

Project Status as of January 1, 2018: This status report to the LCCMR will be sent to cooperators and suppliers of *Cirsium* species to keep them informed. Update included as part of a broader presentation on Canada thistle management at the Iowa Invasive Species Conference. Honey Creek Resort at Rathbun Lake, Moravia, Iowa. March 28 - 29, 2017.

Project Status as of July 1, 2018: This status report to the LCCMR will be sent to cooperators and suppliers of *Cirsium* species to keep them informed. Abstracts have been submitted for a regional conference and will be reported on in the next reporting cycle.

Project Status as of January 1, 2019: Updates on the findings of this project were presented at the Upper Midwest Invasive Species Conference and the North Central Weed Science Society.

M. Marek-Spartz, M., E. Katovich, R. Becker, M. Chandler, and L. Van Riper. 2018. Biological Control of Canada Thistle: Host Range of *Hadroplontus Litura* on Native *Cirsium spp.* Presentation at the Upper Midwest Invasive Species Conference. Rochester Convention Center, Rochester MN. Oct. 15-18, 2018.

Poster: Katovich, E., R. Becker, M. Marek-Spartz. Host Specificity of *Hadroplontus litura* on native *Cirsium* Species. Proc. North Central Weed Science Soc. Hyatt Regency, Milwaukee, WI. Dec. 3–6, 2018. (35)

Becker, R. 2018. Update included as part of a broader presentation: Canada thistle in Minnesota Prairies: Now you see it, now you don't. Webinar hosted by MIPN. International audience (Canada and US). 70 participants. Invited talk, of on 4 in a series. Feb 13 2018. Available online https://www.mipn.org/proceedings/restoration-webinar-series/

Overall Project Outcomes and Results: In North America, *H. litura's* primary host is Canada thistle, although its host range includes the *Cirsium-Silybum-Carduus* complex of the Asteraceae subtribe Carduinae (Zwolfer and Harris 1965). There are no *Carduus* or *Silybum* species native to North America, but there are at least 62 native species of *Cirsium* (Keil 2006). Initial host range testing indicated that *H. litura* fed on native Indian, wavy-leaved and Flodman's thistles (*Cirsium brevistylum, Cirsium undulatum* and *Cirsium flodmanii,* respectively*)* (Zwolfer and Harris 1964, Zwolfer 1965, Zwolfer and Harris 1966). Our results show that *H. litura* was able to complete development on native *Cirsium*, including swamp, Flodman's, field, and tall thistle in no-choice development tests. These *Cirsium* spp. are within the fundamental host range of *H. litura*.

In no-choice oviposition tests, female *H. litura* laid eggs on both Hill's and Pitcher's thistle, but no adults emerged in development tests. However, more than half of Hill's and Pitcher's thistle plants died during the course of this experiment and larval tunneling was documented in dune thistle. It is unclear whether Hill's and Pitcher's died as a result of *H. litura* attack, or whether mortality was caused by other factors. Wavy-leaved thistle plants failed to overwinter at our location for our host range studies so we were unable to complete larval development tests. However, previous studies indicated that *H. litura* completed larval development on wavy-leaved thistle when *H. litura* eggs or larvae where transferred onto plants. (Zwolfer and Harris 1966).

Hadroplontus litura can attack and develop on several of the 62 *Cirsium* species native to North America. As such, Cripps et al. (2011) conclude that *H. litura* probably would not have been approved in today's regulatory climate for release as a biocontrol agent against Canada thistle in North America. Their conclusion is based on the current concern for attack on native non-target species and conflicting reports on the efficacy of *H. litura* as a biocontrol agent against Canada thistle.

Field, tall, Flodman's and swamp thistles are within the fundamental host range of *H. litura* as they completed development on these native *Cirsium* species under no-choice conditions. However, it is unclear whether *H. litura* would accept these *Cirsium* species in field conditions. The ecological host range of *H. litura* would encompass insect behavior in a field setting, where the weevils would exhibit normal host search and acceptance behavior and would typically be a subset of the fundamental host range (Schaffner, 2001). We were unable to find reports in the literature of non-target attack by *H. litura* in the field. Differences in phenology between a host plant, such as Canada thistle and native non-host plants also restrict a biocontrol agent's host range in the field. *Hadroplontus litura* females lay eggs in thistle stems. We found that all native *Cirsium* species had new leaves or emerged shoots prior to Canada shoot emergence in the spring so would have stems or leaves available for *H. litura* attack.

Final Report Summary:

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 291,717 $290,919 proposed amendment Jan 2018 Final $290,919	Civil Service -part of 1 Project Senior Scientist ($123,000), 2 Project Technicians ($60,650), and 2 Junior Scientist $46,000/$49,400) collectively approx.0.9 FTE @ est. 36.8% fringe over 3 years. Student Labor approx. 0.38 FTE @ est., 7.57% fringe over 3 years (Full time summer, 1/4 time during school session - $12,167).
Professional/Technical/Service Contracts:	$3,600	Watering charges and other service charges for greenhouse and field space. Fees set by the

Budget Category	$ Amount	Overview Explanation
	Amended Jan 30 2017 $3,400 Final $3,400	University and amount listed based on past fee structures.
Equipment/Tools/Supplies:	$2,083 Amended Jan 30 2017 $4,283 $5,083 proposed amendment Jan 2018 Amended Jan 30 2019 $ 4926 Final $4926	Temperature probes, field supplies: flags, netting, stakes, pots, potting medium, cages, insect purchases, etc.
Travel Expenses in MN:	$2,600 Amended Jan 30 2017 $ 600 Amended Jan 30 2019 $ 755 Final $755	Travel within Minnesota to collect thistles and *C. litura*, to monitor phenology (development, staging, life-cycles) of the various thistles and *C. litura*, and travel to meet cooperators, sponsors, to present results within Minnesota. Estimate 75% mileage, 15% meals and 10% lodging.
TOTAL ENRTF BUDGET:	**$300,000**	

Explanation of Use of Classified Staff: N/A

Explanation of Capital Expenditures Greater Than $5,000: N/A

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: a total of 3.84 FTEs over the 3 years of funding (approx. 0.9 Civil Service and 0.38 student labor FTEs per year for 3 years). 97.2% of this grant supports Minnesota jobs, 2.8% is spent on supplies and travel to conduct the research.

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
PI is not receiving any salary funding, 5% of PI's time = $22,455 salary and fringe.	$22,455	$3,742.50	The time I spend on this effort is paid for from USDA CSREES funds
State			
University indirect costs $156,000.	$156,000	$26,000	Indirect costs are used for U of M facilities expenses.
TOTAL OTHER FUNDS:	**$178,000**	**$27,742.50**	

VII. PROJECT STRATEGY:
A. Project Partners:
Project Partners Receiving Funds:

- Dr. Roger Becker, PI, Professor, Department of Agronomy and Plant Genetics, University of Minnesota.
- Dr. Elizabeth Katovich, Senior Scientist, Department of Agronomy and Plant Genetics, University of Minnesota.

Roger Becker and Elizabeth Katovich will lead the studies. Both cooperators have worked on previous and current LCCMR sponsored studies for purple loosestrife and garlic mustard biological control.

Project Partners Not Receiving Funds:

- Dr. Laura Van Riper, Terrestrial Species Coordinator, Minnesota Department of Natural Resources, will advise and assist where appropriate in working with the *C. litura* and will facilitate in identifying resources, expertise and MnDNR sites for thistle collections and *C. litura* work.
- Welby Smith, Minnesota Biological Survey, and Dan Wovcha. Minnesota Department of Natural Resources will provide native thistle locations and appropriate collection permits.
- Monika Chandler, Minnesota Department of Agriculture. Will advise and assist where appropriate in working with the *C. litura* and will facilitate procuring, releasing and recovering the weevils.

B. Project Impact and Long-term Strategy: Canada thistle is a common invasive plant in Minnesota impeding management goals in several ecosystems. Utilizing biological control on large infestations would prevent the need to apply herbicide, mow or till these sites reducing negative impacts by improving native forb diversity in prairies, and in general, forb diversity in several ecosystems increasing pollinator nectar and pollen source diversity and abundance.

This proposal is the first step in a long-term implementation strategy for biological control of Canada thistle in Minnesota. Before we can proceed, it is necessary to determine whether *C. litura* attacks native thistles. If the weevil only attacks Canada thistle, we will submit a future proposal to LCCMR for funding towards implementing a long-term Canada thistle biological control program. Activity 3 in this proposal is beginning that process through improved understanding of the phenology of *C. litura* in Minnesota to facilitate rearing and release should we proceed with this biological control effort.

C. Funding History: Have current LCCMR funding via MnDNR for garlic mustard biological control. Biological Control of Garlic Mustard $140,000 ending June 30 2016 and Monitoring Biological Control of Garlic Mustard

$52,214 award ended June 30 2014. These efforts inform and build expertise and capacity to conduct the Canada thistle work, but these funds can not be used directly for the Canada thistle work.

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): Please see attached visual.

X. RESEARCH ADDENDUM: Environment and Natural Resources Trust Fund: Please see attached research addendum.

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than January 1, 2016; July 1, 2016; January 1, 2017; July 1, 2017; January 1, 2018; July 1 2018; and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2015 Project Budget
Final Project Title: Biological Control of Canada Thistle
Legal Citation: M.L. 2015, Chp. 76, Sec. 2, Subd. 06c
Project Manager: Roger Becker
Organization: University of Minnesota
M.L. 2015 ENRTF Appropriation: $ 300,000
Project Length and Completion Date: **4** years, June 30, 2019.
Date of Report: August 16, 2019 Final

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Revised Activity 1 Budget Approved 1/9/2018	Amount Spent	Activity 1 Balance	Revised Activity 2 Budget Approved 1/24/2019	Amount Spent	Activity 2 Balance	Revised Activity 3 Budget Approved 1/24/2019	Amount Spent	Activity 3 Balance	Revised TOTAL BUDGET Approved 1/24/2019
BUDGET ITEM	**Collect and grow thistle plants**			**Determine whether Ceutorhynchus litura attacks thistles native to MN**			**Determine weevil life-cycle and phenology in Minnesota**			
Personnel (Wages and Benefits) Overall	$ 58,343	$ 58,343	$ -	$ 116,132	$ 116,132	$ -	$ 116,444	$ 116,444	$ -	$ 290,919
Civil Service Senior Scientist, .0.4 FTE @ est. 36.8% fringe over 3 years. (Estimated at. $123,000)										
Civil Service Junior Scientist collectively approx.0.2 FTE @ est. 36.8% fringe over 3 years. (Estimated at. $46,000)										
Two Civil Service Technician approx.0.1 FTE each @ est. 36.8% fringe over 3 years. 0.2 FTE per year total. (Estimated at. $60,650)										
Civil Service Junior Scientist, .0.2 FTE @ est. 36.8% fringe over 3 years. (Estimated at. $49,400)										
Student Labor approx. 0.38 FTE @ est., 7.57% fringe over 3 years (Full time summer, 1/4 time during school session). (Estimated $12,167)										
Professional/Technical/Service Contracts										
Watering charges and other service charges for greenhouse and field space. Fees set by the University and amount listed based on past fee structures. (Lab/Med	$ 720	$ 720	$ -	$ 1,313	$ 1,313	$ -	$ 1,367	$ 1,367	$ -	$3400
Equipment/Tools/Supplies										
Temperature probes, field supplies: flags, netting, stakes, pots, potting medium, cages, insect purchases, etc. (Lab/Med Supplies U of M)	$ 438	$ 438	$ -	$ 2,400	$ 2,400	$ -	2088	$ 2,088	$ -	$4926
Travel expenses in Minnesota										

Travel within Minnesota to collect thistles and *C. litura* , to monitor phenology (development, staging, life-cycles) of the various thistles and *C. litura* , and travel to meet cooperators, sponsors, to present results within Minnesota. Estimate 75% mileage, 15% meals and 10% lodging.	$ 499	$ 499	$ -	$ 155	$ 155	$ -	$ 101	$ 101	$ -	$ 755
COLUMN TOTAL	$ 60,000	$ 60,000	$ -	$ 120,000	$ 120,000	$ -	$ 120,000	$ 120,000	$ -	$ 300,000

Final Report to the ENRTF M.L. 2015, Chp. 76, Sec. 2, Subd. 06c
Biological Control of Canada Thistle
August 2019

Elizabeth Katovich and Roger Becker, University of Minnesota

Abstract The invasive plant, Canada thistle (*Cirsium arvense*), is a serious threat to natural and managed ecosystems in Minnesota and is the most prevalent prohibited noxious weed in the state. In 1998, the Canada thistle biocontrol agent, *Hadroplontus litura,* a stem-mining weevil*,* was introduced by the USFWS into a limited area in Minnesota, with a resulting decline in Canada thistle populations.

A program to augment the use of *H. litura* for biological control of Canada thistle is under consideration and could provide cost-effective, long-term management of Canada thistle in Minnesota's natural areas. Although showing a preference for Canada thistle, initial host range testing of *H. litura* in the 1960's indicated that *H. litura* attacked the native*,* wavy-leaved thistle (*Cirsium undulatum*) and Flodman's thistle (*Cirsium flodmanii*). Before promoting or augmenting biocontrol with H. litura in Minnesota, we wanted to clarify whether *H. litura* would attack additional thistles native to Minnesota.

The first objective of our research was to determine whether *H. litura* could feed, oviposit and complete development on *Cirsium* spp. native to Minnesota, clarifying whether the host range of *H. litura* includes Minnesota's native *Cirsium* species. The second objective of this research was to determine the phenology of native thistles in relation to Canada thistle to help determine the ecological host range of *H. litura* on native thistles.

In no-choice tests, female *H. litura* accepted all native thistle species for oviposition. In addition, *H. litura* was able to complete development to the adult stage on swamp (*Cirsium muticum*), field (*Cirsium discolor)*, and tall thistle (*Cirsium altissimum*) and we confirmed initial host range test results of completed development on Flodman's thistle. These *Cirsium* spp. are within the fundamental host range of *H. litura*.

In the remaining species tested; Hills (*Cirsium pumilum var. hillii*) and the federally threatened Pitcher's thistle (*Cirsium pitcher*), no adults were found in development tests. However, more than half of Hill's and Pitcher's thistle plants died during the course of the experiment. Larval tunneling was documented in Pitcher's thistle, but not in Hills thistle. It is unclear whether Hill's and Pitcher's thistle died as a result of *H. litura* attack, or whether mortality was caused by other factors. Wavy-leaved thistle may not be adapted to the environment of the St. Paul location as plants failed to overwinter for our host range studies so we were unable to complete larval development tests for this species.

We determined the relative shoot emergence or shoot initiation among *Cirsium* species in relation to Canada shoot emergence in the spring. Since *H. litura* attacks and lays eggs in thistle shoots, native *Cirsium* species that had delayed spring emergence could temporally escape *H. litura* oviposition. Shoots of the perennials, wavy-leaved, Pitcher's and Flodman's thistles emerged prior to Canada thistle shoots. Biennial rosettes of field, tall and swamp thistle initiated new leaf growth prior to Canada thistle shoot emergence from the soil as well. These results reveal that all tested native thistles likely are not temporally protected since they have shoots available for *H. litura* attack in the spring.

The ecological host range of *H. litura* would also encompass insect behavior in a field setting. Weevils that exhibit normal host search and acceptance behavior would typically be a subset of the fundamental host range. Field, tall, Flodman's and swamp thistles are within the

fundamental host range of *H. litura* as they completed development on these native *Cirsium* species under no-choice conditions. However, we did not determine whether *H. litura* would accept these *Cirsium* species in field conditions, as it was beyond the scope of our studies. In conclusion, we recommend that tests be conducted in the field to document the ecological host range of *H. litura* prior to the continued released of *H. litura* as a biocontrol agent of Canada thistle in Minnesota.

Introduction

Native *Cirsium* species occupy a critical, but often misunderstood position in landscapes across North America (Eckberg et al. 2017). Of significance, native thistle flowers produce a high sugar nectar and pollen source for over 200 species of native pollinators, including a variety of butterflies, bees and other insects (Robertson 1929, Hilty 2015, Eckberg et al. 2017). Native thistle flowers are highly attractive to bumblebees (Lye et al. 2010), especially in late summer and are a pollen and nectar source (Fussell 1992). In addition, native thistle flowers and vegetation provide a food source for a variety insect defoliators and seed feeders (Hilty 2015, Eckberg 2017). Lastly, native thistle seeds provide a food source for numerous species of birds, especially American goldfinches (*Spinus tristis*), which feed heavily on thistle seed during their breeding season (Stokes 1950). This may be attributed to the high moisture content present in the milky stage of thistle seeds which is important for water intake during breeding (Gluck 1985).
***Cirsium* species native to Minnesota.** The University of Minnesota herbarium lists six thistles in the *Cirsium* genus as native to Minnesota. These include three biennials; tall thistle (*Cirsium altissimum*), field thistle (*Cirsium discolor*), and swamp thistle (*Cirsium muticum*), and three perennials: Flodman's thistle, (*Cirsium flodmanii),* wavy-leaved thistle (*Cirsium undulatum)* and Hill's thistle (*Cirsium pumilum* var. *hillii*). Hill's thistle is a monocarpic perennial (Keil 2006) and is listed as a species of special concern in Minnesota (https://www.dnr.state.mn.us/rsg/profile.html?action=elementDetail&selectedElement=PDAST2E1C0). Hill's thistle is found most often in dry prairies and savanna woodlands in Minnesota. Although not endemic to Minnesota, Pitcher's thistle (*Cirsium pitcheri*), is native to the dune ecosystem of the Great Lakes region and is a threatened species (USFWS). Non-target attack of Pitcher's thistle by agents released for the biological control of *Carduus* and *Cirsium* species has long been a concern (Havens et al. 2012).

Canada thistle biology. The ubiquitous invasive perennial, Canada thistle (*Cirsium arvense*) is native to Europe and the Mediterranean (Slotta et al. 2010) and has been introduced world-wide. Introductions into North America from multiple continents may have occurred (Slotta et al 2010). It is considered as one of the worst weeds of agricultural and natural systems (Cripps et al. 2011). In North American, Canada thistle is present in 42 states, 12 Canadian provinces and has a noxious weed status in 46 states (USDA, NRCS. 2014. Accessed 6/24/2019). As summarized by Davis (2018), combination of weed control methods provide greater long-term Canada thistle management in annual or perennial systems than herbicide use alone.

Canada thistle is an herbaceous perennial plant, with aboveground shoots dying back over the winter and underground roots surviving from year to year (Moore 1975). Plants reproduce through seed dispersal and vegetatively spread via underground lateral roots to form large interconnected clonal patches (Moore 1975, Donald 1994). Canada thistle plants are dioecious, with male and female flowers produced on separate plants (Hayden 1934). Flowers are pollinated by honey bees and other native pollinators. Seeds are attached to a plumose achene that can aid in dispersal (Moore 1975, Becker et al. 2008)

Canada thistle biocontrol. In North America, the stem-mining weevil, *Hadroplontus* (formerly *Ceutorhynchus) litura* Fabricius, was first introduced into Canada as a biological control agent for Canada thistle in 1965 (Peschken and Beecher 1973). It was subsequently introduced into the United States in 1972, with the first released in Montana. *Hadroplontus litura* has since become established in a number of western states (Winston et al. 2009). In 1998, *H. litura* was introduced into a limited area in Minnesota, with a resulting long-term decline in Canada thistle populations (Chandler 2009).

***Hadroplontus litura* biology**. Adult *H. litura* overwinter in the soil and leaf litter. In spring, the onset of adult activity is synchronized with the emergence of Canada thistle shoots from the soil. (Zwolfer and Harris 1966, Gramig et al. 2015, Peschken and Wilkinson 1981). Adults initially feed on leaves of the emerging shoots (Zwolfer and Harris 1966, Peschken and Beecher 1973, Rees 1990). Females oviposit in the mid-vein on the underside of leaves. Larvae progress through three instars (Zwolfer and Harris 1966) and successively mine leaf mid-ribs, stems and crowns of Canada thistle plants throughout the spring and summer. Median development times of egg and larval instars can be predicted using thermal time models based on accumulated degree days (Gramig et al. 2015). In North Dakota, development occurred earlier at southern-most locations compared to more northerly sites (Prischmann-Voldseth et al 2016). Third instar larvae emerge from Canada thistle plants in late summer, pupate in the soil, and emerge as adults from July to October, depending on location (Zwolfer and Harris 1966, Peschken and Beecher 1973, Rees 1990). *Hadroplontus litura* is univoltine (one generation per year).

Efficacy of *H. litura* as a biocontrol agent of Canada thistle. There are conflicting reports concerning the efficacy of *H. litura* as a biocontrol agent against Canada thistle. Reed et al. (2006) found that *H. litura* infestations did not reduce thistle stem counts, flowering or overwinter survival in Canada thistle stands on two South Dakota wildlife. In contrast, Rees (1990) report a 75 to 92% reduction of Canada thistle stems infested with *H. litura* larvae at four sites 15 years after release. Underground roots suffered higher winter mortality rates as a consequence of *H. litura* larval mining and adults dispersed 9 km over 15 years. In addition, *H. litura* larval stem tunneling in the spring disrupted the transport of photoassimilates to roots, resulting in reduced levels of free sugars and fructans (Hein and Wilson 2004). However, sugar levels recovered later in the summer.

Efficacy of *H. litura* may increase when combined with releases of other Canada thistle biocontrol agents or management strategies. Peschken and Wilkinson (1981) report that *H. litura* did not control thistle stands alone, but could contribute to a decline in thistle populations when combined with other plant stressors, such as other insects or pathogens. Markin and Larson (2011) document a significant decline in Canada thistle abundance after ten years when *H. litura* was released in combination with the gall forming fly, *Uroprhoa cardui* (Diptera: Tephritidae) and the seed-head weevil, *Larinus planus* (Coleoptera: Curculionidae). Total non-structural carbohydrates were 1.5 times lower with early season sampling of Canada thistle roots after attack by *H. litura*, *L. planus*, *U. cardui*, plus the leaf defoliator, *Cassida rubiginosa* (Coleoptera: Chrysomelidae) (Lui et al. 2000).

Burns et al. (2013) concluded that *H. litura* "was a relatively weak biological control agent", but could suppress Canada thistle stands in combination with competitive species, such as sunflower, *Helianthus annuus.* In addition, competition from the native, cool-season, needle and thread grass (*Hesperostipa comata*) in combination with *H. litura*, reduced root biomass and may compliment restoration methods over each agent alone (Ferro-Serrano et al. 2008). Lastly, the combination of *H. litura* injury and herbicide applications reduced Canada shoot biomass over either control strategy alone (Collier et al. 2007).

Host range of *H. litura*. The host range of a weed biological agent is defined as the set of plant species attacked by the agent (Van Klinken 2000). In North America, *H. litura's* primary host is Canada thistle, although its host range includes the *Cirsium-Silybum-Carduus* complex of the Asteraceae subtribe Carduinae (Zwolfer and Harris 1965). There are no *Carduus* or *Silybum* species native to North America, but there are at least 62 native species of *Cirsium* (Keil 2006). Initial host range testing indicated that *H. litura* fed on native *Cirsium brevistylum, Cirsium undulatum* and *Cirsium flodmanii* (Zwolfer and Harris 1964, Zwolfer 1965, Zwolfer and Harris 1966). Slotta et al. (2012) reported that the host range of Canada thistle biocontrol insects, *Larinus planus* and *Rhinocyllus conicus*, did not follow phylogenetic lines developed for *Cirsium* species, which were derived from native *Cirsium* DNA sequences. Therefore, they recommend that a more comprehensive list of *Cirsium* species should be included in host range testing of Canada thistle biological control insects.

In 1998, *H. litura* was introduced into a limited area in Minnesota, with a resulting decline in Canada thistle populations after a year (Chandler 2009). *Hadroplontus litura* can be purchased commercially for release in Minnesota. Before recommending *H. litura* be released into additional sites in Minnesota, we need to determine whether *H. litura* will attack Minnesota's native thistles. If *H. litura* only develops on Canada thistle, a program to augment and support biological control with *H. litura* could be implemented in Minnesota to provide a cost-effective, long-term management tool for Canada thistle.

The first objective of our research was to determine whether *H. litura* could feed, oviposit and complete development on *Cirsium* spp. native to Minnesota, clarifying whether the host range of *H. litura* includes Minnesota's native *Cirsium* species. The second objective of this research was to determine the phenology of native thistles in relation to Canada thistle to help determine the ecological host range of *H. litura* on native thistles.

Materials and Methods

***Cirsium* plant propagation**.

We collaborated with the Minnesota Biological Survey to locate sources for each native thistle whenever possible, or purchased *Cirsium* thistle seed or plants from local seed sources (Table 1). Since *H. litura* adults actively oviposit in the spring, *Cirsium* plants were established the summers prior to host range testing and overwintered so that they would be available in the spring when adults became active.

Two years prior to each year of testing, seeds of all three biennial *Cirsium* species and the perennials, wavy-leaved and Pitcher's thistle were germinated using two techniques. Field stratification consisted of planting seeds in plug trays filled with a standard commercial potting mix (LC8; 70-80% Canadian sphagnum peat moss, 20-25% perlite, 5-10% vermiculite; Sungro Horticulture, Agawam, MA). Trays were placed outside in November in St. Paul, MN and lightly mulched with straw to overwinter. Mulch was removed in early spring (April in Minnesota) when seedlings emerged. As a backup in case field stratification failed, thistle seeds were also stratified in the lab by adding moistened sand to a 90 mm diameter x 15 mm deep plastic petri dish, adding a layer of seeds, then covering the seeds with additional moist sand. Petri dishes were sealed and placed in a refrigerator at 4 C. After six weeks, seeds were removed and planted in a plug tray filled with the standard potting mix described previously.

In the spring of 2015, 2016 and 2017 (one year prior to testing), all *Cirsium* seedlings propagated via the described procedures, were planted outdoors into 11.4 l pots using a standard potting mix and greenhouse soil (consisting of silt loam:sand:manure:peat at a 1:1:1:1, v/v/v/v ratio) in a 1:1 ratio. Instead of seedlings, Flodman's thistle plants and Canada thistle shoot segments were planted the summer prior to testing using this same pot protocol. Plants were fertilized with a slow-release fertilizer containing macro- and micro-nutrients (Osmotcote Plus, 15-9-12 plus micronutrients, Scotts Company, Marysville. OH) at the recommended rate.

Very few Hill's thistle germinated from seed, despite use of various germination strategies. Since we were unable to establish plants form seedings the preceding year, we collected and transplanted rosettes from the Ordway Prairie, MN during the summers of 2016, 2017 and 2018.

In the autumn prior to testing, these potted thistle plants were overwintered in the field at the University of Minnesota, St. Paul location using a pot-in-pot method (Mathers 2003) to ensure winter survival during Minnesota winters (Katovich unpublished data). This technique consists of digging a hole in the ground, then placing an empty 11.4 l pot into the hole so that the rim of the pot is level with the soil. Next, a potted plant is inserted into the empty pot. This method facilitates easier removal of potted plants the following spring. Plants were lightly mulched with straw for overwintering. This technique is similar to that used by Peschken and Derby (1992) to overwinter potted Canada thistle plants in Regina, Saskatchewan. Multiple plants of each species were established so that replicated host-range field trials could be conducted.

By spring 2017, after overwintering the potted plants established in 2016, no healthy native thistle crowns were present in pots. Holes were in the soil where the crowns used to be, so we concluded that they were foraged by small rodents over the course of winter, even though they were placed in a fenced enclosure. All Canada thistle crowns were undisturbed. As a result, in the fall of 2017, all plants were individually caged with 0.635-cm square mesh galvanized steel hardware cloth that extended outside of the pot into the ground. In the spring of 2018, all overwintered, native thistles were present and survived, with the exception of wavy-leaved thistle. We concluded that wavy-leaved thistle was not able to survive winter temperatures of 2016/2017.

Hadroplontus litura colony establishment.

To establish insect colonies, adult *H. litura* were purchased from Biological Control of Weeds Inc., Bozeman, MT and were received in July of 2015, 2016 and 2017. Once received, adults were released immediately onto caged Canada thistle plants established in 11.4 l pots and maintained outside. *Hadroplontus litura* adults were overwintered outside on the caged Canada thistle plants using the pot-in-pot method. When adults became active in subsequent springs, they were collected from the overwintered plants and used in host range tests.

Host range testing of _Hadroplontus litura_.

To further understand the host range of *H. litura*, we conducted no-choice feeding and oviposition tests, no-choice development tests and single-choice oviposition tests. Host plant acceptance of *H. litura* includes both oviposition choice by females and the ability of larvae to complete development. Once *H. litura* females lay their eggs into a plant stem, developing larvae are unable to change host plants. Consequently, female oviposition determines the potential host range of this weevil species. If a *Cirsium* species was not accepted for oviposition, then it was considered not at risk for *H. litura* larval stem mining and was not included in no-choice larval development testing. All host range tests were conducted in the field on caged plants in April through June, 2016-2018 at the University of Minnesota, St. Paul campus. Details on individual host range tests follow.

No-choice feeding and oviposition tests. No-choice feeding and oviposition tests were conducted in the spring and early summer when *H. litura* females were laying eggs. Procedures were similar to those described by Gerber et al. (2009) for *Ceutorhynchus scrobicollis* host range tests. Adults were collected from overwintered, caged Canada thistle plants described previously. Prior to inclusion in oviposition tests, females were tested to ensure that they were laying eggs using the procedure described by Gerber et al. (2009). Only ovipositing females were used in subsequent experiments. To conduct the no-choice feeding and oviposition test, an excised native *Cirsium* spp. plant leaf was inserted into a hydrated piece of florist foam encased in a self-sealing plastic bag. Leaves were a minimum of 5 cm in length as *H. litura* does not oviposit on those shorter than 5 cm (Zwolfer and Harris 1964). A leaf was placed into a 1 l glass canning jar and covered with nylon mesh secured with a jar ring lid. A mating pair of *H. litura* were placed into each jar. Jars were kept indoors at room temperature near a window so they were exposed to the same photoperiod as outdoors. After one day, leaves and stems were dissected and checked for eggs. Feeding and number of eggs per leaf were recorded. Percent feeding on the leaf was visually estimated. If eggs were not found on the *Cirsium* test plant leaf at the end of one day, it was replaced with a fresh Canada thistle leaf for an additional day and checked for eggs to confirm that the female was still ovipositing. A replication was only counted valid if eggs were sequentially laid in the Canada thistle leaf. Jars containing *H. litura* on an excised Canada thistle leaf were always included as controls when testing native *Cirsium* to ensure that conditions conducive for oviposition were present. A minimum of 10 replications were completed for each species with each individual jar as a replication. Mean percent feeding, number of eggs, and mean standard error values were calculated.

No-choice development tests. No-choice development tests were conducted on all thistle species since they were all accepted for oviposition in no-choice tests or single-choice tests. Caged, potted thistle plants were maintained outdoors in an open area protected by surrounding trees. In spring, active adults were collected from the colonies maintained on caged Canada thistle plants overwintered in the field. Adults were marked with a paint pen to make them easier to recover from test plants, and to differentiate parents from F1 progeny during the no-choice development trials. Prior to inclusion in trials, females were tested for egg laying and only ovipositing females were used in experiments. For each trial, two marked *H. litura* mating pairs were placed on each caged, potted thistle plant and removed after two weeks. Caged plants were monitored for emergence of F-1 progeny later in the season by checking for new adult leaf feeding, or for adults climbing in the interior of the screen cages. Each plant was checked for F1 progeny a minimum of three times and number of progeny was recorded for each plant. At the final collection time, all plants were dissected and checked for larval mining and tunneling. Caged Canada thistle plants were tested separately, but concurrently with native *Cirsium* spp. as controls. A minimum of five replications of each *Cirsium* spp. were tested, with each caged plant a replication.

Single-choice oviposition tests. Single choice oviposition tests were conducted for all native *Cirsium* species using glass canning jars kept indoors at room temperature near a window so they were exposed to the same photoperiod as outdoors. Adult females were presented with an oviposition choice between a native *Cirsium* or Canada thistle leaf. This test is less conservative than no-choice oviposition trials and allows females to choose where they want to deposit eggs. Overwintered *H. litura* were collected from the colony maintained on caged Canada thistle plants after they became active in the spring. Prior to inclusion in tests, all *H. litura* females were placed in an oviposition test on Canada thistle as previously described in the no-choice oviposition test protocol. One mating pair of *H. litura* were placed into a glass jar and simultaneously offered an excised native *Cirsium* and a Canada thistle leaf so that females were able to choose which species to accept for oviposition. After one day, leaves were dissected and the number of *H. litura* eggs recorded, along with presence/absence of feeding. Each exposure period was treated as one replicate. Replicates were only regarded as valid when females laid eggs into Canada thistle or *Cirsium* spp. leaves.

<u>*Hadroplontus litura* and thistle common garden phenology studies.</u>

Phenology of Hadroplontus litura. The phenology of *H. litura* was followed from 2016 to 2018 at our Canada thistle nursery on the University of Minnesota, St. Paul campus. *Hadroplontus litura* adults were added to caged, potted Canada thistle plants during the preceding summer, overwintered with the pot-in-pot technique as described previously. After the first indication of adult activity in the spring, six Canada thistle plants were sampled at weekly intervals. At each sampling time, two stems from each plant with adult feeding damage were dissected. Presence of eggs or first instar larvae were noted. Once first instar larvae were recorded, weekly sampling was discontinued to allow remaining larvae to continue their development. First generation adult (F1s) activity was detected by observing new adult leaf feeding or finding adults crawling on screened cages. At this time, all plants were searched for adults. Phenological events were recorded by day of the year, with day one corresponding to January 1. Cumulative growing degree days (gdd) were calculated from the Midwest Regional Climate Center online data portal using data from the on-site, University of Minnesota, St. Paul reporting station (lat/long: 44.9902/-93.1824; elevation: 296 m)

We used the Midwest Regional Climate Center equation for gdd:

GDD = TMEAN – TBASE, if TMEAN is greater than TBASE

GDD = 0, if TMEAN is less than TBASE

Where: *TBASE* = 0 C

TMEAN = mean temperature, *(TMAX + TMIN)/2*

A base temperature of 0 C beginning on April 1 (day 92 for 2016, and day 91 for 2017 and 2018) (Donald 2000) was used for Canada thistle emergence.

Thistle common garden. A thistle common garden was established at the St. Paul Campus Field Station, St Paul, MN to compare the relative phenology of native C*irsium* and Canada thistle. The garden contained five of the six studied thistle species, with the exception being Hill's thistle. Hill's thistle seeds germinated at very low rates, so they were prioritized for use in host range testing, the primary focus of our study. The common garden experiment was established in July, 2015 and designed as a randomized complete block with six replications. Each thistle species was present once in each block. Due to high thistle mortality, in 2016, two additional replications were added to compensate for expected loss of plants from season to season.

Thistle species were propagated as described previously and transplanted into the garden the year prior to data collection. The soil type was a Waukegan silt loam with 6.8% organic matter and pH of 6.7. In July, 2015, plants were first transplanted into the field, with each plot consisting of one plant. Plants were spaced 1.2 m apart and watered as needed. In the fall of 2015, 2016 and 2017, thistle seeds were planted in each plot to establish plants that would develop the appropriate stages for testing the following year. Additional seedlings were transplanted each spring to replace plants that did not survive the winter, however data was not collected on these plants until the following spring and summer. In summers of 2015 and 2016, the area was cultivated with a hand driven mechanical cultivator and manually weeded for weed control within and between plots. Due to high winter kill in the winter of 2016/2017, in spring 2017 low-profile warm and cool season grasses were seeded over the area to provide cover for thistles, reduce the weed pressure, and to catch snow to insulate thistles during the winter.

In springs of 2016 through 2019, dates of new leaf emergence on biennial rosettes and shoot emergence of perennials were recorded beginning as soon as the snow had melted. In the summer of 2016, 2017 and 2018, number of leaves, length of longest leaf, number of buds and flowers were recorded weekly until all flowers were mature, which occurred by mid-September.

Results and Discussions

We conducted three different host range tests on native *Cirsium* thistles with *H. litura*: no-choice oviposition tests, no-choice larvae development tests, and single-choice oviposition tests. These tests are listed from least to most conservative in predacity nontarget thistle injury, but are least to most representative of expected nontarget effects in the real world.

***Hadroplontus litura* host range tests**

No-choice oviposition tests. Under no-choice conditions, female *H. litura* accepted all native thistle species for oviposition (Table 2), with the exception of Hill's thistle which was not tested so it could be included in single-choice oviposition tests discussed later. The majority of eggs were laid in the leaf midrib or leaf petiole. A minority of eggs, less than 10%, were found in the leaf blade. Eggs were laid singly or in clusters. From these results, we conclude that *H. litura* females can accept these native *Cirsium* species for oviposition. However, the oviposition tests do not determine whether the native *Cirsium* species can support *H. litura* larval development to the adult stage so we next conducted no-choice development tests.

No-choice development tests. Unmarked F1 adults were recovered on caged Flodman's, tall, field and swamp thistle plants at the same time that unmarked F1 *H. litura* were found in caged Canada thistle control plants. (Table 3). From this we conclude that *H. litura* larvae were able to complete development to the adult weevil on these native thistle species in no-choice tests. These *Cirsium* species are within the fundamental host range of *H. litura*.

Unfortunately, two of five Pitcher's thistle plants died of undetermined causes during the course of this experiment. No F1 *H. litura* adults were recovered from the dead plants nor from the three remaining live caged Pitcher's thistle plants. However, larval tunneling was documented in one Pitcher's thistles of the three live (Figure 1). Although F1 adults were not present on the three remaining Pitcher's thistle plants, larval tunneling in the crown of one plant warrants additional studies to determine whether Pitcher's thistle is within the fundamental host range of *H. litura*.

Three of five Hill's thistle plants died of undetermined causes during the course of this experiment. No plant tissue remained of these three plants so we could not dissect to determine whether larvae or larval tunneling could have contributed to mortality in Hill's thistle. Upon dissection of the two surviving plants, no larval tunneling was found in roots or crowns. Thus, no larval development was observed on Hill's thistle, but based on these limited results, and the undetermined nature of death in three of five plants, additional tests are needed before a determination that *H. litura* will not develop on Hill's thistle can be made.

Single-choice oviposition tests. In single-choice oviposition tests, where *H. litura* females were able to choose which host to accept for oviposition, eggs were deposited on all native thistles tested (Table 4). Wavy-leaved thistle was not included as no plants were available for testing due to high mortality during rearing. Compared with Canada thistle leaves, there were more eggs deposited on field thistle leaves and a similar number deposited on Flodman's thistle leaves. Eggs were present in the remaining species, but when given a choice, *H. litura* preferred Canada thistle with approximately 70 to 75% of eggs laid on Canada thistle plants. From these results, we conclude that *H. litura* females will accept all tested species for oviposition, even in the presence of Canada thistle. However, Canada thistle is clearly preferred for oviposition over Hill's, Pitcher's, swamp and tall thistle.

Conclusions. *Hadroplontus litura* host range tests. Our results show that *H. litura* was able to complete development on swamp, Flodman's, field, and tall thistle in no-choice development tests. These *Cirsium* spp. are within the fundamental host range of *H. litura*. In no-choice oviposition tests, female *H. litura* laid eggs on both Hill's and Pitcher's thistle, but no adults emerged in development tests. However, more than half of Hill's and Pitcher's thistle plants died during the course of this experiment and larval tunneling was documented in Pitcher's thistle. It is unclear whether Hill's and Pitcher's died as a result of *H. litura* attack, or whether mortality was caused by other factors. Wavy-leaved thistle plants failed to overwinter for our host range studies so we were unable to complete larval development tests for this species. However, previous studies indicated that *H. litura* completed larval development on wavy-leaved thistle when *H. litura* eggs or larvae where transferred onto plants. (Zwolfer and Harris 1966). Wavy-leaved thistle does not appear to successfully overwinter in St. Paul, as it did also not survive in our common thistle garden.

<u>Thistle common garden and *Hadroplontus litura* phenology studies</u>

We to characterize the comparative phenologies of *H. litura* with the native *Cirsium* thistles in common garden studies to explore the potential for nonsynchronous phenologies to offer protection from *H. Litura* attack. To do so, we conducted the following studies.

***Hadroplontus litura* phenology study.** The mean dates of first Canada thistle shoot emergence in the thistle common garden at St. Paul, MN were April 18th, April 15th, and May 5th, in 2016, 2017, and 2018, respectively (Table 5). The later date for first emergence in 2018 was most likely due to cold temperatures associated with a blizzard on April 15, 2018.

First activity of *H. litura* in the spring ranged from mid-April (2016) to early May (2018) on caged and overwintered Canada thistle plants, coinciding with the emergence of Canada thistle vegetative shoots (Table 5). We first observed *H. litura* eggs on April 26th, May 8th, and May 10th in 2016, 2017, and 2018, respectively, approximately two to three weeks after first shoot emergence each year (Table 5). Larvae were first observed on May 19th,May 8th, and May 24th, in 2016, 2017, and 2018, respectively. However, head capsule measurements indicated that multiple instars were present so larvae development likely began before we first observed it. F1 adult emergence was first observed on June 15th, June 19th, and June 14th in 2016, 2017, and 2018, respectively. In spring, overwintering adult *H. litura* activity coincided with the emergence of Canada thistle shoots. Similar results are reported by Zwolfer and Harris 1966, Gramig et al. 2015, and Peschken and Wilkinson 1981. In general, *H. litura* develop earlier at more southern latitudes and later at more northern latitudes (Prishmann-Voldseth 2016). At sites in eastern North Dakota (48.7016 to 46.3628), eggs were found from mid-May to the beginning of June, a period of two to three weeks (Prishmann-Voldseth 2016. Gramig et al. (2015) reported an egg medium development time, when 50% of a cohort were in the egg stage, as when 235 degree days had accumulated after the soil temperature warmed to 9 C in eastern North Dakota. At our St. Paul, MN site (44.9902ºN), *H. litura* eggs were recorded from late April to mid-May, earlier than those reported by Prishmann-Voldseth in eastern North Dakota (2016) which is expected as the St. Paul field site is located at a lower latitude.

F1 adults are cryptic and very difficult to recover in the field (Peschken and Beecher 1973, Gamig et al. 2015). At the St. Paul site, F1 adults were collected in screen-caged plants in mid-June, similar to dates reported near Bozeman, MT (near 45.6778º (Rees 1990) and earlier than the August emergence recorded in the most northern location documented, Regina, Saskatchewan (near 50.4547) (Peschken and Wilkinson 1981).

Phenology of Cirsium species in a common garden. It was difficult to establish and maintain all native thistles in the common garden. Swamp thistle did not establish well in the garden most likely because as its name implies, it grows best in moist areas near marshes and wetlands (Eckberg et al. 2017). Tall and swamp thistles did not survive the winter of 2016/2017. Wavy-leaved thistle established during the years of planting, but the majority of first year plants did not survive the winter. Pitcher's thistle plants that survived the first winter flowered the following summer, although it can take two to eight years to flower in the sand dunes of its native habitat (Havens et al. 2012, Eckberg et al. 2017). Canada, Flodman's and field thistle established most successfully at our site.

Spring vegetative shoot/leaf emergence. The purpose of this study was to determine the relative shoot emergence or shoot initiation among *Cirsium* species in relation to Canada shoot emergence in the spring. In spring, the first possible data collection date available to record shoot emergence varied among years and was determined by snow melt. The first observation dates for 2016 and 2017 were March 15th and March 20th, respectively. In 2018, we had a blizzard in St. Paul, MN on April 15 and the snow did not melt from the field until later in April. Consequently, the first observation for 2018 was delayed until April 25th.

Mean day of the year recorded for first Canada thistle emergence in years 2016, 2017 and 2018 was 108, 105 and 125, respectively, which occurred on April 17th, April 15th, and May 5th, respectively (Data not shown). Cumulative growing degree days for first Canada thistle emergence were 204, 222 and 330 for 2016, 2017 and 2018, respectively (Figure 2). Creating a model of Canada thistle emergence using a nonlinear logistic dose-response regression model, Donald (2000) predicted that between 1% and 80% of Canada thistle shoots would emerge between 197 and 587 GDD using a base air temperature of 0 C. Our results fall into the range described by Donald (2000).

Vegetative shoots of the perennial Flodman's thistle emerged earlier than Canada thistle. All shoots emerged prior to the first possible observation dates in mid- to late-March in 2016 and 2018. However, only 25% of shoots had emerged in 2017 on March 20th, but by the end of March, all shoots had emerged (maximum emergence is 1.0) (Figure 3). Shoots of other perennial species, wavy-leaved and Pitcher's thistles emerged by mid- to-late March (data not shown).

We began recording growth and emergence at the first possible observation date as soon as snow melted from the field site. All the biennial thistle rosettes, field, tall and swamp thistles, overwintered and had initiated growth of new leaves prior to the first possible observation date (Figures 4, 5 and 6). with one exception. In 2016, only 80% of tall thistle rosettes had initiated leaves by the time snow melted.

So, would differences in relative shoot emergence or shoot initiation among *Cirsium* species in relation to Canada shoot emergence in the spring offer protection *from H. litura* attack? Since *H. litura* attacks and lays eggs in thistle shoots, native *Cirsium* species that had delayed spring emergence could escape *H. litura* oviposition. Shoots of the perennial wavy-leaved (data not shown), Pitcher's (data not shown) and Flodman's thistles (Figure 3) emerged prior to Canada thistle shoots (Figures 2). Biennial rosettes of field (Figure 4), tall (Figure 5) and swamp thistle (Figure 6) all initiated new leaf growth prior to Canada thistle shoot emergence from the soil as well. These results reveal that all tested native thistles have shoots or newly emerging leaves on rosettes available for *H. litura* attack in the spring.

First bud and flowering. Canada thistle is a long-day species (Link and Kommedahl 1958) and only flowers when a daylength of 14 to16 hours is attained (Hunter and Smith 1972). Dates of first bud observation at St. Paul, MN were June 1st, 5th, and 20th in 2016, 2017 and 2018 respectively (data not shown). Daylengths for these dates were 15 hours 23 minutes, 15 hours 28 minutes, and 15 hours, 36 minutes in 2016, 2017, and 2018 respectively (lat/long: 44.9902/-93.18). Buds on Flodman's thistle plants, also a perennial *Cirsium* species, were first observed on June 1st, 2016 and June 2nd, 2017 (data not shown) calendar dates and daylengths similar to those of Canada thistle. In 2018, flowering shoots did not develop in Flodman's thistle plants. This is most likely due to the fact that these plants were seedlings the previous year and generally require a couple of years of growth to flower. The length of time between first bud appearance and first flower, defined as the time when ray florets were expanding from the receptacle, was approximately one month for both Flodman's and Canada thistle. (Figures 7 and 8).

The remaining perennial species, wavy-leaved and Pitcher's thistle, formed buds and flowered earlier than the other species (Figures 9 and 10). Pitcher's thistle plants flowered the second year after germination.

In contrast, the length of the bud stage for the biennial tall, swamp and field thistles ranged from one and a half to two months (Figures 11, 12 and 13) All of these species formed buds in late-May to early-June, but did not flower until late-July to early-August. In tall and swamp thistle, apical mining and the subsequent damage to apical shoot meristems by the artichoke plume moth *Platyptillia carduidactyla* may have delayed bud production. As a result of *P. carduidactyla* apical mining, flower buds were initiated on axillary shoots to compensate for damaged apical flower buds (Adhikari and Russell 2014).

The common garden studies show that although biennial thistles flowered later than the perennial thistles, the length of the bud stage overlapped with bud production and flowering of Canada thistle. As a result, any of the biennial thistles could potentially be attacked by the accidentally introduced *Larinus planus*, based on bud and flowering phenologies (Louda and O'Brien 2002, Winston et al. 2009).

Overall Conclusions

In North America, *H. litura's* primary host is Canada thistle, although its host range includes the *Cirsium-Silybum-Carduus* complex of the Asteraceae subtribe Carduinae (Zwolfer and Harris 1965). There are no *Carduus* or *Silybum* species native to North America, but there are at least 62 native species of *Cirsium* (Keil 2006). Initial host range testing indicated that *H. litura* fed on native Indian, wavy-leaved and Flodman's thistles (*Cirsium brevistylum, Cirsium undulatum* and *Cirsium flodmanii,* respectively*)* (Zwolfer and Harris 1964, Zwolfer 1965, Zwolfer and Harris 1966). Our results show that *H. litura* was able to complete development on native *Cirsium*, including swamp, Flodman's, field, and tall thistle in no-choice development tests. These *Cirsium* spp. are within the fundamental host range of *H. litura*.

In no-choice oviposition tests, female *H. litura* laid eggs on both Hill's and Pitcher's thistle, but no adults emerged in development tests. However, more than half of Hill's and Pitcher's thistle plants died during the course of this experiment and larval tunneling was documented in dune thistle. It is unclear whether Hill's and Pitcher's died as a result of *H. litura* attack, or whether mortality was caused by other factors. Wavy-leaved thistle plants failed to overwinter at our location for our host range studies so we were unable to complete larval development tests. However, previous studies indicated that *H. litura* completed larval development on wavy-leaved thistle when *H. litura* eggs or larvae where transferred onto plants. (Zwolfer and Harris 1966).

Hadroplontus litura can attack and develop on several of the 62 *Cirsium* species native to North America. As such, Cripps et al. (2011) conclude that *H. litura* probably would not have been approved in today's regulatory climate for release as a biocontrol agent against Canada thistle in North America. Their conclusion is based on the current concern for attack on native non-target species and conflicting reports on the efficacy of *H. litura* as a biocontrol agent against Canada thistle.

Field, tall, Flodman's and swamp thistles are within the fundamental host range of *H. litura* as they completed development on these native *Cirsium* species under no-choice conditions. However, it is unclear whether *H. litura* would accept these *Cirsium* species in field conditions. The ecological host range of *H. litura* would encompass insect behavior in a field setting, where the weevils would exhibit normal host search and acceptance behavior and would typically be a subset of the fundamental host range (Schaffner, 2001). We were unable to find reports in the literature of non-target attack by *H. litura* in the field.

Differences in phenology between a host plant, such as Canada thistle and native non-host plants also restrict a biocontrol agent's host range in the field. *Hadroplontus litura* females lay eggs in thistle stems. We found that all native *Cirsium* species had new leaves or emerged shoots prior to Canada shoot emergence in the spring so would have stems or leaves available for *H. litura* attack. In conclusion, we recommend that field tests be conducted where search and acceptance behavior can occur to document the ecological host range of *H. litura* prior to the continued release or promotion of *H. litura* as a biocontrol agent of Canada thistle in Minnesota.

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Table 1. ***Cirsium*** **thistle species included in** ***Hadroplontus litura*** **host range testing. All native species are present in Minnesota except Pitcher's thistle (*****Cirsium pitcher)*****, which is native and present east of Minnesota. Canada thistle (*****Cirsium arvense*****) is a non-native invasive species.**

Scientific name	Common name	Life cycle	Status	Seed/plant source
Cirsium arvense (L.) *Scoop*	Canada thistle	perennial	Prohibited Noxious Weed-MN	St. Paul, MN (44.989920, -93.185503)
Cirsium altissimum (L.) Spreng.	tall thistle	biennial	none	Cumberland, Iowa (41.274186, -94.870336)
Cirsium discolor (Muhl. ex Willd.) Spreng.	field thistle	biennial	none	Maplewood, MN (44,929148, -92.997039)
Cirsium flodmanii (Rydb.) Arthur	Flodman's thistle	perennial	none	Morning Sky Greenery (45.607745, -95.856771)
Cirsium muticum Michx.	swamp thistle	biennial	none	Prairie Moon Nursery (43.903211, -91.637046) and Burnham Wildlife Management Area, Polk County, MN (47.630295, -96.35160)
Cirsium pumilum var. hillii (Canby) Fernald	Hill's thistle	monocarpic perennial	Species of Special Concern-MN	Ordway Prairie, MN (45.444663, -95.244426)
Cirsium undulatum (Nutt.) Spreng.	wavy-leaved thistle	perennial	none	Germplasm Resources Information Network (GRIN)
Cirsium pitcheri	Pitcher's thistle	monocarpic perennial	Threatened species-USFWS. Native to Ontario, WI, MI, IL, IN	Chicago Botanic Garden (Lake Michigan area, original source not known)

[1]Reported as Decimal Degrees

Table 2. Results of *Hadroplontus litura* no-choice oviposition tests on caged *Cirsium* species in the field. St. Paul, MN. 2016

				Number of eggs		
Species[1]	**Scientific name**	**Number of replications**	**Mean percent feeding**	**Total**	**Mean**	**± Mean SE**
Canada thistle	*Cirsium arvense*	74	2.2	309	4.2	0.4
field thistle	*Cirsium discolor*	10	2.4	77	7.7	1.3
Flodman's thistle	*Cirsium flodmanii*	10	2.2	56	5.6	0.9
Pitcher's thistle	*Cirsium pitcheri*	10	0.8	41	4.1	1.0
swamp thistle	*Cirsium muticum*	10	1.1	89	8.9	1.4
tall thistle	*Cirsium altissimum*	10	3.0	106	10.6	1.9
wavy-leaved thistle	*Cirsium undulatum*	10	1.0	45	4.5	1.0

[1]Note: Hill's thistle failed to establish so were not available for testing in 2016.

Table 3. Results of *Hadroplontus litura* no-choice larval development tests on caged *Cirsium* species in the field. St. Paul, MN. Trials conducted from 2016 to 2018.

		Number of replications		Numbers of adults emerged		
Species[1]	Scientific name	Total	With adult emergence[1]	Total	Mean	Range
Canada thistle	*Cirsium arvense*	10	8	27	2.7	0 to 7
field thistle	*Cirsium discolor*	8	6	112	14.0	0 to 43
Flodman's thistle	*Cirsium flodmanii*	5	1	9	1.8	0 to 9
Pitcher's thistle	*Cirsium pitcheri*	5	0	0	0.0	0
Hill's thistle	*Cirsium pumilum* var. *hillii*	5	0	0	0.0	0
swamp thistle	*Cirsium muticum*	7	5	7	1.0	0 to 2
tall thistle	*Cirsium altissimum*	5	2	6	1.2	0 to 5

[1]Sum of alive and dead adults

Table 4. Results of single-choice oviposition tests with *Hadroplontus litura* on caged *Cirsium* species grown in the field, St. Paul, MN. Trials conducted from 2017 to 2018.

Species	Scientific name	No. of replications	Mean egg number per plant		Distribution of eggs (%)	
			Native thistle	Canada thistle	Native thistle	Canada thistle
field thistle	*Cirsium discolor*	6	4.8	2.5	66	34
Flodman's thistle	*Cirsium flodmanii*	7	2.4	2.9	46	54
Hill's thistle	*Cirsium pumilum* var. *hillii*	5	0.6	3.2	16	84
Pitcher's thistle	*Cirsium pitcheri*	7	1.1	4.1	22	78
swamp thistle	*Cirsium muticum*	5	1.0	2.6	28	72
tall thistle	*Cirsium altissimum*	6	1.5	4.8	24	76

Table 5. ***Hadroplontus litura*** **phenology monitored in the field on caged Canada thistle (*Cirsium arvense*) plants. University of Minnesota. St. Paul, MN. 2016, 2917 and 2018. Date indicates when life stage was first recorded.**

Year	*Hadroplontus litura* eggs			*Hadroplontus litura* larvae			*Hadroplontus litura* F1 adults			Canada thistle		
	Date	Day of the year	Cumulative GDD[1]	Date	Day of the year	Cumulative GDD	Date	Day of the year	Cumulative GDD	Mean first shoot emergence	Day of the year	Cumulative GDD
2016	April 26	117	374	May 19	140	826	June 15	167	1769	April 18	108	204
2017	May 8	128	611	May 8	128	611	June 19	170	1969	April 15	105	222
2018	May 10	130	485	May 24	144	894	June 14	165	1751	May 5	125	330

[1]Cumulative growing degree days calculated with a base temperature of 0 C, starting on April 1 for each respective year

Figure 1. *Hadroplontus litura* tunneling in Pitcher's thistle (also known as dune thistle) (*Cirsium pitcher*) crowns. July, 2018. St. Paul, MN

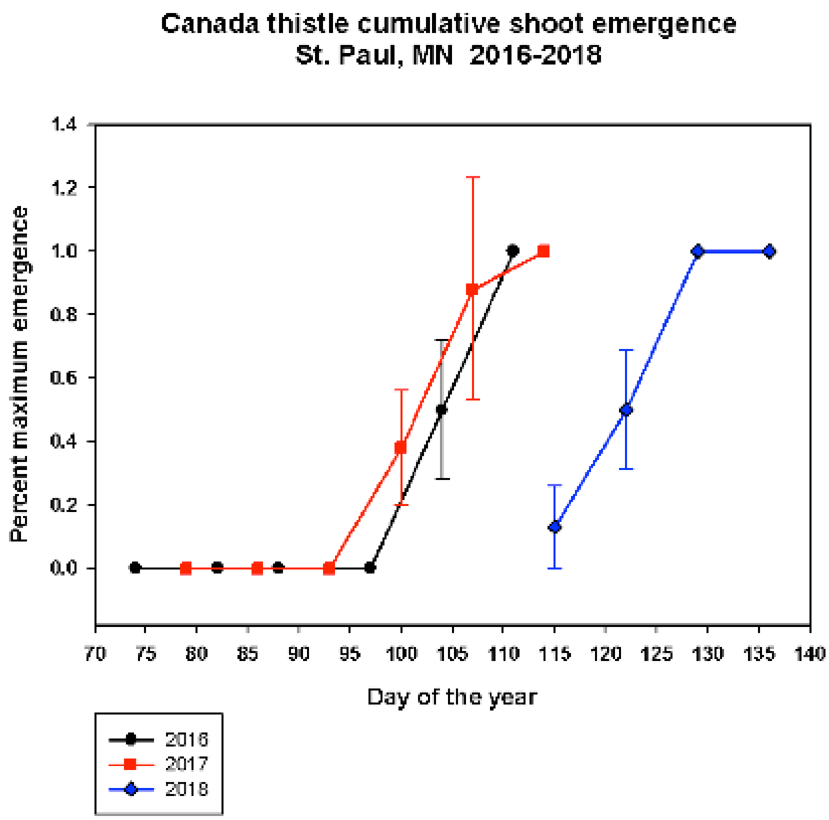

Figure 2. Date of perennial, Canada thistle (*Cirsium arvense*) vegetative shoot emergence in the spring in thistle common garden. St. Paul, MN. 2016, 2017, and 2018. Percent maximum emergence of 1.0 is equivalent to 100% shoot emergence. Days of the year are calendar days with January 1 as Day 1.

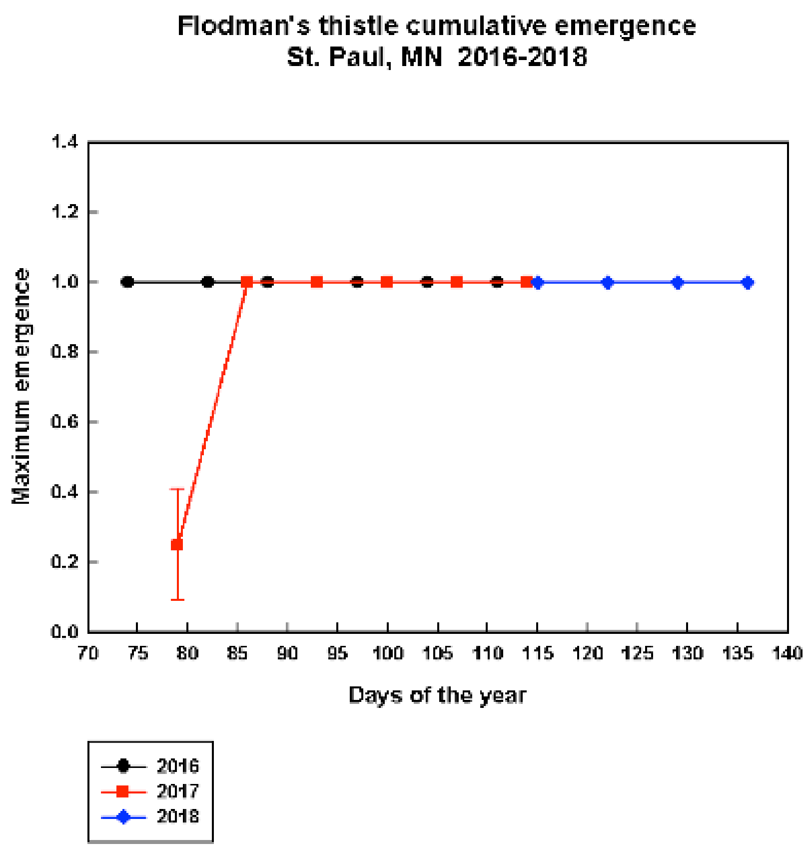

Figure 3. Date of perennial, Flodman's thistle (*Cirsium flodmanii*) vegetative shoot emergence in the spring in thistle common garden. St. Paul, MN. 2016, 2017, and 2018. Percent maximum emergence of 1.0 is equivalent to 100% shoot emergence. Days of the year are calendar days with January 1 as Day 1.

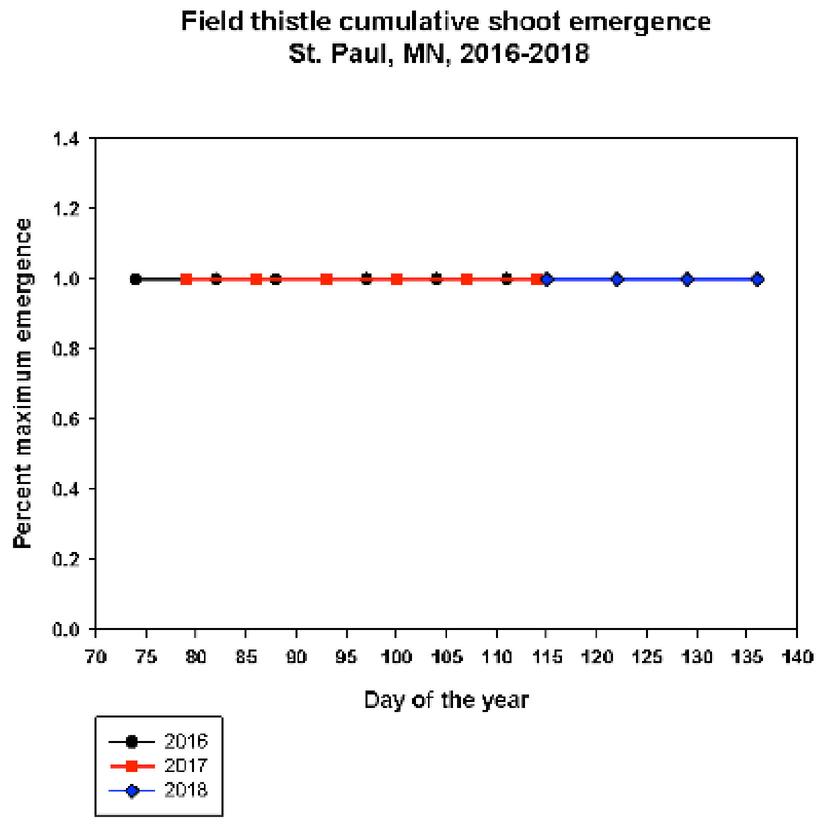

Figure 4. Date in the spring when new leaves on biennial, field thistle (*Cirsium discolor*) rosettes first recorded in thistle common garden. St. Paul, MN. 2016, 2017, and 2018. Percent maximum emergence of 1.0 is equivalent to 100% shoot emergence. Days of the year are calendar days with January 1 as Day 1.

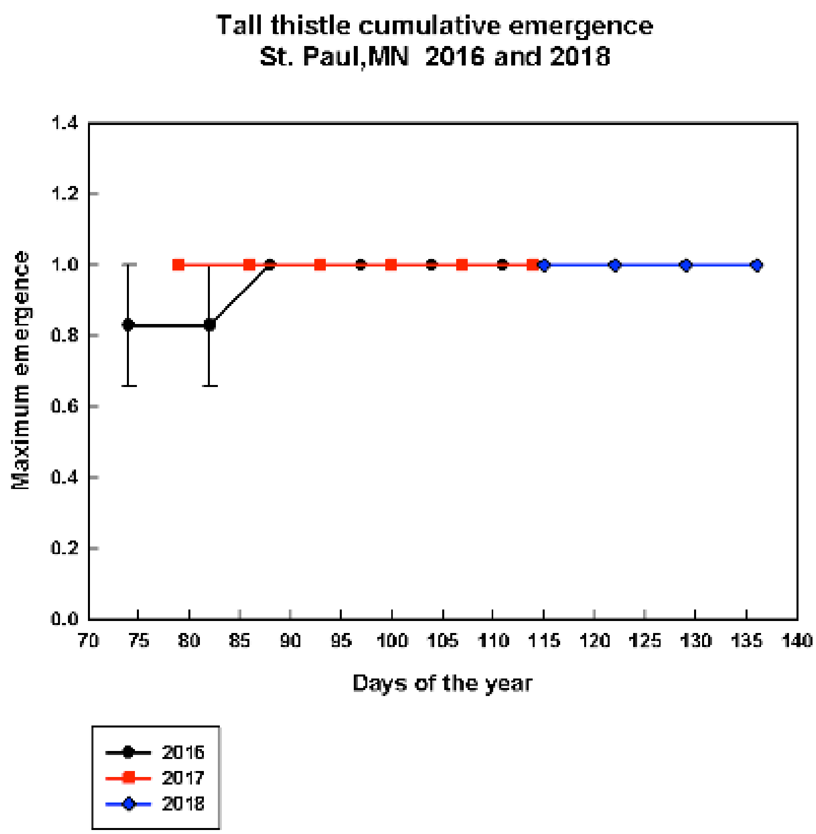

Figure 5. Date in the spring when new leaves on biennial, tall thistle (*Cirsium altissimum*) rosettes first recorded in thistle common garden. St. Paul, MN. 2016, 2017, and 2018. Percent maximum emergence of 1.0 is equivalent to 100% shoot emergence. Days of the year are calendar days with January 1 as Day 1.

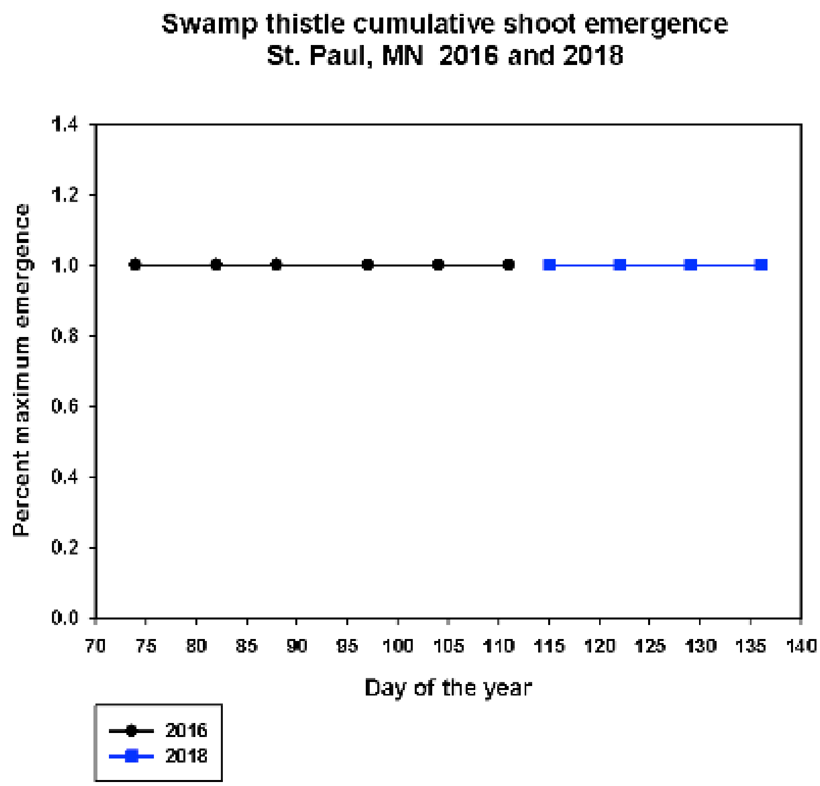

Figure 6. Date in the spring when new leaves on biennial swamp thistle (*Cirsium muticum*) rosettes first recorded in thistle common garden. St. Paul, MN. 2016 and 2018. Percent maximum emergence of 1.0 is equivalent to 100% shoot emergence. Days of the year are calendar days with January 1 as Day 1.

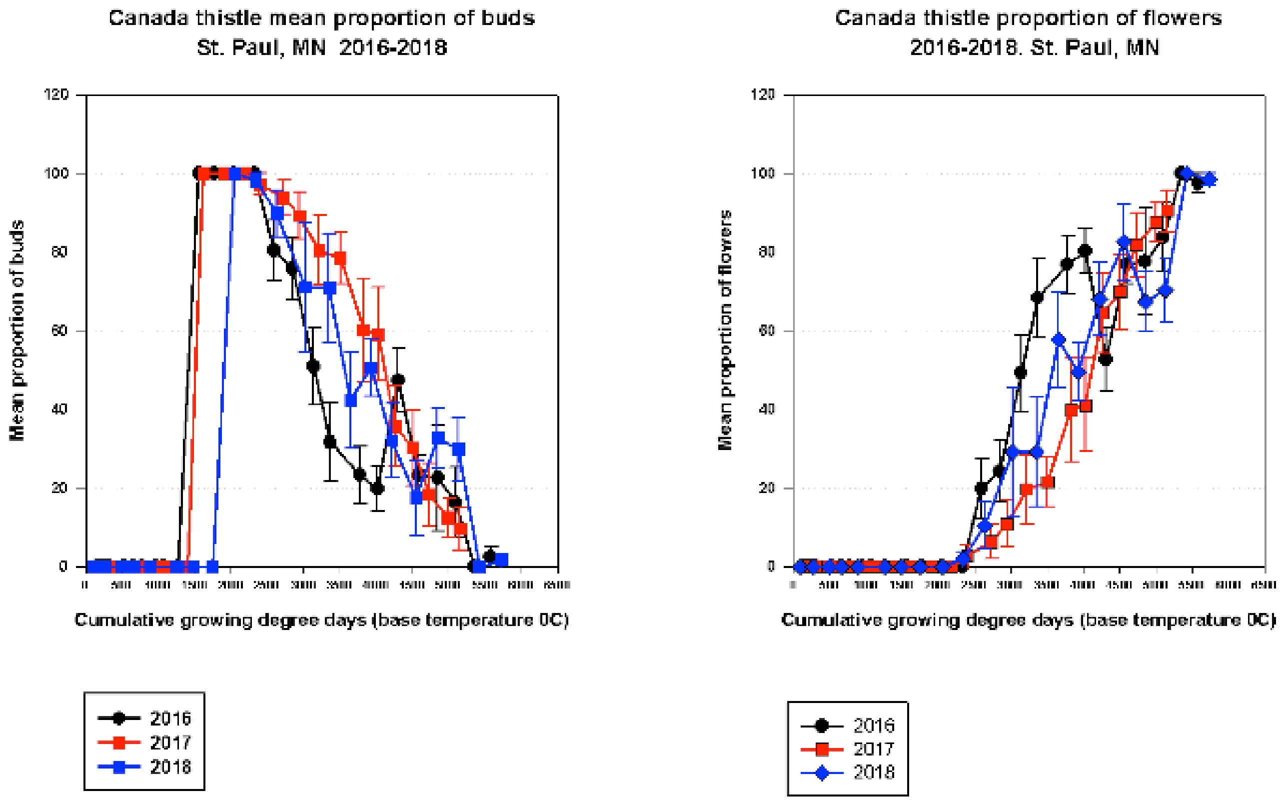

Figure 7. Mean percent buds or flowers in perennial Canada thistle by cumulative growing degree days (base temperature 0 C) and days of the year for bud and flowering in a thistle common garden. St. Paul, MN. Flowers include partial, full and mature flowers. 2016, 2017, and 2018.

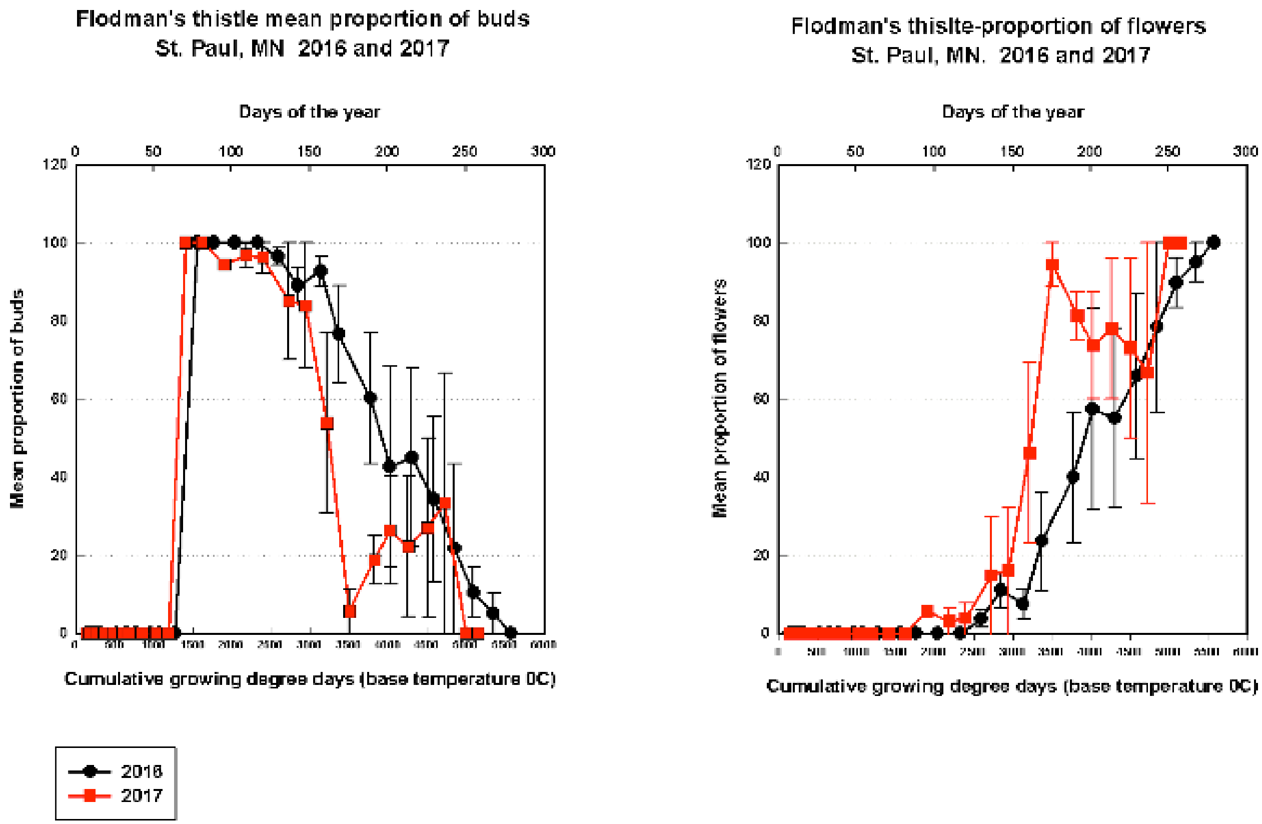

Figure 8. Mean percent buds or flowers in perennial Flodman's thistle (*Cirsium flodmanii*) thistle by cumulative growing degree days (base temperature 0 C) and days of the year for bud and flowering in a thistle common garden. St. Paul, MN. Flowers include partial, full and mature flowers. 2016 and 2017.

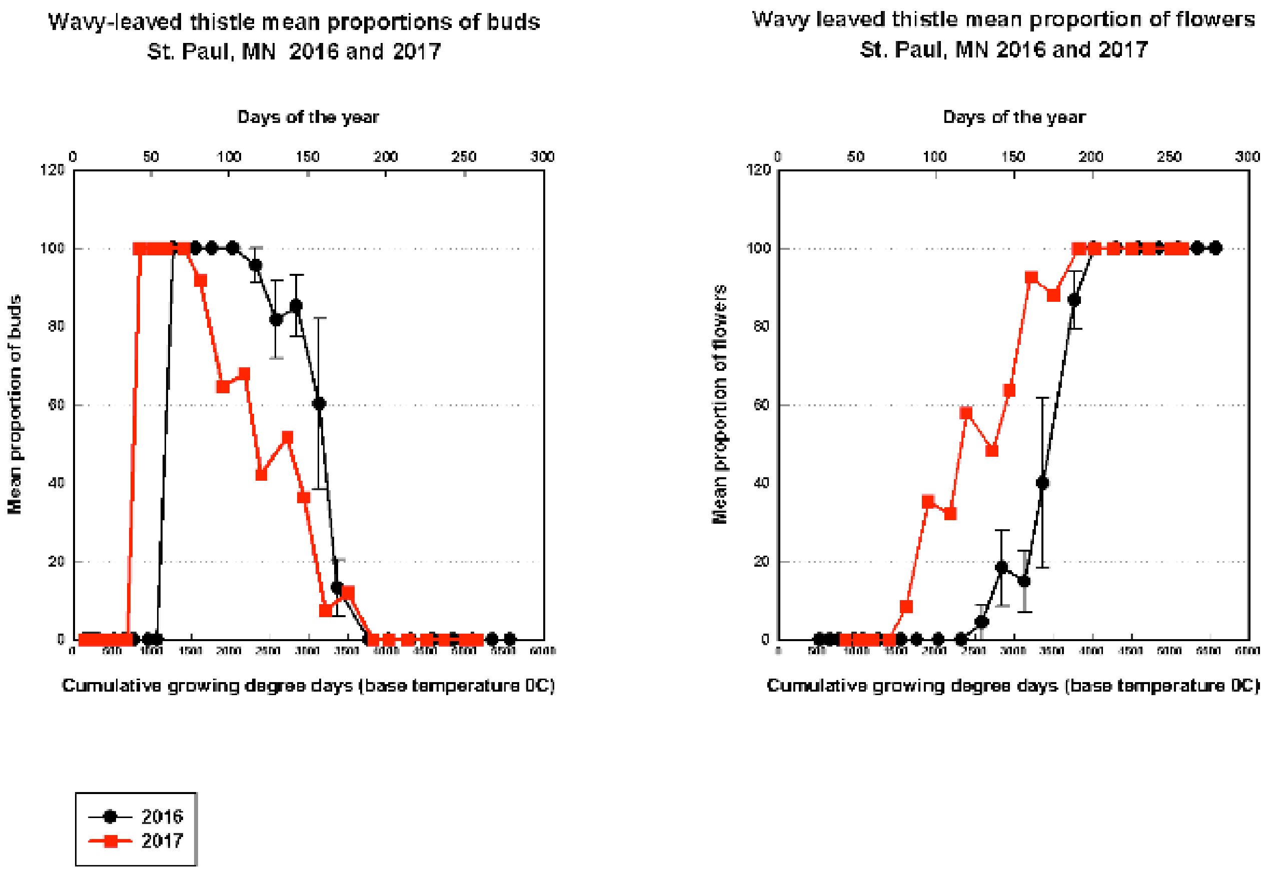

Figure 9. Mean percent buds or flowers in perennial wavy-leaved thistle (*Cirsium undulatum*) thistle by cumulative growing degree days (base temperature 0 C) and days of the year for bud and flowering in a thistle common garden. St. Paul, MN. Flowers include partial, full and mature flowers. 2016 and 2017.

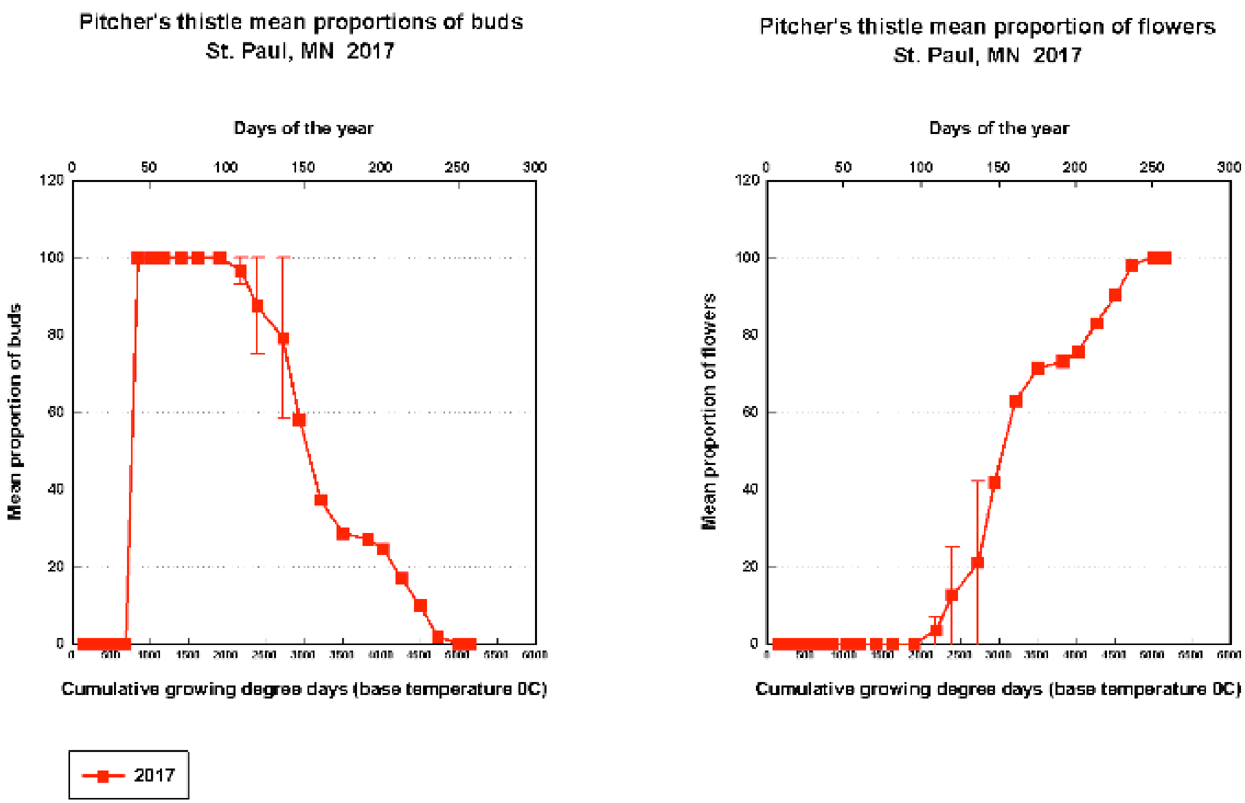

Figure 10. Mean percent buds or flowers in Pitcher's (*Cirsium pitcheri*) thistle by cumulative growing degree days (base temperature 0 C) and days of the year for bud and flowering in a thistle common garden. St. Paul, MN. Flowers include partial, full and mature flowers. 2017.

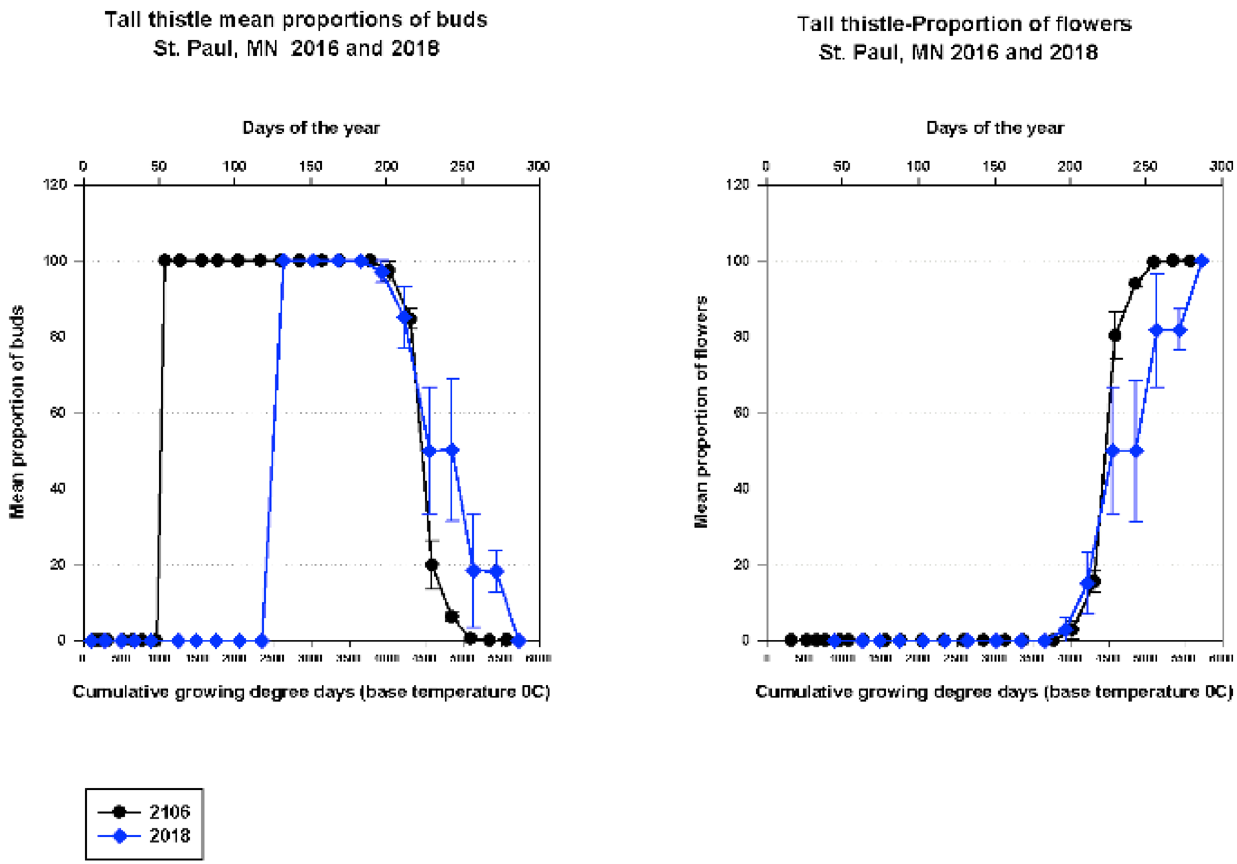

Figure 11. Mean percent buds or flowers in biennial tall thistle (*Cirsium altissimum*) thistle by cumulative growing degree days (base temperature 0 C) and days of the year for bud and flowering in a thistle common garden. St. Paul, MN. Flowers include partial, full and mature flowers. 2016 and 2018.

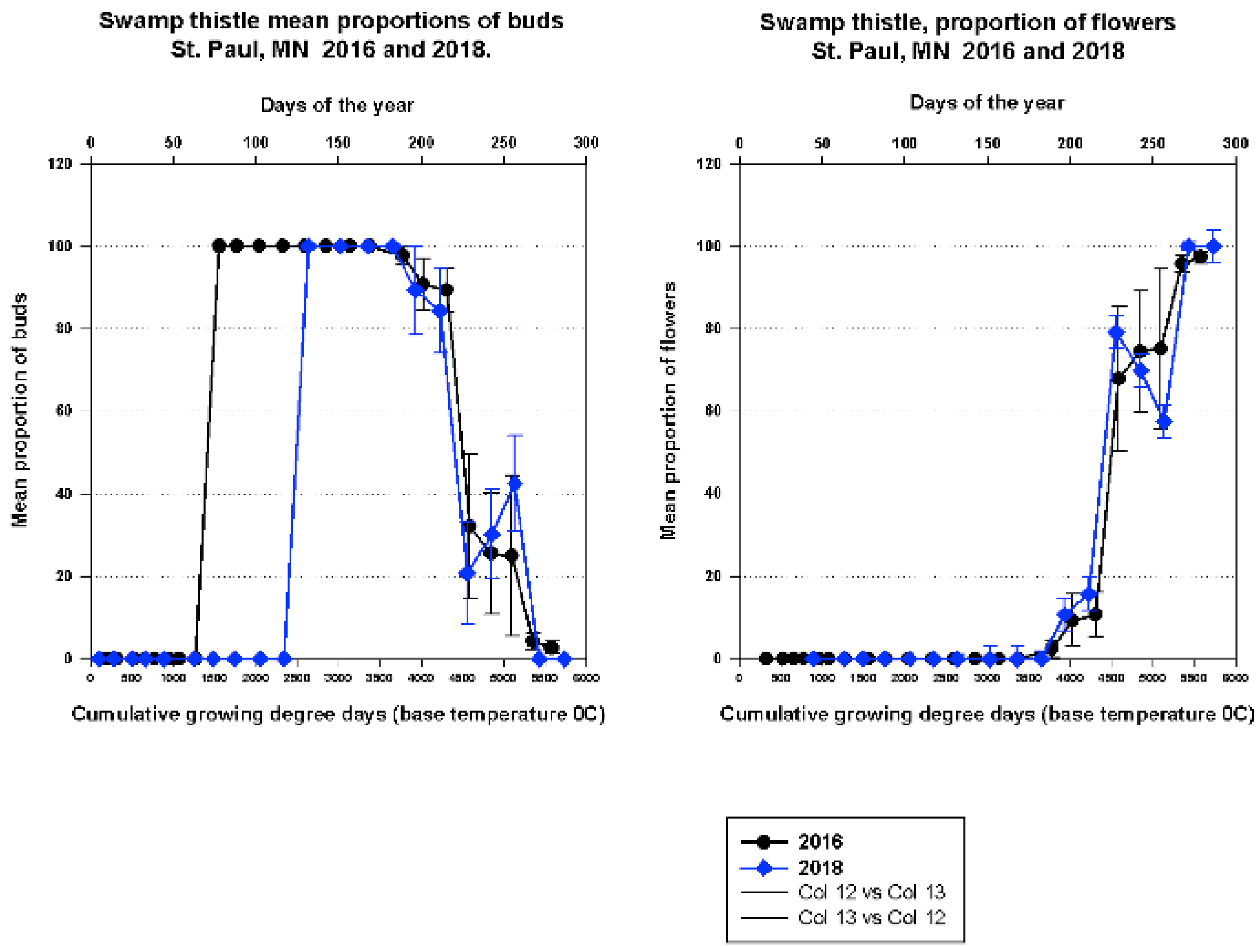

Figure 12. Mean percent buds or flowers in biennial swamp thistle (*Cirsium muticum*) thistle by cumulative growing degree days (base temperature 0 C) and days of the year for bud and flowering in a thistle common garden. St. Paul, MN. Flowers include partial, full and mature flowers. 2016 and 2018.

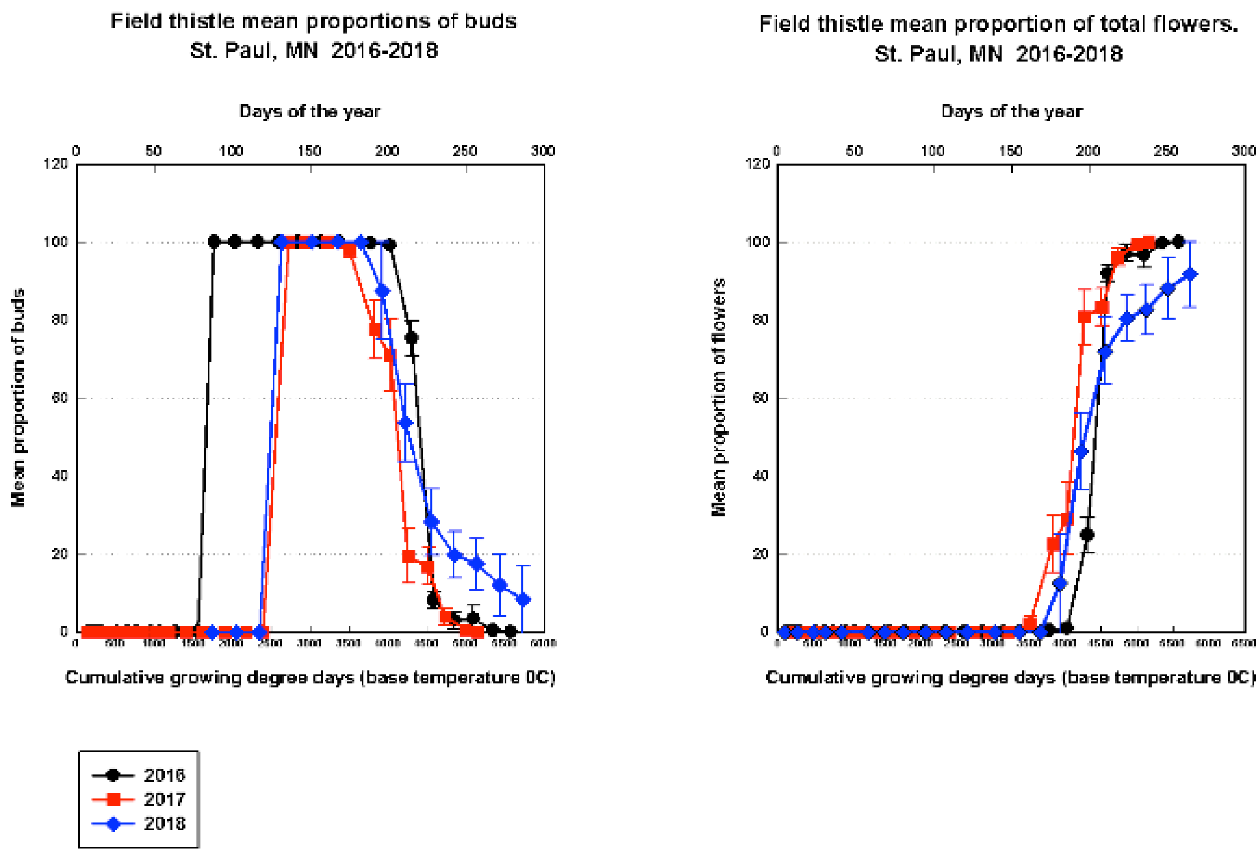

Figure 13. Mean percent buds or flowers in field thistle (*Cirsium discolor*) thistle by cumulative growing degree days (base temperature 0 C) and days of the year for bud and flowering in a thistle common garden. St. Paul, MN. Flowers include partial, full and mature flowers. 2016, 2017 and 2018.