Final report 1/1/12 to 12/31/12, by Lincoln Smith, USDA-ARS, Albany, CA

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Development of a New Biological Control Agent for Yellow Starthistle to test host specificity of weevil *Larinus filiformis*.

Executive Summary

A combination of laboratory and field experiments were conducted to evaluate the host plant specificity of *Larinus filiformis*, a weevil native to eastern Turkey that develops inside flower heads of yellow starthistle. The weevil has been difficult to maintain through its full life cycle in the laboratory. However, host specificity experiments to date indicate that the weevil has a strong preference for yellow starthistle. Of 12 nontarget species or varieties tested in multiple choice experiments oviposition occurred almost exclusively on yellow starthistle; however, eggs were found once on *Centaurea americana* and three times on *Ce. cyanus*. Larvae were not able to develop on either of these nontarget plants. The nontarget species most likely to be at risk have been tested, which indicates that this weevil is extremely specific.

Field garden experiments were conducted in Turkey near Trabzon in 2011 and at Igdir in 2012. Unusually rainy weather caused many flower heads to rot at Trabzon; however, weevil attack was recorded only in yellow starthistle. Igdir had a much more suitable climate, and 14 plant species or varieties were tested. *Larinus filiformis* was found attacking only yellow starthistle; however, some unidentified immature weevil specimens from nontarget plants remain to be identified by DNA analysis. These are very likely to be other species of weevils that were observed in some of these plants: *Larinus minutus*, *L. syriacus* and *L. turbinatus*. We took advantage of the Igdir field garden experiment to collect data on a prospective biological control agent of Scotch thistle, *Larinus latus*, which did not attack any of the nontarget plants.

The combined results indicate that *L. filiformis* is specific to yellow starthistle. Additional testing should be done to complete the host plant test list before submitting a petition to USDA-APHIS to request a release permit. Adults reared from the Igdir field experiment are being overwintered in our containment lab to use for these experiments next spring.

Introduction

Yellow starthistle (YST, *Centaurea solstitialis*, Asteraceae) is a winter annual forb originating from the Mediterranean Basin that has invaded about 8 million ha of North American rangeland, primarily in California, Oregon, Washington and Idaho, and is spreading eastward (Sheley *et al.* 1999, Pitcairn *et al.* 2006). Although yellow starthistle plants can be killed by a number of herbicides (DiTomaso et al. 2006a), it is difficult to achieve lasting control because seeds in the soil can persist for several years (Joley et al. 2003). Mowing, controlled grazing and controlled burning can be effective when timed appropriately but are difficult to plan and execute (Thomsen *et al.* 1994, 1997, Benefield *et al.* 1999, DiTomaso et al. 2006b). Classical biological control, introducing species of insects or pathogens that attack only yellow starthistle, has the best prospect of economically reducing YST populations over large areas (Smith 2007a). To date, six species of insects and one fungal pathogen have been introduced (Turner *et al.* 1995, Pitcairn et al. 2004, Woods et al. 2010). Two insects, *Eustenopus villosus* and *Chaetorellia succinea*, are now widespread and abundant, and appear to be reducing YST populations in some regions (Pitcairn et al. 2008), but they are not abundant at higher elevations (Yacoub 2005). USFS lands that are at risk of invasion by YST are generally at elevations higher than those occupied by these insects. Foreign exploration for new agents in eastern Europe and western Asia indicates that the most suitable prospective agent for higher elevations is the seed head weevil, *Larinus filiformis* (Smith *et al.* 2005, Cristofaro *et al.* 2006, Gültekin *et al.* 2008a, b). *Larinus filiformis* is very common in eastern Turkey, where it attacks about 75% of YST flower heads (Gültekin *et al.* 2008b). Climate matching based on its known range in Eurasia suggests that *L. filiformis* will be well adapted to many areas where YST habitat occurs on USFS land (Gültekin *et al.* 2008b).

The purpose of this project was to test the specificity of *L. filiformis* in field and laboratory experiments to determine if it is sufficiently specific to be safe for introduction as a classical biological control agent.

A. Laboratory Tests of Host Specificity

2011 Laboratory Tests

One hundred adult *Larinus filiformis* collected by M. Cristofaro and L. Gültekin in Eastern Turkey were received in the Albany containment laboratory in June 2010. All these insects died within a month, as did individuals that were held in Italy, suggesting that they were either stressed by confinement in large numbers during shipping or may have been infected by a pathogen. A shipment of 8 adults was received in June 2011, which were used immediately for host specificity testing. Individual females that were ovipositing were placed in a screen cage (60 x 60 x 90 cm) in a greenhouse with 4 to 5 test plant species, one of which was yellow starthistle. Trials were run for either 2 or 4 days, and 12 nontarget plant species or varieties were tested during July 2011. Flower heads were examined for adult feeding damage and oviposition. Any flower heads containing eggs were held to determine if the insects could complete development.

Larinus filiformis consistently oviposited on yellow starthistle, placing an average of 1.4 eggs per day (Fig. 1). In total, one egg was placed on *Ce. americana*, out of nine trials, and four eggs on *Ce. cyanus*, in three out of five trials. Females oviposited an average of 1.8 (± 0.3 SE) eggs per day during 2-day trials, but only 1.2 (± 0.2) eggs per day during 4-day trials, which suggests that there was not enough yellow starthistle available for oviposition sites during the longer trials. Oviposition on nontarget host plants occurred only during the 4-day trials, which is when there were insufficient yellow starthistle flower heads. No larvae developed on either *Ce. americana* or *Ce. cyanus*, but an average of 67% of eggs on yellow starthistle produced adults. The relatively high oviposition rate on *Ce. cyanus* (3 occurrences in 5 trials) suggests that this plant may warrant further testing. However, it should be noted that although this introduced plant is an ornamental, it is also considered to be invasive in some parts of the USA. *Centaurea stoebe* is the closest taxonomic relative to the target weed that was tested, *Ce. cyanus* and *Ce. montana* are next closest, progressively followed by safflower, *Ce. americana*, *Saussurea americana*, and the most distant are *Carduus nutans* and the three *Cirsium* species. Of these, the only species that are native to North America are *Ce. americana* (American basket flower), *Cirsium brevistylum* (clustered thistle) and *Ci. hydrophilum* (Suisun thistle).

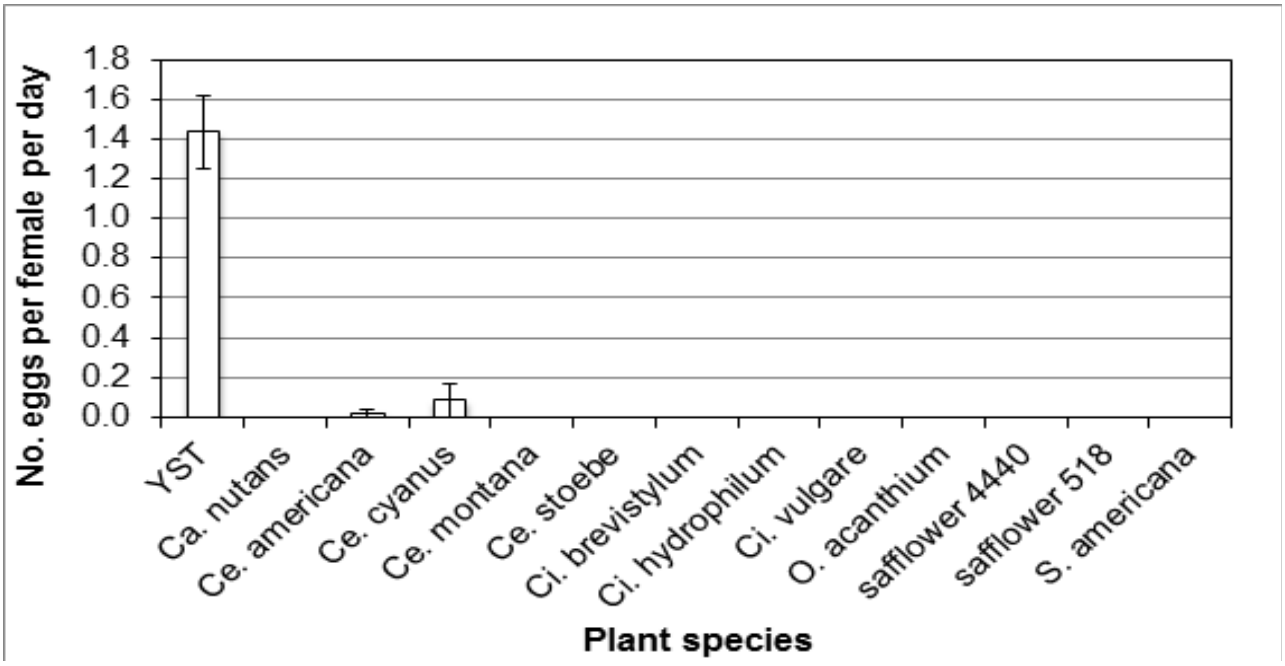

Figure 1. Specificity of *Larinus filiformis* as indicated by the number of eggs oviposited on plants in a multi-choice cage experiment in the USDA containment greenhouse (mean ± SE). One female was placed in a cage with 1 yellow starthistle plant and 3 to 4 nontarget plants of different species for either 2 or 4 days. A total of one egg was placed on *Centaurea americana* (out of 9 trials) and four eggs on *Ce. cyanus* (3 times out of 5 trials), but no larvae developed on either of these plants.

2012 Laboratory Tests

Adults reared from yellow starthistle inside the containment laboratory in early fall of 2011 were held to use for 2012 experiments. After about two weeks they were moved into a cold incubator (5°C, constant darkness) to hibernate until ready for use in spring host specificity tests. On June 12, 2012, adults were transferred to a sleeve box with ambient light conditions (>14 hours of light, diurnal temperature range 20-30°C) and held with cut leaves and immature flower buds of yellow starthistle to feed and develop eggs. To determine what size flower buds and the number consumed, individual females were placed in a cup containing a bouquet of yellow starthistle stems that had flower buds of different developmental stages. The flower buds were counted and classified based on their developmental stage (Maddox 1981), which ranged from very small (Bu1) to very large (Bu4), and included blooming (F1) and post-bloom (F2) flower heads. The stems were replaced every 2 to 6 days. Because the relative abundance of the various flower bud types may affect the number eaten, we calculated an "electivity index" (Chesson 1983) which adjusts for variation in relative abundance. The index ranges from +1 (100% attack) to -1 (0% attack), and 0 indicates neutral preference.

The adults survived for 28 to 46 days during the feeding experiment; however, none of the females started to oviposit. This suggests that there was not enough suitable food for the females to develop eggs. Adults had greatest preference (highest electivity index) for immature flower buds (Bu1 to Bu3) (Fig. 2). However, in our experiment they consumed more Bu1 than the other stages because this stage was usually most abundant. Often more than 50% of Bu2 and Bu3 were attacked, but never was more than 80% of any stage of flower bud attacked. While this

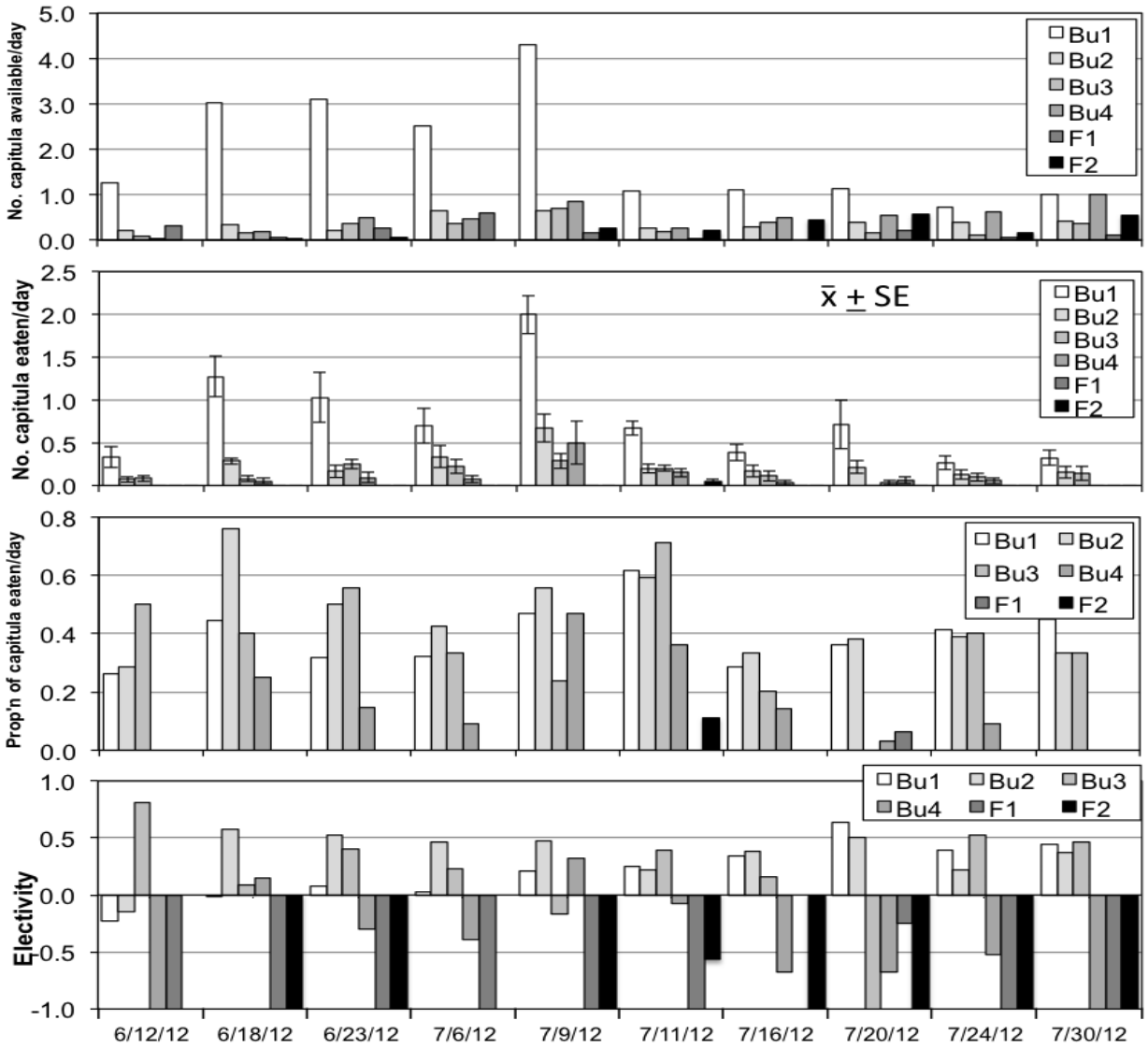

Figure 2. Feeding by adult *Larinus filiformis* after termination of overwintering conditions in the containment laboratory. One female was placed in a cup with a bouquet of yellow starthistle stems containing flower buds of different developmental stages for 2 to 6 days. Unopened flower buds ranged from small (Bu1) to large (Bu4), and included open flowers (F1) and post-bloom flower heads (F2) (Maddox 1981). Electivity is an index that ranges from +1 (strong preference) to -1 (strong aversion), and 0 indicates neutral preference (Chesson 1983). would suggest that the weevils did not lack food, it is possible that the confined conditions and closely bunched flower buds prevented them from feeding more. Under natural conditions, flower buds are far apart, and the weevils would have to fly from plant to plant, whereas in the this experiment there was not enough space to permit flying.

The original intention was to run a series of choice and no-choice host specificity experiments; however, this could not be done because none of the females started to oviposit.

B. Field Tests of Specificity in Turkey

Experiments were conducted near Trabzon (on the Black Sea) in 2011 and at Igdir (eastern Turkey) in 2012. Trabzon was an effective location to rear test plants because of the cool rainy winter weather. However, unusually heavy precipitation during the summer of 2011 caused many of the flower heads to rot on the plants. In

2012 the experiment was performed at Igdir, which has hot dry summer weather, to avoid loss of data due to rotting.

2011 Field Experiment

Adult *Larinus filiformis* were collected near Igdir and released in the field garden on June 8 and June 25, 2011 (Fig. 3). Adults were still present in the garden on July 10. The garden contained over 80 plants representing 7 species or varieties of test plants. Numbers and developmental stages of flower heads were recorded on June 10 (Fig. 4). Senescing flower heads were collected on July 10, July 20 and Aug. 9 and were either dissected at the site or were shipped to the quarantine laboratory in Albany, CA for later dissection (Fig. 5).

In Albany, CA, we dissected yellow starthistle flower heads collected from the field garden at Trabzon on July 10, 2011, which indicated an attack rate of 13% by weevils, presumably *Larinus filiformis* (Table 1). However, only 1 adult later emerged from other flower heads held in a bag, probably because summer rains caused microbial decay of the flower heads.

Although 2011 was an unusually rainy year at Trabzon, we decided that it would be more reliable to do the 2012 garden experiment at a drier location to avoid rotting of flower heads. Our Turkish cooperator, Levent Gültekin, identified a new location, at a research station near Igdir, for performing the 2012 field experiment. This location has indigenous populations of yellow starthistle and *Larinus filiformis* and much less rain during the summer.

Figure 3. Arrangement of plants in Trabzon garden, May 23, 2011 (left to right: artichoke, safflower-linoleic, safflower-oleic, *Ci. brevistylum*/YST, *Ce. americana*, safflower-linoleic, safflower-oleic, *Ce. cyanus*, YST, artichoke). Release of *Larinus filiformis* in Trabzon garden on June 8, 2011.

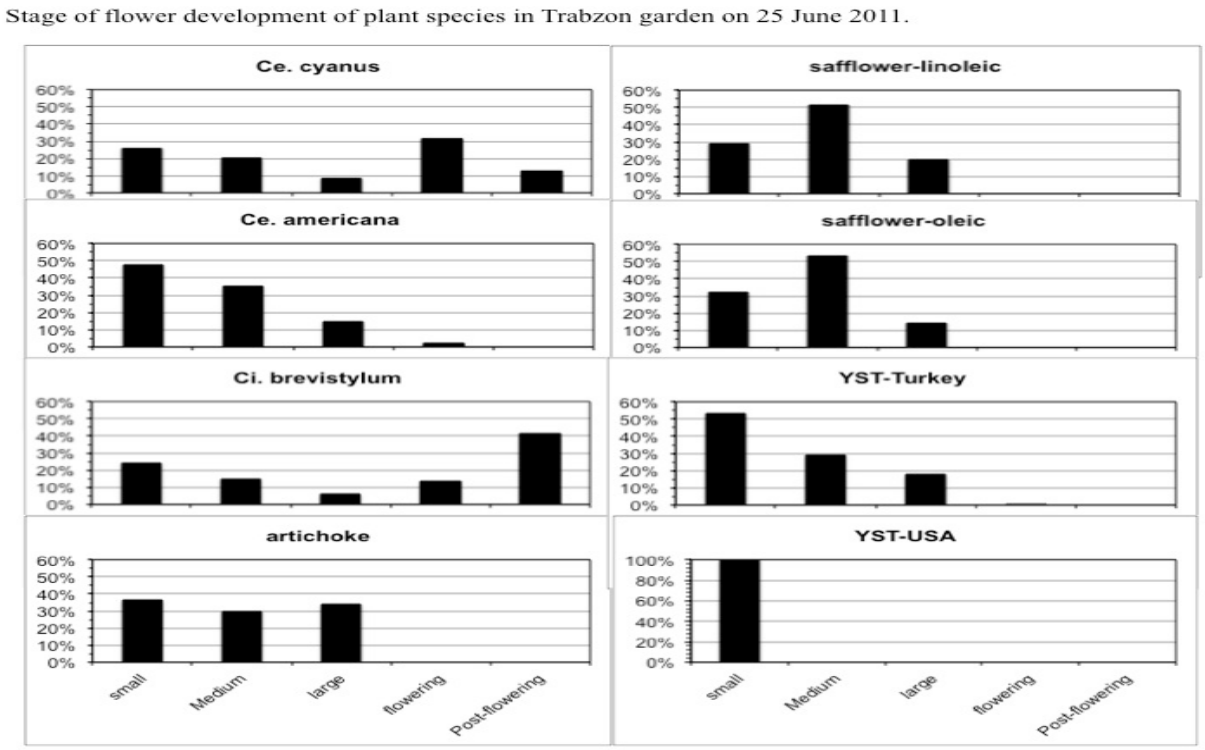

Figure 4. Proportion of flower heads at different developmental stages at the time of releasing adult *Larinus filiformis*, on 10 June 2011, in the field garden at Trabzon, Turkey. Adults oviposit in large flower heads of yellow starthistle (YST) before they begin to flower. All the test plants had capitula of appropriate stages to assess their risk to attack by the weevil. Plants of the USA accession of YST were small and delayed in development compared to the Turkish plants.

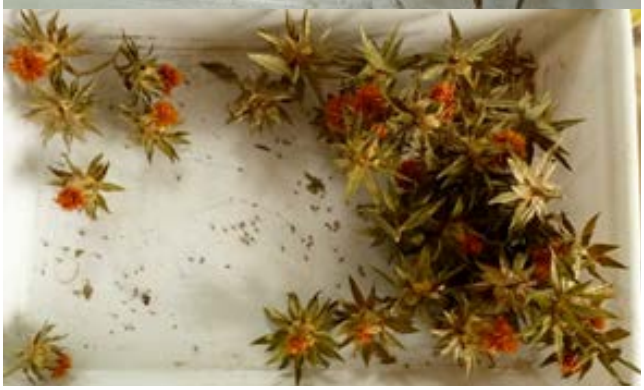

Figure 5. Final harvest of flower heads at field garden at Trabzon, Turkey on 8-10 Aug. 2011. All plants were then pulled up and burned to prevent future establishment of any unwanted plants.

Table 1. Attack rates of flower heads (FH) dissected on July 10, 2011 in the field garden at Trabzon, Turkey
Infestation rate by

	No. FH	weevil	fly	moth	disease
yellow starthistle-TK	231	13%	0%	0%	11%
artichoke	52	0%	32%	26%	2%
safflower-oleic	200	0%	4%	2%	17%
safflower-linoleic	280	0%	5%	1%	17%
Centaurea americana	77	0%	97%	0%	0%
Centaurea cyanus	341	0%	47%	0%	0%

2012 Field Experiment

Our Turkish cooperator, Levent Gültekin, identified a new location, at a research station in Igdir, for performing the 2012 field experiment. Igdir is in eastern Turkey, which is drier than Trabzon and has indigenous populations of yellow starthistle and Scotch thistle (*Onopordum acanthium*). It is also close to natural populations of *Larinus filiformis*. An agreement was established with the director to use the site and to obtain help to cultivate the plot and water the plants. Although this location is much more suitable for conducting the field experiment, it is too cold during winter to grow most of the nontarget biennial plants. Eighteen species test plants were grown in pots near Trabzon Turkey during the winter of 2011-2012, as was done for the preceding field experiment. Over 670 plants were started, of which 260 survived for use in the field experiment. Dr. Gültekin made four trips to Trabzon to supervise maintenance of the biennial plants and to start the annual plants for the 2012 experiment. During the winter, all the *Centaurea americana* plants died as did most of the *Cirsium brevistylum* and *Cirsium rhothophilum*. The remaining test plants were transported to Igdir in April and transplanted into the field garden on April 20-21. Plants were weeded and irrigated as needed, and the size and number of flower heads was recorded every two weeks (Fig. 6). Dr. Gültekin collected adult *L. filiformis* near Igdir, and he released 34 in the field garden on May 19 and 37 on June 2.

Figure 6. Igdir garden on June 16, 2012. Plants from foreground to background: Scotch thistle, yellow starthistle, safflower, *Cirsium hydrophilum*, safflower, yellow starthistle, safflower, *Centaurea cyanus*, *Cirsium loncholepis*, *Cirsium occidentale*, Scotch thistle, artichoke.

Adults were seen on yellow starthistle plants on May 20 (6 adults), June 2 (16 adults) and June 16 (15 adults), indicating the minimum period that were present in the garden. Fourteen adult *Larinus latus*, a prospective biological control agent of Scotch thistle, were collected and released in the garden on June 17.

Flower heads were collected as they matured to permit any insects to complete development, but to catch adults before they emerged. Mature flower heads were collected on June 16, June 28, July 16 and August 1 to dissect and rear out insects (Figs. 7 and 8, Table 2). Flower heads were also collected from *Cirsium arvense* (bull thistle) and *Acroptilon repens* (Russian knapweed) growing next to the garden and from *Onopordum acanthium* (Scotch thistle) growing on the road outside the experiment station. When possible, 200 flower heads were dissected for each plant species. Additional flower heads of safflower, bachelor's button and yellow starthistle were held in containers for adult emergence and to process later. All adult insects were preserved for identification, viable pupae and large larvae were transferred to artificial diet to produce adults, and immature or damaged insects were preserved in acetone for DNA analysis. These specimens will be processed during the winter.

Figure 7. Harvesting safflower flower heads in Igdir Field garden on July 17, 2012. All flower heads were harvested for dissection when they matured. By August 3 all flower heads had been harvested and all plants were destroyed. Adult *Larinus filiformis* emerging from yellow starthistle (lower left); damage by *Larinus latus* to Scotch thistle (lower right).

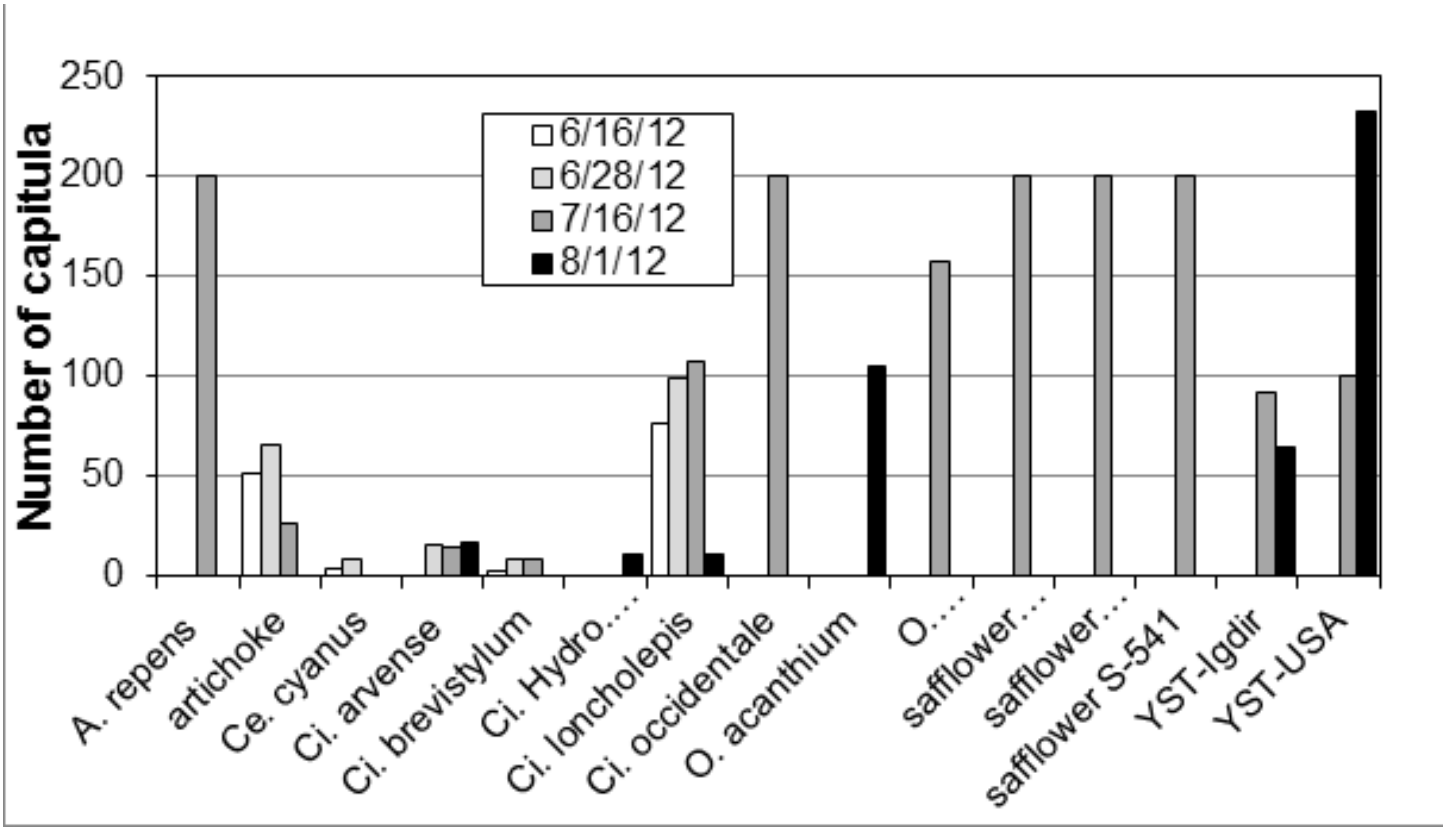

Figure 8. Number of flower heads dissected on four sample dates. All plants of C*entaurea americana* and *Cirsium rhothophilum* died before flowering. 'YST-Igdir' and 'YST-USA' are yellow starthistle plants from Turkey and California, respectively.

A variety of insects attacked flower heads of the various plant species (Fig. 9). There was no attack on *Cirsium brevistylum*, which flowered early and produced only 13 flower heads. Lepidoptera, flies and gall-formers (tephritid flies and cynipid wasps) attacked flower heads of many of the plants. Weevil damage was observed in Russian knapweed (*Acroptilon repens*), bachelor's button (*Centaurea cyanus*), *Cirsium loncholepis*, *Ci. hydrophilum vaseyi*, Scotch thistle (*O. acanthium*), safflower and yellow starthistle. However, *Larinus filiformis* was only found in yellow starthistle, and *Larinus latus* was only found in Scotch thistle (Fig. 10). *Larinus curtus* was found in 3% of yellow starthistle. *Larinus turbinatus*, which is known to have a fairly broad host range, was found in 10% of *Ci. loncholepis* and 2% of *Ci. hydrophilum vaseyi*. Unidentified weevil specimens collected in *Ce. cyanus*, *Ci. loncholepis*, *Ci. hydrophilum vaseyi*, safflower and yellow starthistle will be processed for identification this winter.

About 11,500 yellow starthistle flower heads collected from the Igdir garden were taken to the USDA containment laboratory. So far, 54 adult *L. filiformis* have been reared and are being maintained to use for experiments in spring 2013.

Table 2. Plants available for infestation and infestation by *Larinus filiformis* in the Igdir field garden in 2012.

Plant species	No. plants 5/20/12	No. flower heads 6/17/12	No. flower heads dissected	No. flower heads infested by *L. filiformis*
artichoke-USA	26	46	54	0
Centaurea cyanus	16	1,465	229	0
Cirsium arvense	–	–	200	0
Cirsium brevistylum	1	5	13	0
Cirsium hydrophilum vaseyi	19	81	117	0
Cirsium loncholepis	32	136	201	0
Cirsium occidentale	7	1	11	0
Cirsium rhothophilum	4	0	0	–
Onopordum acanthium, road	20	0	157	0
Onopordum acanthium-Igdir	20	231	105	0
safflower-cw4440	23	692	200	0
safflower-Hartmann	16	647	200	0
safflower-s541	24	724	200	0
yellow starthistle-Igdir	20	2,221	156	26
yellow starthistle-USA	24	2,478	333	33
Total	252	8,727	2,376	59

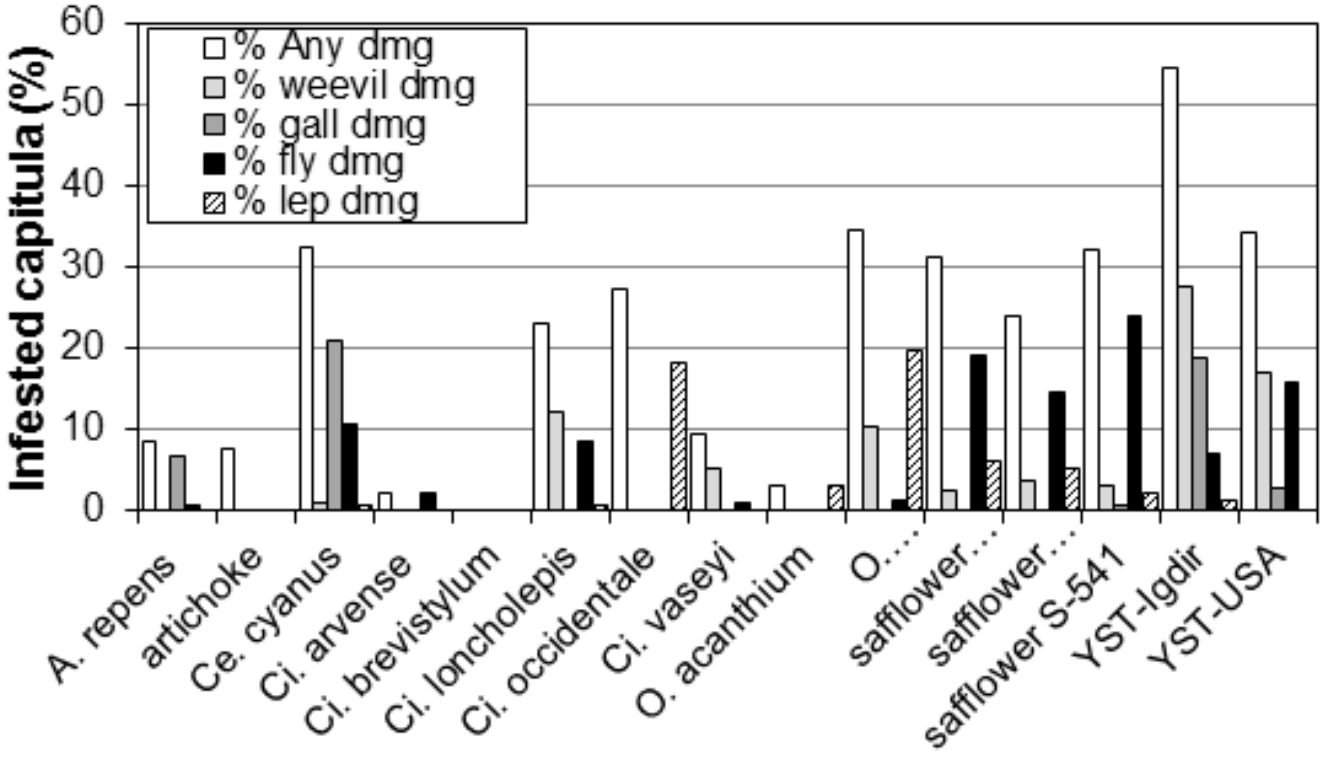

Figure 9. Percentage of dissected flower heads that were infested by insects. Several species of weevils were found in addition to the two species that were released (see text), as well as moths ('lep') and flies. Galls were produced by either flies (*Urophora* sp.) or wasps (Cynipidae). Specimens are being evaluated by DNA and morphological analysis to determine their identification.

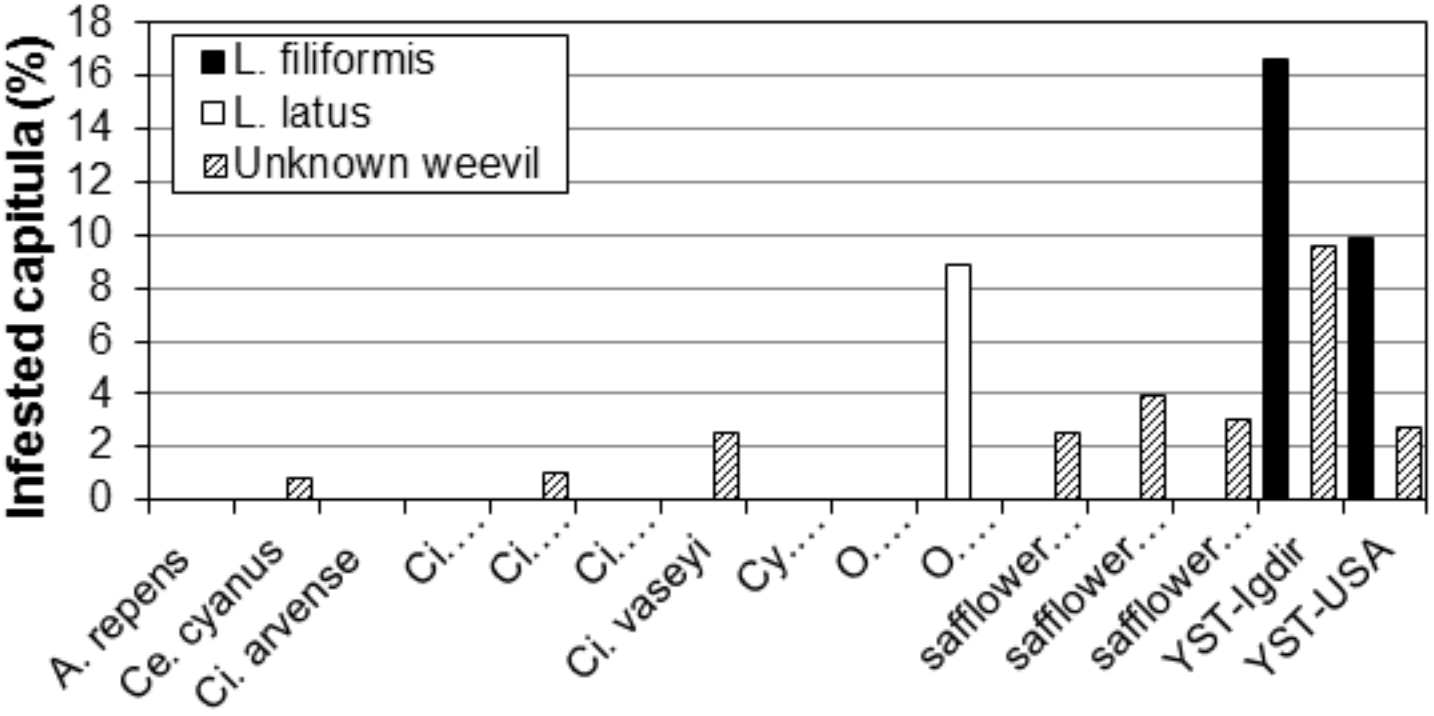

Figure 10. Percentage of dissected flower heads that were infested by *Larinus filiformis*, a prospective biological control agent of yellow starthistle, *Larinus latus*, a prospective agent of Scotch thistle (*Onopordum acanthium*), and unidentified weevils. DNA and morphological analysis is being conducted on unidentified weevil specimens. Weevils from the nontarget plant species are likely to be *Larinus syriacus*, *L. turbinatus*, or *L. minutus*.

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