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16. ABSTRACT
 Yellow starthistle and tumbleweed (Russian thistle) are two of the most important weeds on California Department of Transportation (Caltrans) rights-of-way. They occur in high densities, out-competing desirable grasses and increasing risk of wildfire and soil erosion. Transportation rights-of-way serve as corridors that can aid the spread of weed seeds by vehicles. Weed-infested rights-of-way can also be a source of seeds that might contribute to the spread of weeds to neighboring properties. Both these plants are alien to North America and have previously been targeted for biological control, but the introduced agents have not reduced these weeds in California. Release of new agents can provide widespread and perpetual reduction of weed populations without further expenditures.

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Enhanced Biological Control of Yellow Starthistle and Tumbleweed (Russian Thistle)

Final Report

by

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9 July, 2006

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EXECUTIVE SUMMARY

We conducted evaluations of host plant specificity and potential efficacy of prospective insect and mite biological control agents of two invasive alien weeds, yellow starthistle and tumbleweed. The biological control agents are alien to the U.S. and must be evaluated before it is possible to obtain permission to release them.

Yellow starthistle. We completed evaluation of host plant specificity of the rosette weevil from Turkey (*Ceratapion basicorne*). We tested 51 species of nontarget plants including 4 economic species and 24 species native to California. The results indicate that the weevil poses no risk to native plants, but it may cause some damage to bachelor's button (which is both an invasive weed and an ornamental flower). This damage is no worse than that caused by other previously approved biological control agents. Under greenhouse conditions, weevil infestation reduced growth of well-watered, fertilized plants by 23%. Impact under field conditions could be greater. We submitted a "petition" to the Technical Advisory Group (TAG) of U.S. Department of Agriculture, Animal and Plant Health Inspection Service (USDA-APHIS) for *Ceratapion basicorne* in Jan. 2006 (Appendix 1). TAG has representatives from all the U.S. federal land management agencies, Canada, and Mexico. The petition is still being evaluated by TAG. No release can be made until APHIS issues a permit. In the meanwhile, we began evaluation of the flea beetle, *Psylliodes chalconera*, from Russia. We have tested 55 plant species and expect to complete testing this summer. We produced a short manual on biological control of yellow starthistle (Appendix 3).

Tumbleweed. We completed evaluation of host plant specificity of the blister mite from Greece (*Aceria salsolae*). We tested 36 species of nontarget plants including 6 economic species and 25 species native to California. The results indicate that the mite only multiplies on the target weed and its close weedy relatives. Under laboratory conditions, mite infestation reduced growth of well-watered, fertilized plants by 66%. We submitted a "petition" to USDA-APHIS Technical Advisory Group (TAG) for *Aceria salsolae* in Dec. 2004 (Appendix 2). TAG recommended approval of the mite for release in Aug. 2005. We wrote a draft Environmental Assessment and submitted an official request for a release permit to USDA-APHIS in Sept. 2005. The permit application is still being processed. No release can be made until APHIS issues a permit. In the meanwhile, we continued evaluation of two other candidates. Tests in France determined that the bug, *Piesma salsolae*, is not specific enough to be safe for release, and work on this agent has been discontinued. We evaluated the seed-feeding caterpillar, *Gymnancylla canella*, for three years and find that it is more specific. We hope to complete testing this fall.

Steps required before making releases:

1. Scientist submits petition to TAG.
2. TAG recommends approval to release.
3. Scientist submits permit application to USDA-APHIS.
4. APHIS writes an Environmental Assessment and consults with U.S. Fish and Wildlife Service.

If they conclude that there will be no significant negative impact (FONSI), then APHIS can issue a permit for the scientist to release the agent. The state of California must also permit the release.

This regulatory process can take one or more years. More information is available at:

<http://www.aphis.usda.gov/ppq/permits/tag/>

Key Words:

yellow starthistle, *Centaurea solstitialis*, Russian thistle, tumbleweed, *Salsola tragus*, *Salsola iberica*, biological control, invasive alien weed, weed control

1. PROJECT DESCRIPTION**Need for Project**

Yellow starthistle and tumbleweed (Russian thistle) are two of the most important weeds on California Department of Transportation (Caltrans) rights-of-way. They occur in high densities out-competing desirable grasses, and increase risk of wildfire and soil erosion. Transportation rights-of-way serve as corridors that can aid the spread of weed seeds by vehicles. Weed-infested rights-of-way can also be a source of seeds that might contribute to the spread of weeds to neighboring properties. Both these plants are alien to North America and have previously been targeted for biological control, but the introduced agents have not reduced these weeds in California (Wagenitz 1975, Turner et al. 1995, Sheley et al. 1999). Discovery of new prospective agents in Eurasia provides new opportunities to achieve successful, long-term control of these weeds (Rosenthal et al. 1994, De Lillo and Sobhian 1996, Smith 2004). The cost-benefit ratio of successful biological control projects is usually very favorable; examples range from 1: 2 to 1: 1675 (Cruttwell-McFadyen 1998).

Yellow starthistle (*Centaurea solstitialis*, Asteraceae) infests about 14 million acres in California, primarily in rangeland and road rights-of-way (Pitcairn et al. 2006). It is toxic to horses, causing the lethal disease, nigropallidal encephalomalacia (Cordy 1978), reduces plant biodiversity and the spines interfere with cattle grazing and human recreation. Six species of insect biological control agents have been introduced to California, two of which are now widespread and are beginning to reduce seed production (Pitcairn et al. 2005). The rust, *Puccinia jacea* var. *solstitialis*, was first released in California in 2003 and is being released throughout the state by California Department of Food and Agriculture (CDFA). Although the rust is beginning to infect the weed, it does not appear to be causing much damage (Smith 2004). Developing new biological control agents that attack the root, stems and leaves of the plant will complement the impact of the other biological control agents, and will help to provide long-term control and reduce the need to apply herbicides.

Tumbleweed (Russian thistle, *Salsola tragus*, Chenopodiaceae) is a major alien weed infesting roadsides and other disturbed sites in California (Young, 1989, Goeden and Pemberton 1995). Windblown tumbleweeds careening across roads may increase the possibility of automobile accidents. Tumbleweed is an important off-season host of the beet leafhopper, which transmits curly top virus, which attacks several hundred varieties of ornamental and commercial crops, causing severe damage (Bennett 1971). Tumbleweed is native to southern Russia and western Siberia, but now occurs across much of Europe and Asia (Botschantzev 1969). Two moths were introduced for biological control in the 1970s; however, predators and parasites prevent them from becoming abundant enough to control the weed (Goeden and Pemberton 1995). Developing new biological control agents will help reduce both the size and number of tumbleweeds and reduce the need to apply herbicides.

Increasing the effectiveness of biological control will help Caltrans meet its commitment to manage these weeds and reduce usage of herbicides by 80% by 2012, without increasing maintenance activities.

Project Goal

To release natural enemies that are highly specific to yellow starthistle and tumbleweed to reduce the weed populations to innocuous levels.

Project Objectives

- 1) discover promising arthropod biological control agents in Eurasia,
- 2) test them to determine if they are safe,
- 3) test them to determine if they are likely to impact the target weed,
- 4) obtain state and federal permission to release them,
- 5) provide information to Caltrans on biological control of yellow starthistle and tumbleweed.

We evaluated the most promising agents (a root-feeding weevil for yellow starthistle, and a blister mite for tumbleweed) in quarantine laboratory to determine their host plant specificity. Depending on research progress, we expanded our work to include additional agents for these two weeds (a seed-feeding caterpillar on tumbleweed and a flea beetle on yellow starthistle). When sufficient information was collected, we prepared and submitted a petition to USDA-APHIS to request permission to introduce the new agents. When permission is granted, we will multiply and release the agents. We provided Caltrans with information regarding the detection, identification and use of the currently established agents and any new agents for yellow starthistle and tumbleweed.

2. PROJECT ACHIEVEMENTS

Yellow starthistle

1. Completed evaluation of host plant specificity of the rosette weevil from Turkey (*Ceratapion basicorne*). We tested 51 species of nontarget plants including 4 economic species, 9 varieties of safflower, and 24 species native to California (Fig. 9, Appendix 1, Publication 2). The results indicated that the weevil poses no risk to native plants, but it may cause damage to bachelor's button (which is both an invasive weed and an ornamental flower).
2. Evaluated the potential impact of the rosette weevil on growth of yellow starthistle. Under greenhouse conditions, weevil infestation reduced growth of well-watered, fertilized plants by 23% (Appendix 1). Impact under field conditions could be greater.
3. Submitted a "petition" to the Technical Advisory Group (TAG) of USDA-Animal and Plant Health Inspection Service (APHIS) for *Ceratapion basicorne* in Jan. 2006 (Appendix 1). The petition is still being evaluated by TAG. No release can be made until APHIS issues a permit.
4. Began evaluation of the flea beetle, *Psylliodes chalconera* which was discovered in Russia during foreign exploration (Publication 1). We have tested 55 plant species and expect to complete testing this summer.
5. Produced a short manual on biological control of yellow starthistle (Appendix 3).

Tumbleweed

6. Completed evaluation of host plant specificity of the blister mite from Greece (*Aceria salsolae*). We tested 36 species of nontarget plants including 6 economic species and 25 species native to California (Table 2, Appendix 2). The results indicated that the mite poses no risk to native or economic plants.
7. Completed evaluation of potential impact of the blister mite on growth of tumbleweed (Appendix 2). Our results show that under laboratory conditions, mite infestation reduced growth of well-watered, fertilized plants by 66%. This corroborates the observation that

infested plants (5.5 inches tall) in the field in Turkey were less than half the size of uninfested plants (14 in.) (Sobhian et al. 1999).

8. Submitted a "permit" to USDA-APHIS Technical Advisory Group (TAG) for *Aceria salsolae* in Dec. 2004 (Appendix 2). In Aug. 2005, TAG recommended approval to release the mite. I wrote a draft Environmental Assessment and submitted an official request for a permit from USDA-APHIS to release the mite in Sept. 2005. The permit application is still being processed. No release can be made until APHIS issues a permit.
9. Began evaluation of the seed-feeding caterpillar, *Gymnancyla canella*, from France, and have tested 31 plant species. We hope to complete testing this fall.

Publications in Scientific Journals

1. Cristofaro, M. M. Yu. Dolgovskaya, A. Konstantinov, F. Lecce, S. Ya. Reznik, L. Smith, C. Tronci, and M. G. Volkovitsh. 2004. *Psylliodes chalconeris* Illiger (Coleoptera: Chrysomelidae: Alticinae), a flea beetle candidate for biological control of yellow starthistle *Centaurea solstitialis*. In: Proceedings of the XI International Symposium on Biological Control of Weeds (eds. Cullen, J.M., Briese, D.T., Kriticos, D.J., Lonsdale, W.M., Morin, L. and Scott, J.K.) pp. 75-80. CSIRO Entomology, Canberra, Australia. (= Appendix 5)
2. Uygur, S., L. Smith, F. N. Uygur, M. Cristofaro, and J. Balciunas. 2005. Field assessment in land of origin of host specificity, infestation rate and impact of *Ceratapion basicorne* a prospective biological control agent of yellow starthistle. *Biocontrol* 50(3): 525-541.
3. Smith, L. 2005. Host plant specificity and potential impact of *Aceria salsolae* (Acari: Eriophyidae), an agent proposed for biological control of Russian thistle (*Salsola tragus*). *Biological Control* 34(1): 83-92. (similar content to Appendix 2)
4. Smith, L., R. Hayat, M. Cristofaro, C. Tronci, G. Tozlu and F. Lecce. 2006. Assessment of risk of attack to safflower by *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of *Centaurea solstitialis* (Asteraceae). *Biological Control* 36(3): 337-344. (= Appendix 4)

Other Publications

5. Smith, L. 2004. Proposed Field Release of the Blister Mite, *Aceria salsolae* de Lillo and Sobhian (Acari: Eriophyoidea) from Greece, for Biological Control of Russian thistle (*Salsola tragus* L.) in the United States. Petition 04-06 to USDA-APHIS Technical Advisory Group (TAG). 60 pp. (Submitted 12/17/04. TAG recommended approval for release, 8/8/05.) (= Appendix 1)
7. Smith, L. 2005. Environmental Assessment: "Field Release the Blister Mite, *Aceria salsolae* (Acari: Eriophyidae), for Biological Control of Russian thistle (tumbleweed), *Salsola tragus* (Chenopodiaceae)". submitted to USDA-APHIS 11/2/05.
8. Smith, L. M. Cristofaro, R. Yu. Dolgovskaya, C. Tronci and R. Hayat. 2005. Status of new agents for biological control of yellow starthistle and Russian thistle. California Invasive Plant Council Meeting, Oct. 6-8, 2005, Chico, CA. pp. 22-26.
9. Smith, L. and A. E. Drew. 2005. Biological Control of Yellow Starthistle. USDA, Agricultural Research Service, Albany, California. 16 p. (= Appendix 3)
10. Smith, L. 2006. Proposed Field Release of the Weevil, *Ceratapion basicorne* (Coleoptera: Apionidae), from Turkey for Biological Control of Yellow Starthistle (*Centaurea solstitialis*) in the United States. Petition 06-01 to USDA-APHIS Technical Advisory Group (TAG). 138 pp. (Submitted 1/26/06.) (= Appendix 1)

3. PROJECT DESIGN

The following is a brief description of the procedures. See Appendices for more details on background and methods (Appendices 1 and 4 for the yellow starthistle rosette weevil, *C. basicorne*, and Appendix 2 for the tumbleweed mite, *A. salsolae*).

The plants species that were chosen to be tested for host plant specificity followed specific TAG guidelines (USDA-APHIS 1998). Special attention was made to evaluate the plant species most likely to be at risk (due to similarity to the target weed); economic species in the same family as the target weed; and native species, including threatened or endangered species, in the same family (see Appendices 1 and 3: Experimental Methodology and Analysis: Test Plant List and the internal Appendix "Host Plant Test List"). The test lists were compiled with the assistance of botanists, and were reviewed by TAG and USFWS with the specific objective of answering any concerns about nontarget effects to native or economic species. The petitions submitted to TAG also address expected impacts of releasing the agents on other animals, human health and economy, and the abiotic environment (Appendices 1 and 2: Potential Environmental Impacts). The petition is designed to provide the information necessary for writing an Environmental Assessment (EA), which is critical for obtaining a Finding of No Significant Impact (FONSI), which is required before a release permit can be issued.

Foreign Exploration and Evaluation

Foreign exploration to discover new agents is being conducted in regions from which the weeds originated and that have climates similar to target regions in the U.S. For yellow starthistle this is in Turkey, and for tumbleweed this is in Kazakhstan and Tunisia (Appendices 1 & 2, Target Weed Information). The project partially supported exploration trips conducted by Biotechnology and Biological Control Agency (BBCA) in Kazakhstan and Tunisia, and field evaluations of the yellow starthistle rosette weevil in Turkey. BBKA is a nonprofit research organization directed by Massimo Cristofaro, who is a Senior Research Scientist at the Institute of New Technology for Energy and the Environment (ENEA C.R. Casaccia), a national research center near Rome, Italy. BBKA coordinated and helped scientists at Ataturk University in Erzurum, Turkey to conduct three years of field experiments to determine if the weevil, *Ceratapion basicorne*, attacks safflower. The project also supported research conducted by Çukurova University, in Adana, Turkey to determine if *C. basicorne* attacks other plants in the field and to measure the impact of the weevil on yellow starthistle plants in the field.

Host Specificity Evaluation of Yellow Starthistle Agents

Research focussed on the weevil, *Ceratapion basicorne*, which develops in root crowns of yellow starthistle rosettes (Fig. 1). This insect was collected in Turkey and evaluated in the USDA-ARS quarantine laboratory in Albany, CA (Smith and Drew in press). Because the insect normally has only one generation per year, we had to develop methods to rear it and condition it to complete reproductive dormancy during a shorter time period. We obtained seed of nontarget test plants, many of which are native species. For the latter, we often had to develop methods to germinate and grow the plants. Insects were tested under no-choice conditions, in which a fecund female was trapped on a plant for 5 days to feed and lay eggs (Fig. 2; Appendix 1, Experimental Methodology and Analysis). We tested 51 species of nontarget plants including 9 varieties of safflower. If there were signs of damage or insect development, then the plants were also tested under choice conditions (Fig. 3), which are more realistic.

The low oviposition rate on safflower under choice conditions prompted us to conduct field experiments. Such experiments can only be done in a country where the insect already occurs,

so we did these experiments in Turkey. For three years we conducted experiments at three sites to determine if the weevil would attack safflower in the field. We grew yellow starthistle and safflower plants and transplanted them at the field sites (Fig. 4; Appendix 4, Methods). By monitoring insect development, we harvested the plants just as the weevil larvae were completing development, and held the plants to rear out the adult insects for identification. Cooperating scientists at Ataturk University and BBKA played a key role to accomplish this research.

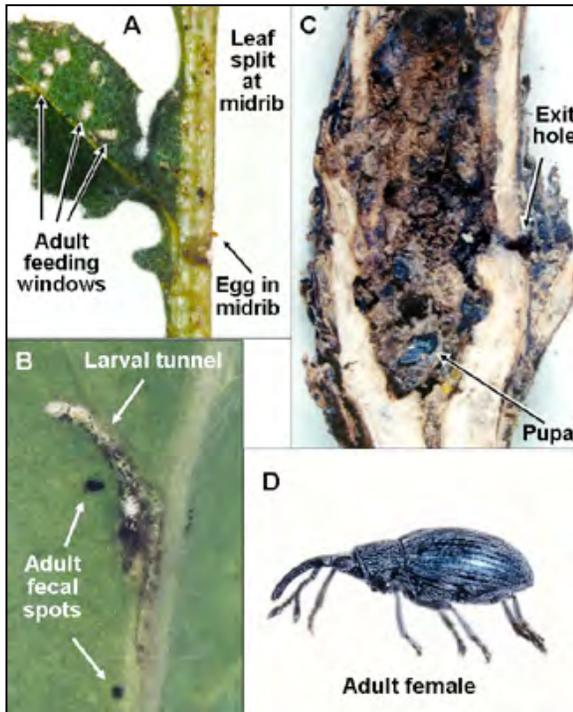


Figure 1. Adult *Ceratapion basicorne* and damage caused by adults feeding on leaves and larvae tunneling in leaves and root crown of yellow starthistle.



Figure 2. No-choice oviposition host specificity test. Individual females were held inside a plastic tube attached to the leaf of a test plant for 5 days. A small crumpled paper towel provided a hiding place for the insect.



Figure 3. Choice oviposition host specificity tests were conducted in a sleevebox (door open for photograph) inside the quarantine laboratory. Individual females were released in a sleevebox with leaves of 4-5 species of test plants. Tests were run for 5 days. A small crumpled paper towel (center) provided a hiding place for the insect.

Host Specificity Evaluation of Tumbleweed Agents

Research focussed on the mite, *Aceria salsolae*, which develops in leaf shoots and buds of tumbleweed (Figs. 5, 6). This mite was collected in Greece and evaluated in the USDA-ARS quarantine laboratory in Albany, CA (Appendix 2, Biological Control Agent Information; and Publication 3). We had to develop methods to rear and evaluate the mite under containment, so that it could not escape quarantine (Appendix 2, Experimental Methodology and Analysis). We obtained seed of nontarget test plants, many of which are native species. For the latter, we often had to develop methods to germinate and grow the plants. Mites were tested under no-choice conditions, in which an infested sprig of tumbleweed was put on a test plant (Fig. 7). After 5 weeks, the test plant was examined under a microscope and we "extracted" any mites using a procedure that involved washing the plants and capturing the mites on a filter paper.

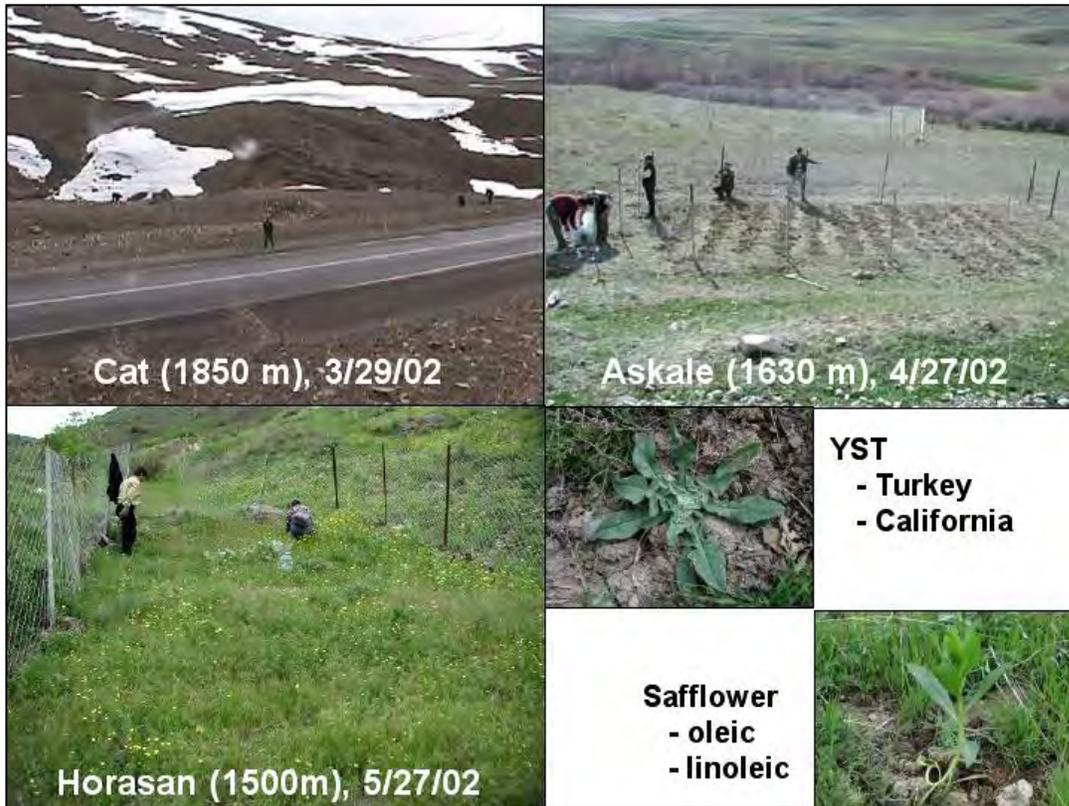


Figure 4. Field experiments were conducted at three sites in eastern Turkey during three years to determine if *Ceratapion basicorne* attacks safflower under field conditions. The weevil was never reared from safflower despite high infestation of nearby yellow starthistle plants.

Impact of Yellow Starthistle Agent

Impact of the weevil, *Ceratapion basicorne*, was evaluated by infesting potted yellow starthistle plants and rearing them inside the quarantine greenhouse until the adult insects emerged (see Appendix 1). Plants were dried and weighed at the end of the experiment.

Impact of Tumbleweed Agent

Impact of the mite, *Aceria salsolae*, was evaluated by infesting potted tumbleweed plants and rearing them inside a sleevebox in the quarantine mite room for 14 weeks (see Appendix 2, Publication 3). Plants were dried and weighed at the end of the experiment.

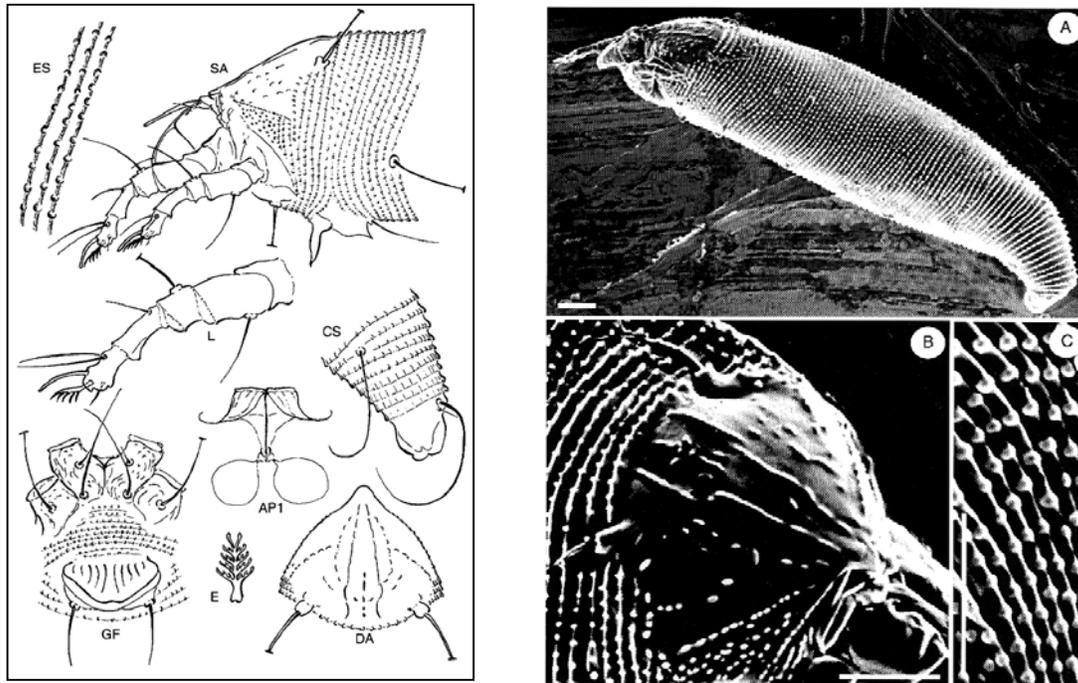


Figure 5. Illustration and scanning electron micrograph of the Salsola mite, *Aceria salsolae* (de Lillo & Sobhian 1996).



Figure 6. Damage caused by *Aceria salsolae*. Mite feeding kills meristems of *Salsola tragus* after about 3 weeks (left photo). Comparison of uninfested cutting of *Salsola tragus* (left) to an severely stunted infested plant (right) collected in Greece (right photo).



Figure 7. Test plants were infested with the Salsola mite, *Aceria salsolae*, for 4 weeks. Plants were then examined under a microscope for damage and mite survival and reproduction. Any mites were extracted from the plants with a soapy solution and counted.

4. PROJECT RESULTS AND DISCUSSION

Foreign Exploration and Evaluation

BBCA conducted exploration trips in Turkey, Kazakhstan and Tunisia to discover new agents for tumbleweed. The weevil, *Larinus filiformis*, was discovered attacking yellow starthistle flowerheads in eastern Turkey, and Levent Gultekin (Attaturk University) conducted preliminary studies on its biology. Many new prospects for tumbleweed were discovered during the first trip into Kazakhstan in 2004 (Fig. 8, Table 1). The leaf-feeding weevil, *Anthypurinus biimpresus*, was discovered in Tunisia, and BBCA is beginning to conduct preliminary studies on its biology.

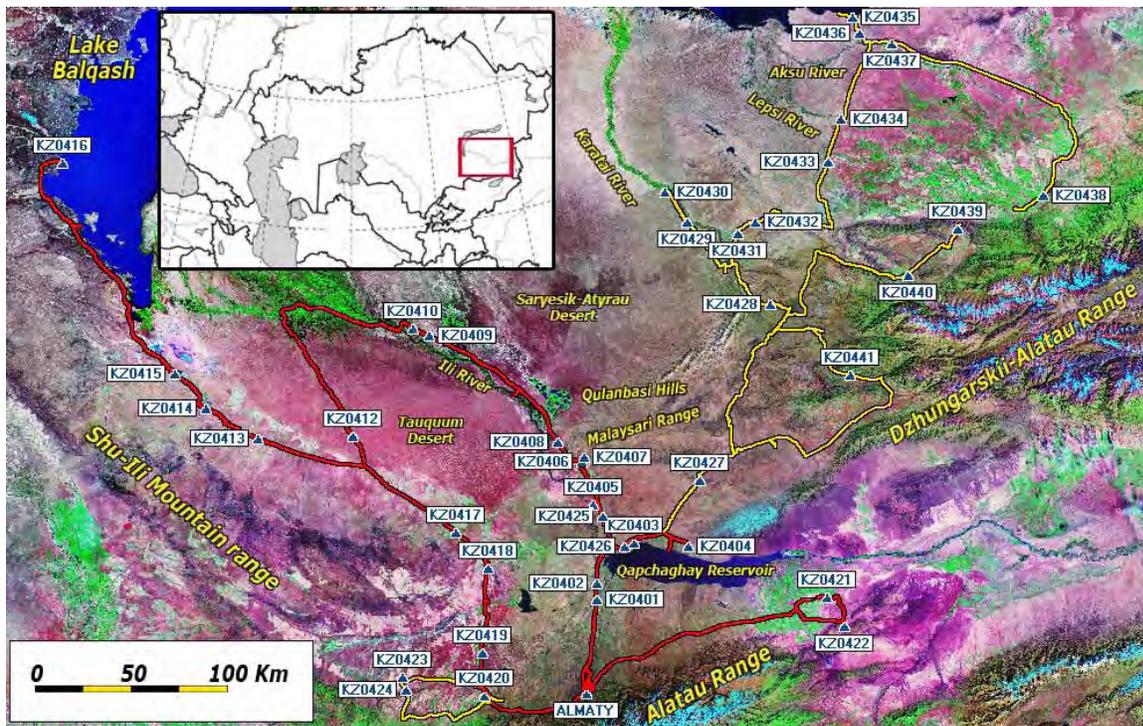


Figure 8. Map of collection sites during exploration in Kazakhstan, June 23 – July 15 2004.

Host Specificity Evaluation of Yellow Starthistle Agents

Ceratapion basicorne. We tested a total of 51 species of host plants, including 24 native species and 4 economic species (Appendix 1, Results and Discussion). Nativity of plant species, and additional data, are presented in Table 6 in Appendix 1. In no-choice oviposition tests, *C. basicorne* females oviposited on 94% of plant species in the subtribe Centaureinae, including *Carthamus tinctorius* (safflower) and the native species *Centaurea americana* and *Ce. rothrockii* (Fig. 9). Eggs were observed on only three plants outside the tribe Cardueae: *Liatris punctata*, *Gazania rigens* and *Pluchea odorata*; however, larvae damaged none of these plants. The no-choice results indicate no risk of larval damage to plants outside the tribe Cardueae. Plants in the tribe Cardueae were further tested in choice experiments, which showed some risk to bachelor's button (*Ce. cyanus*), Napa thistle (tocalote, *Ce. melitensis*), and safflower. However, three years of field experiments conducted in Turkey indicated no risk of attack to safflower under field conditions, despite high rates of attack on nearby yellow starthistle (Appendix 4).

In the sleevebox choice experiment, adult feeding and oviposition by *C. basicorne* was significantly greater on yellow starthistle than on any of the eight other nontarget species tested (Fig. 10). About 74% of eggs were deposited on yellow starthistle, 20% on *Ce. cyanus* (bachelor's button), 5% on *Ce. melitensis*, 3% on *Sa. americana* and 1% on safflower. These results indicate that *C. basicorne* females are more attracted to YST than to bachelor's button or any of these other nontarget test plants. However, bachelor's button appears to be at risk of some attack, at least under these confined laboratory conditions. A subset of the sleevebox choice trials that tested safflower varieties against YST indicates a low risk of oviposition

Table 1. Species of insects found on tumbleweed in Italy, Kazakhstan, Tunisia and Turkey

Coleoptera: Curculionidae (weevils)

- Anthypurinus biimpressus* - Tunisia 2004 unknown biology c
Baris memnonia (Boheman) - unknown biology (Artemisia?) c
Baris sulcata (Boheman) c*
Baris convexicollis Boheman. Develops on several species of Chenopodiaceae, including *Halocharis hispida* (Schrenk) Bunge, *Suaeda confusa* Iljin, *Suaeda* spp., *Salsola soda* L., *Climacoptera transoxana* (Iljin) Botsch., and *Bassia hysopifolia* (Pall.) O. Kunze
Baris memnonia Boheman. On ruderal Chenopodiaceae in NE Turkey. In Middle Asia, on *Atriplex centralasiatica* Iljin.
Baris przewalskyi Zaslavskii. Known from *Camphorosma* in Kazakhstan.
Conorhynchus candidulus Faust c*
Conorhynchus (Pycnodactylus) arabs (Olivier) c*
Conorhynchus conirostris (Gebler). Feeds and apparently develops on many Chenopodiaceae.
Cosmobaris scolopacea (Germar) c*. Develops on many Chenopodiaceae, recorded as a pest of sugarbeet.
Cycloderes pilosulus Fabricius. Apparently polyphagous, never common on Chenopodiaceae.
Elasmobaris signifera (Faust) – affine sp in Turkey on Chenopodiaceae c*
Lixus polylineatus Petri – found in Turkey on Chenopodiaceae c*
Lixus rubicundus Zoubkoff c*. Develops on many Chenopodiaceae, recorded as a pest of sugarbeet.
Microlarinus ?rhinocylloides Hochhuth. Hosts unknown.
Philernus gracilitarsis (Reitter). On many annual and perennial Chenopodiaceae.
Piazomias sp. Feeds also on Artemisia in Kazakhstan; apparently polyphagous.
Salsolia morgei Bajtenov c**
Temnorhinus elongatus (Gebler). Hosts unknown, apparently polyphagous on Chenopodiaceae. Recorded as a pest of sugarbeet.
Ulobaris loricata (Boheman). Develops on many Chenopodiaceae, recorded as a pest of sugarbeet.

Coleoptera: Chrysomelidae (leaf beetles)

- Chaetocnema breviscula* Faldermann. Feeds on many Chenopodiaceae, recorded as a pest of sugarbeet.
Cassida parvula Boheman. Feeds on many Chenopodiaceae.

Lepidoptera: Pyralidae (moth caterpillar)

- Gymnancyla* n. sp. in Sicily discovered by M.Cristofaro 2004.
-

c = weevils det. By E. Colonnelli (all on salsola)

* = oligophagous or polyphagous species (eat few or many different plant species)

** = monophagous species (known to eat only one plant species)

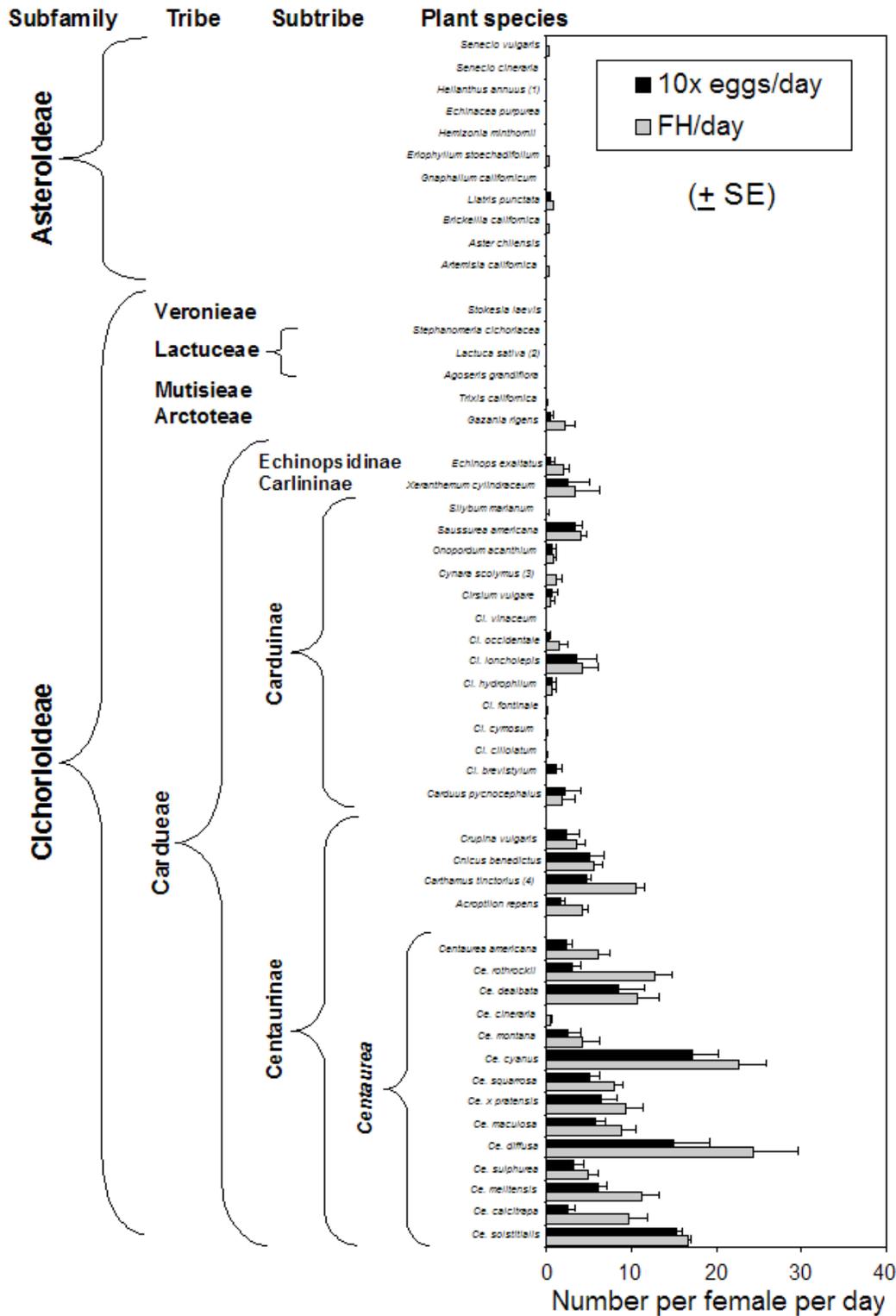


Figure 9. Mean number of *Ceratopion basicorne* eggs (times 10) and mean number of adult feeding holes per day on test plants in the no-choice host specificity experiment. Individual females were held inside a plastic tube attached to the leaf of a nontarget test plant for 5 days (on yellow starthistle for 2-3 days). (error bar = SE).

to 5 of 9 varieties tested (Fig. 11). These experiments were conducted under confined artificial conditions, and in the field the insect is likely to be much more selective (Clement and Cristofaro 1995, Sheppard 1999). Because safflower is a commercial crop field testing was done (see below) to determine if this plant is at risk under natural conditions. Very low attack rates on *Sa. americana* and *Ce. melitensis* indicates a small risk to these plants of adult feeding and oviposition. However, larvae did not develop on *Sa. americana* in the no-choice experiments, so any possible damage to this plant should be very limited. Larvae can develop on *Ce. melitensis* (tocalote, Napa thistle), but this plant is a noxious weed, and such attack is welcome. There was no attack on *Ci. loncholepis*, *Ce. rothrockii* or *Ce. sulphurea*, so these plants are not likely to be at risk in the field.

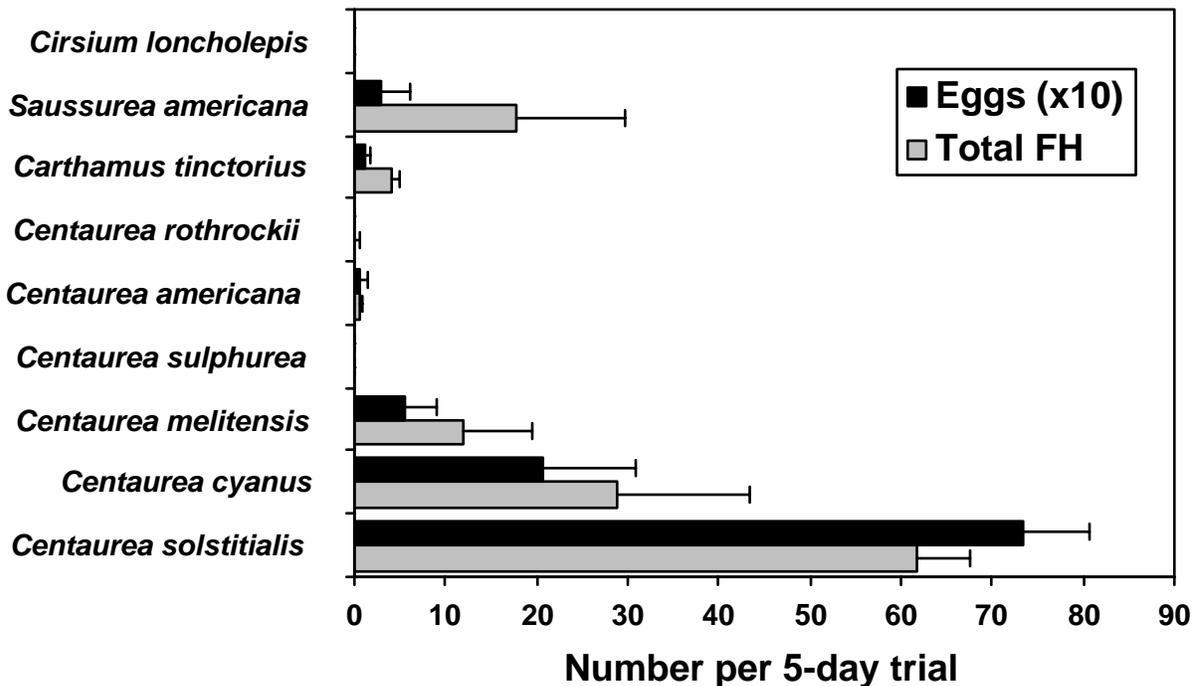


Figure 10. Oviposition and adult feeding by *Ceratopion basicorne* on nontarget species during choice oviposition experiments in sleeveboxes (one female for 5 days exposed to cut leaves of 4-5 plant species at a time, always including yellow starthistle). Number of eggs was multiplied by 10 for visibility on the same scale; FH = number of adult feeding holes, each ca. 1 mm²; error bars = SE. *Car. tinctorius* = safflower, *Ce. cyanus* = bachelor's button, *Ce. solstitialis* = yellow starthistle (target weed).

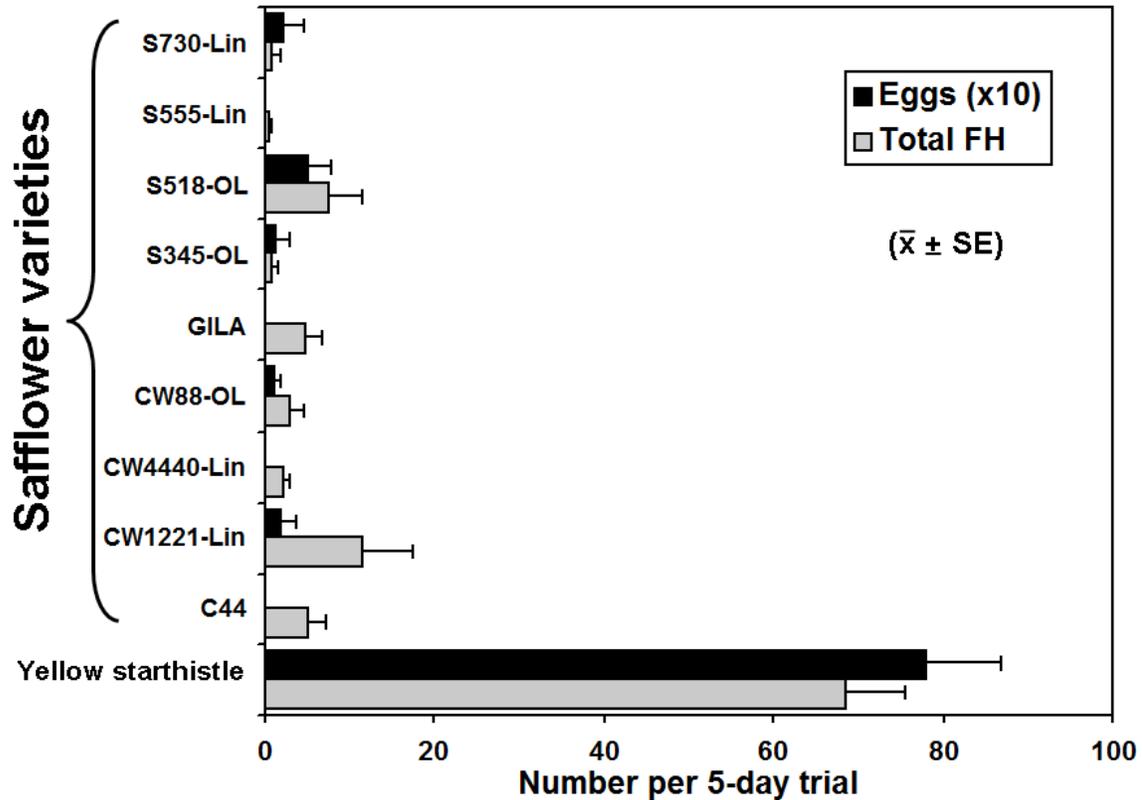


Figure 11. Oviposition and adult feeding by *Ceratopion basicorne* on safflower varieties during choice oviposition experiments in sleeveboxes (one female for 5 days exposed to cut leaves of yellow starthistle and 3 safflower varieties at a time). Number of eggs was multiplied by 10 for visibility on the same scale; FH = number of adult feeding holes, each ca. 1 mm². Safflower variety codes: C = Cargill, CW = CalWest, S = SeedTec, Lin = linoleic, OL = oleic.

Host Specificity Evaluation of Tumbleweed Agents

No live mites were found on any of the nontarget test plants outside the genus *Salsola* after 4 weeks in laboratory experiments, and none of the nontarget plants showed any sign of feeding damage (Table 2). Nativity of plant species is indicated in "Appendix 2" within Appendix 2. All "positive control" cuttings of *Salsola tragus* type A were infested with high numbers of mites indicating that all the test plants were adequately challenged. *Aceria salsolae* successfully reproduced on *Salsola tragus* type A, type B, type C, *S. paulsenii* lax-form, *S. paulsenii* spinose-form, and *S. collina*, all of which are considered to be noxious weeds, and all of which are in the *Salsola* section *kali* subsection *kali* (Rilke 1999). Other experiments and field observations in Europe indicate that the mite also attacks *S. kali* (de Lillo & Sobhian 1996, Sobhian et al. 1999). The mite could not reproduce on *Salsola soda* (in the same genus but more distantly related), *Halogeton glomeratus* (different genus but same tribe), *Suaeda calceoliformis* and *S. moquinii* (different tribe but same subfamily), or *Sarcobatus vermiculatus* (another tribe but same subfamily). The small number of mites extracted from *Salsola soda*, *Suaeda moquinii*, and *Gomphrena globosa* all appeared to be dead.

Table 2. Infestation of test plants 4-5 weeks after transfer of at least 20 mites

Plant species	No. plant replicates	Plants infested (%)	No. mites per infested test plant	
			Test plant	Positive control
Family Chenopodiaceae				
Subfamily Chenopodioideae				
<i>Atriplex canescens</i>	8	0	0	67
<i>Atriplex elegans</i>	15	0	0	126
<i>Atriplex confertifolia</i>	10	0	0	124
<i>Atriplex truncata</i>	9 ^a	0	0	917
<i>Grayia spinosa</i>	13	0	0	247
<i>Kochia americana</i>	9	0	0	798
<i>Kochia scoparia</i>	3	0	0	108
<i>Krascheninnikovia lanata</i>	9	0	0	224
<i>Spinacia oleraceae</i> (Spinach):				
Space (Bejo)	20	0	0	261
Bolero	9	0	0	211
Bossanova	9	0	0	291
Clermont	9	0	0	149
Spin	9	0	0	93
<i>Suckleya suckleyana</i>	10	0	0	565
<i>Zuckia (Grayia) brandegeei</i>	9	0	0	227
<i>Beta vulgaris</i> :				
Sugarbeet:				
Rifle, Spreckles/ Holly	9	0	0	268
Owyhee (Novartis)	9	0	0	829
8757, Beta Seeds	9	0	0	98
NB7R	9	0	0	93
Table beet:				
Red Ace Hybrid	9	0	0	134
Swiss chard:				
Lucullus	9	0	0	62
Rhubarb	9	0	0	167
<i>Chenopodium ambrosioides</i>	9	0	0	299
<i>Chenopodium berlandieri</i>	9	0	0	263
<i>Chenopodium fremontii</i>	9	0	0	247
<i>Chenopodium quinoa</i>	9	0	0	252
<i>Monolepis nuttalliana</i>	8	0	0	591

Table 2. (continued)

Plant species	No. plant replicates	Plants infested (%)	No. mites per infested test plant	
			Test plant	Positive control
Subfamily Salicornioideae				
<i>Allenrolfea occidentalis</i>	9	0	0	525
<i>Salicornia bigelovii</i>	18 ^b	0	0	361
<i>Salicornia maritima</i>	9	0	0	1107
<i>Sarcocornia utahensis</i>	9	0	0	46
Subfamily Salsoloideae				
<i>Halogeton glomeratus</i>	9	0	0	170
<i>Salsola collina</i>	9	100	106	695
<i>Salsola tragus</i> type A	136 ^a	100	378	--
<i>Salsola tragus</i> type B	9 ^a	100	*	
<i>Salsola tragus</i> type C	9 ^a	100	*	
<i>Salsola paulsenii</i> lax-form	9 ^a	100	*	
<i>Salsola paulsenii</i> spinose-form	9 ^a	100	*	
<i>Salsola soda</i>	9 ^a	22	1 ^c	232
<i>Sarcobatus vermiculatus</i>	9	0	0	191
<i>Suaeda calceoliformis</i>	5	0	0	206
<i>Suaeda moquinii</i>	18 ^b	11	6 ^c	748
Subfamily Polycnemoideae				
<i>Nitrophila occidentalis</i>	9	0	0	657
Family Amaranthaceae				
Subfamily Amaranthoideae				
<i>Amaranthus hypochondriacus</i>	9	0	0	706
<i>Amaranthus palmeri</i>	9	0	0	850
<i>Amaranthus pumilus</i>	9	0	0	67
<i>Celosia huttonii</i>	10	0	0	608
Subfamily Gomphrenoideae				
<i>Gomphrena globosa</i>	9	44	1 ^c	175
Family Aizoaceae				
<i>Sesuvium verrucosum</i>	9	0	0	288
Family Caryophyllaceae				
<i>Arenaria hookeri</i>	9	0	0	93
Family Nyctaginaceae				
<i>Abronia villosa</i>	9	0	0	670

^a plant cuttings were used instead of potted plants.

^b half were plant cuttings and half potted plants.

^c all mites were dead, and there was no sign of feeding damage.

* mite densities were not counted but were similar to those on *S. tragus* type A.

Impact of Yellow Starthistle Agent

In the quarantine laboratory experiment, infestation by weevil larvae caused up to a 23% reduction in size of well-watered, fertilized potted plants grown in a greenhouse (Fig. 12; $F_{(2, 57)} = 3.39$, $P < 0.04$) (Appendix 1: p. 31). The mean number of insects per plant was 3.1 (± 1.5 sd) in the low infestation treatment and 4.2 (± 3.0) in the high (which is less than was planned). In the field in Turkey, up to seven larvae have been found in yellow starthistle roots (Uygur *et al.* 2005). So, if this insect attains high densities typical for a successfully established biological control agent, it is likely to cause more damage than we observed in the quarantine impact experiment. In the field, where plants compete for water, nutrients, and light, weevil infestation may have greater impact on plant size, survivorship and reproduction. Furthermore, adult feeding on plant leaves in the spring may cause significant damage if weevil populations become very large, as was observed for *Larinus minutus* on diffuse knapweed (Piper 2004). In a field study of naturally occurring yellow starthistle plants in Turkey, plants infested by *Ceratapion* had 15% lower seed fertility than uninfested plants (Uygur *et al.* 2005). Although it is difficult to predict how much impact *C. basicorne* will have on yellow starthistle populations in North America, it is clear that the weevil has potential to affect the plant. Furthermore, because *C. basicorne* feeds on the rootcrown and leaves, it is not likely to directly interfere with the previously released flowerhead insects, which do not feed on these plant parts.



Figure 12. *Ceratapion basicorne* impact study was conducted inside the Albany quarantine laboratory. Yellow starthistle plants at the rosette stage were exposed to ovipositing females, then plants were held in the greenhouse until completion of larval development. Plants were enclosed in mesh bags during the last 2 weeks to prevent possible escape of emerging adults. Mean weight of infested plants was 23% lower than of uninfested plants.

Impact of Tumbleweed Agent

In the quarantine experiment, mite populations established on all of the infested *Salsola tragus* plants, and signs of damage appeared within 4 weeks (Appendix 2: p. 20; Publication 3). The mite population increased exponentially during 25 weeks (Fig. 13). Infested plants (353 ± 96 cm [SE]) were 34% as large as uninfested plants ($1,051 \pm 258$ cm) based on combined length of all branches at 25 weeks after infestation (Fig. 14). Infested plants (3.4 ± 1.0 g) had 41% as

much biomass as the uninfested plants (8.2 ± 2.5 g). None of the plants had started flowering, so no impact on seed production was observed. The experimental light intensity ($140 \mu\text{mole/sec PAR}$) was much lower than natural outdoor light (which is up to $1900 \mu\text{mole/sec}$), and the temperature and humidity were more moderate than typical outdoor conditions. So, the experimental plants tended to be lankier and less stiff than in the field. So far, we have not been able to grow plants to maturity in the confines of the mite quarantine room, which hampers our ability to measure impact on seed production. In Europe, mites are found on dramatically stunted plants (Fig. 6). In a field study in Afyon, Turkey, infested plants were less than half the size (13.4 cm tall) of uninfested plants (35 cm) (Sobhian et al. 1999). The maximum size of naturally infested plants (30 cm tall) was half that of uninfested plants (60 cm) at the end of August. We have not tested the mite's ability to impact *S. tragus* type B, *S. collina* or *S. paulsenii* for impact, but will do it as soon as we have improved our experimental conditions. In the absence of other data, we would expect the mite to cause similar or less damage to these other *Salsola* species.

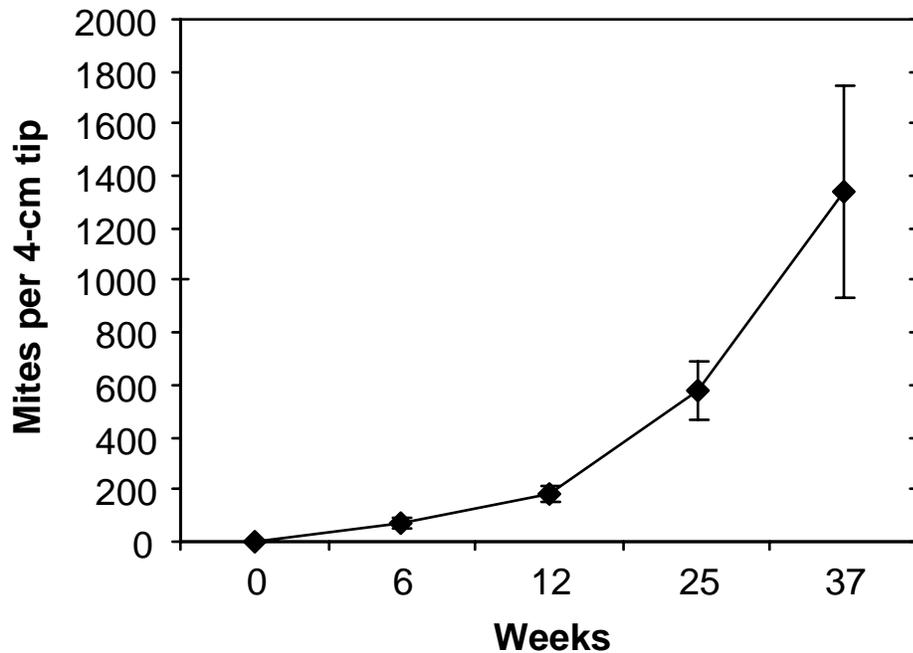


Figure 13. Population growth of mite *Aceria salsolae* on *Salsola tragus* plants during impact study (number per 4-cm branch tip, \pm SE).



Figure 14. Comparison of growth of uninfested *Salsola tragus* (left) to infested plants (right) 14 weeks after infestation by the mite *Aceria salsolae* (grown under artificial lights inside quarantine laboratory).

5. CONCLUSIONS

The yellow starthistle weevil, *Ceratopion basicorne*, will not damage or develop on any plant other than a few species in the subtribe Centaureinae, all of which are alien weeds in North America. It is possible that the weevil will cause some damage to the stems of bachelor's button, creating a small bump where the larva develops. However, the weevil has a short season when it is capable of laying eggs (April to May), and it is adapted to attacking rosettes, which bachelor's button does not form. Furthermore, this insect has not been reported to be a pest of ornamental bachelor's button in Eurasia, where this insect is native. Thus, any damage to bachelor's button is expected to be infrequent and minor. Weevil infestation should reduce growth of yellow starthistle by at least 23% and may increase mortality. We believe that our results are sufficient to demonstrate that the insect is safe and likely to impact yellow starthistle. We submitted a TAG petition to USDA-APHIS in Jan. 2006 (Appendix 1), requesting permission to release *C. basicorne*. This is a major step towards obtaining a regulatory permit to release a new agent for yellow starthistle. Such an agent is expected to complement the impact of other established agents and provide self-sustaining long-term control of the weed. This should help reduce the amount of herbicide applied to control yellow starthistle.

The tumbleweed mite, *Aceria salsolae*, is able to feed and reproduce only on a few closely related species in the *Salsola* section *kali* subsection *kali* (Rilke 1999) including: *Salsola tragus* (type A), *S. tragus* type B, *S. kali*, *S. paulsenii* and *S. collina*. It was not able to reproduce on or damage any of the 36 nontarget species tested, including *Salsola soda*, which is in a different section of the genus from *S. tragus*. The mite stunts the growth of tumbleweed and should reduce plant size by 34 to 50% and probably reduces seed production. We believe that our

results are sufficient to demonstrate that the mite is safe and likely to impact tumbleweed. We submitted a TAG petition to USDA-APHIS in Dec. 2004 (Appendix 2), TAG gave it a positive review, and we submitted a formal request to USDA-APHIS for a permit to release *A. salsolae*. This is a major step towards obtaining a regulatory permit to release a new agent for tumbleweed (Russian thistle). Such an agent is expected to provide self-sustaining long-term reduction in plant size and seed production of tumbleweeds. This should help reduce the amount of herbicide applied for its control and reduce the occurrence of tumbleweed-associated automobile accidents.

This research has made significant advances to the discovery and development of new biological control agents for yellow starthistle and tumbleweed. Petitions have been submitted for two agents. Research has also been conducted on two follow-up agents: *Psylliodes chalconera* for yellow starthistle and *Gymnancyla canella* for tumbleweed. Discovery of beetles attacking tumbleweed in Kazakhstan (*Baris* spp. and *Salsolia morgei*) and Tunisia (*Anthypurinus biimpresus*) greatly increase our chances of finding a agent that will successfully control tumbleweed.

Future Work To Do

Yellow starthistle

Complete evaluation of *Psylliodes chalconera* and submit a petition to TAG.

Develop methods to multiply populations of *Ceratapion basicorne* and *Psylliodes chalconera* in quarantine laboratory so that they can be released sooner at more sites.

Release *Ceratapion basicorne* and evaluate its impact on yellow starthistle (cannot be done until permit is issued by APHIS - possibly in 2007).

Release *Psylliodes chalconera* and evaluate its impact on yellow starthistle (cannot be done until permit is issued by APHIS - possibly in 2008).

Tumbleweed

Complete evaluation of *Gymnancyla canella* and submit a petition to TAG.

Develop methods to measure tumbleweed populations and begin collecting data at future release sites.

Release *Aceria salsolae* and evaluate its impact on tumbleweeds (cannot be done until permit is issued by APHIS - possibly in 2007).

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7. APPENDICES

1. *Ceratapion basicorne* TAG Petition

Smith, L. 2006. Proposed Field Release of the Weevil, *Ceratapion basicorne* (Coleoptera: Apionidae), from Turkey for Biological Control of Yellow Starthistle (*Centaurea solstitialis*) in the United States. Petition 06-01 to USDA-APHIS Technical Advisory Group (TAG). 138 pp. submitted 1/26/06.

2. *Aceria salsolae* TAG Petition

Smith, L. 2004. Proposed Field Release of the Blister Mite, *Aceria salsolae* de Lillo and Sobhian (Acari: Eriophyoidea) from Greece, for Biological Control of Russian thistle (*Salsola tragus* L.) in the United States. Petition 04-06 to USDA-APHIS Technical Advisory Group (TAG). 60 pp. submitted 12/17/04. (TAG recommended approval for release, 8/8/05.)

3. Yellow Starthistle Manual

Smith, L. and A. E. Drew. 2005. Biological Control of Yellow Starthistle. USDA, Agricultural Research Service, Albany, California. 16 p.

4. *Ceratapion basicorne* evaluation in Turkey

Smith, L., R. Hayat, M. Cristofaro, C. Tronci, G. Tozlu and F. Lecce. 2006. Assessment of risk of attack to safflower by *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of *Centaurea solstitialis* (Asteraceae). *Biological Control* 36(3): 337-344.