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Assessment of risk of attack to safflower by *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of *Centaurea solstitialis* (Asteraceae)

L. Smith [a,*], R. Hayat [b], M. Cristofaro [c], C. Tronci [c], G. Tozlu [b], F. Lecce [c]

[a] *USDA-ARS Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710, USA*
[b] *Ataturk University, Faculty of Agriculture, Plant Protection Department, 25240 TR Erzurum, Turkey*
[c] *ENEA C.R. Casaccia, s.p. 25, Via Anguillarese 301, 00060 S. Maria di Galeria, Rome, Italy*

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Abstract

Ceratapion basicorne (Coleoptera: Apionidae) is a prospective biological control agent of yellow starthistle (*Centaurea solstitialis*, Asteraceae: Cardueae), which is an important invasive alien weed in the western United States. Previous studies have shown that it is possible for this insect to oviposit on and complete development on safflower (*Carthamus tinctorius*) under no-choice laboratory conditions; however, it has never been reported as a pest of safflower. Field experiments were conducted at three sites in eastern Turkey during 3 years to evaluate the risk of attack on safflower by this insect in its native range. At two sites where *C. basicorne* was the only apionid observed, no safflower plants were attacked despite high attack rates on yellow starthistle test plants (48–98% of plants infested). At a third site, where *C. basicorne* and three other species in the same genus; *C. scalptum*, *C. orientale*, and *C. onopordi* were present, 8–26% of safflower plants were infested, but none of the insects reared from safflower during 3 years were *C. basicorne*. Other authors have reported rearing *C. basicorne* from field-collected plants of only *Ce. solstitialis*, *Ce. cyanus*, *Ce. depressa*, and *Cnicus benedictus*. Our results indicate that *C. basicorne* does not attack safflower under field conditions and that its introduction would not pose a risk to this crop.
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Keywords: Host plant specificity; Classical biological control; Nontarget plant; Risk assessment

1. Introduction

Yellow starthistle (*Centaurea solstitialis* L., Asteraceae: Cardueae) is an important invasive alien weed in the western US, where it infests over 8 million ha (Duncan, 2001; Sheley et al., 1999). It is an annual plant that germinates in the late fall or early spring, grows as a rosette until May, then bolts and flowers from June until it dies of drought or frost (Maddox, 1981). The plant originates from the Mediterranean region and it is the target of a biological control program (Cristofaro et al., 2002; Maddox, 1981; Piper, 2001; Pitcairn et al., 2004; Turner et al., 1995). *Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae) was identified as a prospective agent (Rosenthal et al., 1994; Zwölfer, 1965). Alonso-Zarazaga (1990) provides a detailed morphological description, and Wanat (1994) lists taxonomic synonyms. The weevil is widely distributed in Europe and western Asia, overlapping the distribution of *Ce. solstitialis* (Alonso-Zarazaga, 1990; Wanat, 1994). Adults oviposit on rosettes in the early spring, and larvae develop in the root crown where they pupate (Clement et al., 1989; Smith and Drew, in press; Uygur et al., 2005). Adults emerge in late May to June and aestivate and hibernate until the following spring. In the wild, *C. basicorne* has been reared only from *Ce. solstitialis*, *Centaurea cyanus* L., *Centaurea depressa* M. Bieb., and *Cnicus benedictus* L. (Alonso-Zarazaga, 1990; Campobasso et al., 1999; Wanat, 1994). No-choice laboratory studies confirmed that the insect has a narrow host

* Corresponding author. Fax : +1 510 559 5737.
E-mail address: lsmith@pw.usda.gov (L. Smith).

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range, but it can oviposit on, and some larvae can complete development on, safflower (*Carthamus tinctorius* L., Asteraceae: Cardueae) (Clement et al., 1989). This raised concern that this insect may not be host-specific enough to introduce as a biological control agent (Clement, 1990).

Safflower is not widely cultivated in countries where *Ce. solstitialis* and *C. basicorne* are known to occur (e.g., Turkey, Greece, Italy, and France) (FAO, 2005). Although the crop has an ancient history in the Mediterranean, modern production of safflower is limited because of several pests, especially the safflower fruit fly, *Acanthiophilus helianthi* (Rossi) [= *A. eluta* (Meigen)] (Tephritidae), which can damage up to 95% of the flower heads (Anon., 1963). Reports of other pests of safflower in the Mediterranean region include: *Heliothis peltigera* Schiff. (Noctuidae), *Chaetorellia carthami* Stackelberg, *Ch. jaceae* R.D., *Terellia luteola* Wiedemann, *Urophora mauritanica* Macquart (Tephritidae), *Larinus grisescens* Gyll., *Larinus syriacus* Gyll., *Larinus orientalis* Cap., and *Larinus ovaliformis* Cap. (Curculionidae) on the flower heads; and *Lixus speciosus* Mill. (Curculionidae), *Agapanthia* sp. (Cerambycidae), four *Chloridea* spp., *Plusia gamma* L. (Noctuidae), *Pyrameis cardui* L. (Nymphalidae), and *Cassida palaestina* Reiche (Chrysomelidae) on vegetative parts (Avidov and Kotter, 1966; Bytinski-Salz, 1952; El-Sheikh et al., 1990; Freidberg, 1996; Garali et al., 2004; Logozzo and Alba, 1990; White et al., 1990). However, none of these publications mentioned the presence of an apionid, such as *C. basicorne*.

Safflower is a significant crop in the western US where about 70,000 ha are planted producing about 124,000 metric tons of seed (USDA, 2005). About 85% of this production is in California, with the rest primarily in Montana, North Dakota, South Dakota, Utah, and Idaho (Purdue University, 2005). Because safflower is abundant in California, which is the state where *Ce. solstitialis* infests the most land (Duncan, 2001), it is important to be certain that *C. basicorne* will not attack the plant under field conditions, especially when the insect is abundant. Laboratory choice oviposition experiments indicate that *C. basicorne* is much less likely to oviposit on safflower when *Ce. solstitialis* is present than under no-choice conditions (Clement et al., 1989; L. Smith, unpublished data). However, because of the potentially high economic injury that could be caused by introduction to the western US of an insect that can attack safflower, we wanted to obtain better information to determine the likelihood that safflower would be attacked by *C. basicorne*. Field choice experiments are known to provide results that are more predictive of post-release risk to nontarget plants (Briese, 1999; Clement and Cristofaro, 1995). This paper describes experiments conducted to determine the risk of attack to safflower plants in the field.

2. Methods

2.1. 2002

The experiments were conducted at three sites near Erzurum, Turkey:

Askale—(39° 58.712′N, 40° 33.783′E, 1580 m elevation) abandoned cultivated field in alluvial soil near a stream.

Horasan—(40° 07.543′N, 42° 29.941′E, elevation 1501 m) rocky south-facing slope below cliffs beside a stream.

Çat—(39° 34.929′N, 40° 54.210′E, 1814 m elevation) rocky field near the top of a ridge.

Centaurea solstitialis was naturally present at all three sites and over 80% of the *Ce. solstitialis* plants at each site were infested by *Ceratapion* in 2001 (Cristofaro et al., 2002). At each site, a wire mesh fence 1.5 m tall was built to protect the experimental plot (about 6 × 12 m) from disturbance by livestock. Each site was visited every other week, starting 11 April, 2002, to monitor for the presence of *C. basicorne* to help determine when to transfer test plants to the field.

Test plants were grown from seed: *Ce. solstitialis* (US) collected in Davis, California in 2000, *Ce. solstitialis* (TK) collected at each site (Horasan, Çat, and Askale) in 2001, and safflower (*Carthamus tinctorius*) cultivars CalWest-1221 (linoleic) and Seedtec-317 (oleic). Test plants were grown indoors before transplanting to the field because of the cold winter conditions in this part of Turkey. *Ce. solstitialis* seeds were planted on February 4 and safflower on March 8, 2002. Safflower seeds were planted 4 weeks after *Ce. solstitialis* to produce plants of similar size during exposure to oviposition. Plants were grown in 10 × 12 cm pots beside windows at 20–25 °C. The plants were "hardened" before transfer to the field by moving them to an unheated greenhouse for 5 days (10–23 °C), then opening it during the day for 2 days, and then opening it day and night for 7 days. Test plants were transplanted in the field on April 24, 26, and 27 at Çat, Horasan, and Askale, respectively. *Ce. solstitialis* plants were 11 weeks old (rosettes with ⩾4 leaves) and safflower was 7 weeks old (10–15 cm tall) when transplanted in the field. The plants were placed in small holes dug with minimal disturbance to existing vegetation. Plants were arranged in 12 rows, each containing one plant type, and the rows alternated in a regular pattern (*Ce. solstitialis* (US), oleic safflower, *Ce. solstitialis* (TK), linoleic safflower), repeated three times. Each row contained 10 plants (eight at Horasan), spaced 40 cm apart within row and 80 cm between rows. Any other naturally occurring *Ce. solstitialis* plants within the plots were removed, but those outside were left undisturbed. We monitored the plants every 2–3 weeks and watered them 2–3 times, as needed. Plants that died were replaced between May 8 and 15. All test plants were harvested and destroyed before they could produce any seed to prevent possible establishment of alien plants in Turkey.

We monitored the phenological development of *C. basicorne* in wild *Ce. solstitialis* plants in the area surrounding the plots every week. When the first pupae were observed, we harvested all the test plants. Test plants were harvested on June 1, 24, and July 5 at Horasan, Çat, and Askale, respectively. The leaves and upper stems were removed and the remaining root and lower stem were placed in a zip-lock plastic bag that had a screen panel for ventilation. The bags

were held at room temperature (20–25 °C) and examined weekly to collect emerging adults which were pinned and labeled. All adults were identified by either Enzo Colonnelli (University of Rome "La Sapienz," Italy) or Boris Korotyaev (Russian Academy of Sciences, St. Petersburg).

2.2. 2003

We repeated the experiment at Askale and Çat to rear out more adults for identification. *Ce. solstitialis* seeds were planted on 23 January 2003 and safflower on 8 March 2003. All were grown in a growth chamber at 20 ± 5 °C and 12 h:12 h light–dark photoperiod. In mid April the temperature was lowered to 10 ± 5 °C for acclimatization. Plants were transferred to the field on May 3 and 4 at Çat and Askale, respectively. Plants were arranged in alternating rows of 10 as in 2002, with a total of 30 replicates of each of four plant types at Çat, and 30 *Ce. solstitialis* (TR) and 60 oleic plants at Askale. More oleic replicates were placed at Askale because *Ceratapion* spp. attack on safflower had previously been observed at this site and oleic was hardier at this site than linoleic. Plants that died were replaced between May 7 and 9 at Çat, and May 11 and 13 at Askale. *Ce. solstitialis* plants outside the experimental plots were monitored every 7–10 days for the occurrence of *C. basicorne* feeding holes, eggs, and adults starting on 9 May 2003. Test plants were collected on June 16 and 19 at Çat and Askale, respectively, and were held in separate bags, as in 2002, to rear out adults.

2.3. 2004

We repeated the experiment using larger plots of safflower to increase the ability to detect possible low levels of *C. basicorne* infestation. The experiment was conducted at Horasan and Askale, using the same sites prepared for the previous experiments. We tested two host plant species: safflower (Seedtec-317, oleic; 250 replicates) and *Ce. solstitialis* (seed from test sites; 40 replicates). Plants were grown as in 2003, were germinated on 18 March 2004, and were transferred to the field on April 26 and 27 at Horasan and Askale, respectively. Safflower plants were arranged in a block of five rows of 50 plants each that was flanked on each of the two long sides by a row of 20 *Ce. solstitialis* plants.

We sampled 20–40 wild *Ce. solstitialis* plants from the area surrounding each plot, weekly starting April 14, to monitor both plant development and *C. basicorne* abundance and phenology. As soon as the first *C. basicorne* pupae were observed in the wild *Ce. solstitialis* plants, we harvested all the test plants (on June 1 at Horasan and on June 18 at Askale). At Horasan, all safflower plants (250) were dissected in the field to confirm the absence of any insect infestation (as was observed in previous years). At Askale, 80 safflower plants were immediately dissected, and all immature insects were preserved in acetone for eventual identification by DNA analysis. The remaining safflower plants were individually placed in sealed plastic bags, and held at room temperature and natural light for adult emergence. All 40 *Ce. solstitialis* test plants from each site were held in plastic bags for adult emergence.

2.4. Statistical analysis

Differences in proportion data were tested by chi-square tests ($\alpha = 0.05$). Standard error bars presented in the figures were calculated using untransformed data in ANOVA. Standard error for a single proportion was estimated following methods of Gottelli and Ellison (2004).

3. Results and discussion

3.1. 2002

The first signs of *Ceratapion* adult feeding on foliage of wild *Ce. solstitialis* plants were observed on April 12, 18, and 29 at Çat, Horasan, and Askale, respectively, which was 5–12 days before we put the test plants in the field. Apionid larvae were found in wild *Ce. solstitialis* plants throughout the period of exposure at all three sites (Fig. 1). These were probably all *Ceratapion* spp. because no other genus of weevil has been reared from *Ce. solstitialis* root crowns in this region at this time of year (Uygur et al., 2005; M.C. pers. obs.). Other curculionid weevils known to attack *Ce. solstitialis* stems and root crowns, such as *Lixus filiformis* (Fabr.), *Lixus lutescens* Cap., *Lixus scolopax* Boheman, and *Cyphocleonus morbillosus* (Fabricius) are distinctly different, and

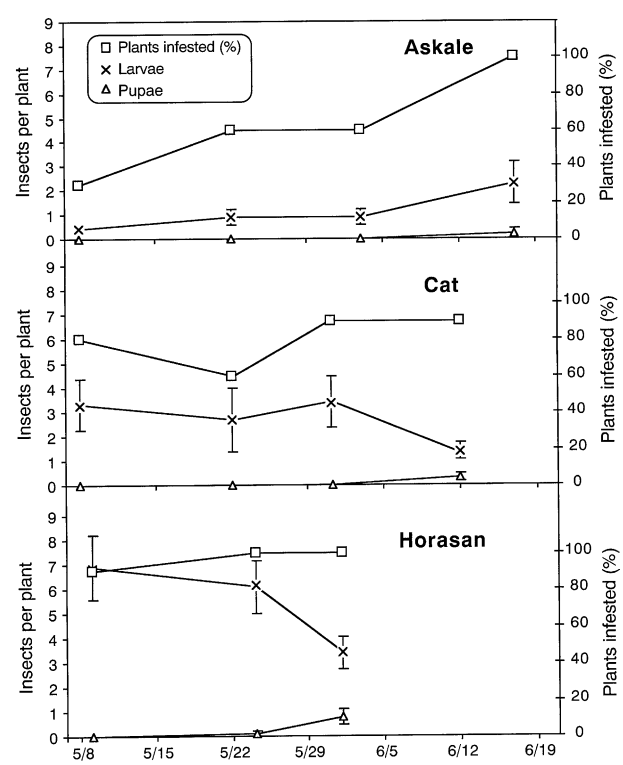

Fig. 1. Seasonal changes in infestation rate and developmental stage of *C. basicorne* on naturally growing *Ce. solstitialis* plants at three sites in 2002 (±SE; $n = 10$).

were never found at these sites during these studies. *Ceratapion* pupae first appeared on June 1, 12, and 17 at Horasan, Çat, and Askale, respectively, and we harvested the plants on June 1 and 24 and July 5. Infestation of the *Ce. solstitialis* test plants was between 48 and 92% (California and Turkish plants combined) at the three sites, indicating that there was a substantial infestation rate to challenge the safflower plants (Table 1). Infestation of Turkish *Ce. solstitialis* was higher than that of Californian *Ce. solstitialis*, based on proportion of plants infested, at all three sites. The cause of this difference is unknown, but could be due to differences in plant size or chemistry, which were not measured. Clement (1994) reported similar differences in susceptibility to attack by capitula insects for Italian and American accessions of *Ce. solstitialis*. The infestation rate of wild *Ce. solstitialis* (90–100%) was not significantly higher than that of the Turkish *Ce. solstitialis* test plants at any of the sites, suggesting that the artificial rearing of the test plants did not reduce suitability or attractiveness to apionids. We obtained 61, 101, and 133 apionids from *Ce. solstitialis* at Horasan, Çat, and Askale, respectively, but some were immature and others were in poor condition for taxonomic determination. All the adults that could be identified were *C. basicorne* (5, 15, and 15 from Horasan, Çat, and Askale, respectively). Many of the harvested plants began to rot, which prevented us from rearing many of the insects to adult. No safflower plants were infested by internal feeding insects at either Horasan or Çat. Seven oleic and five linoleic safflower plants were infested at Askale. We reared *Ceratapion scalptum* (Mulsant and Rey), *Ceratapion orientale* (Gerstaecker), and *Ceratapion onopordi* (Kirby) from these safflower plants, but no *C. basicorne*. Hymenopteran parasitoids were rarely found in the weevil tunnels: three individuals in two *Ce. solstitialis* plants at Askale (3/133 = 2.3% of apionids parasitized), two individuals in one *Ce. solstitialis* plant at Çat (2/101 = 2.0% of apionids), and none at Horasan (of 61 apionids).

Table 1
Infestation of root crowns or lower stems of test plants by apionid weevils (including larvae, pupae, and adults) in 2002

Site	Test plant			
	Centaurea solstitialis		Safflower	
	California	Turkish	Oleic	Linoleic
Plants infested (%)[1]				
Horasan	83.3 b	100.0 a	0.0 c	0.0 c
Çat	27.9 b	66.7 a	0.0 c	0.0 c
Askale	58.6 b	86.7 a	19.0 c[2]	15.8 c[3]
Number of insects per infested plant (±SE)[4]				
Horasan	1.4 ± 0.2 a	1.5 ± 0.2 a		
Çat	2.3 ± 0.5 a	2.8 ± 0.4 a		
Askale	2.6 ± 0.5 a	2.4 ± 0.3 a	1.8 ± 0.8 a	1.0 ± 0.0 a
Number of plants sampled				
Horasan	24	24	22	23
Çat	26	30	25	13
Askale	29	30	21	19

C. basicorne was reared only from *Ce. solstitialis*.
[1] Values followed by the same letter in the same row are not significantly different (chi-square test, $P < 0.05$).
[2] Adults identified: 4 *C. scalptum*, 1 *C. orientale*, and 2 *C. onopordi*.
[3] Adults identified: 2 *C. scalptum*.
[4] Values followed by the same letter in the same row are not significantly different (Fisher's protected LSD, $\alpha < 0.05$).

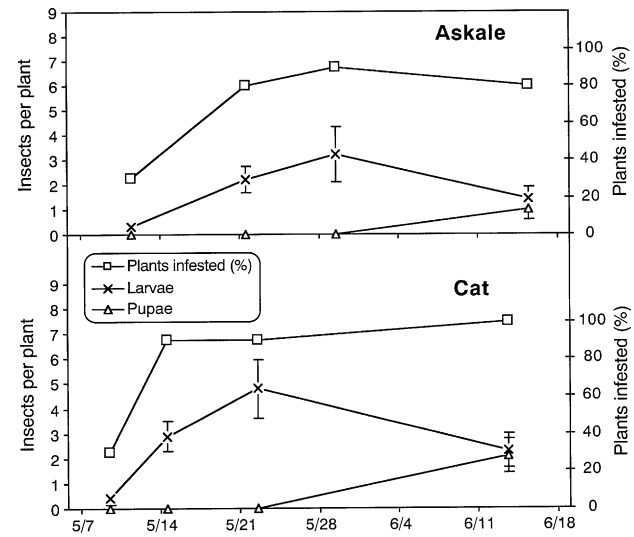

Fig. 2. Seasonal changes in infestation rate and developmental stage of *C. basicorne* on naturally growing *Ce. solstitialis* plants at two sites in 2003 (±SE; $n = 10$).

3.2. 2003

Signs of *C. basicorne* adult feeding were first observed on April 15 and 20 at Çat and Askale, respectively. Test plants were transplanted to the field on May 3–4, and eggs were seen on nearby wild *Ce. solstitialis* plants by May 9–11 at both locations. Pupae first appeared on June 13–15, and test plants were collected on June 16 and 19 at Çat and Askale, respectively (Fig. 2). Repeated sampling of wild *Ce. solstitialis* plants showed that oviposition and larval development occurred during the exposure period of the test plants and that natural infestation rates were very high (80 and 100% at Askale and Çat, respectively). Infestation of *Ce. solstitialis* test plants by *Ceratapion* ranged from 37 to 45% at Çat (Table 2), which was lower than that observed on wild *Ce. solstitialis* plants (100%). Infestation of *Ce. solstitialis* test plants at Askale (76%) was not significantly different from that of wild plants (80%). No safflower plants were infested at Çat, and three plants were infested at Askale. Because many plants began to rot while being held for adults to emerge, many of the adult insects could not be identified, including the three reared from safflower. All the identifiable insects from *Ce. solstitialis* were *C. basicorne*.

3.3. 2004

Signs of *C. basicorne* oviposition were first observed on April 14 and 15 at Askale and Horasan, respectively (Figs. 3 and 4). Test plants were transplanted to the field on April 26 and 27 at the two locations. *Ceratapion* pupae were

Table 2
Infestation of root crowns or lower stems of test plants by apionid weevils (including larvae, pupae, and adults) in 2003

Site	Test plant			
	Ce. solstitialis (US)	*Ce. solstitialis* (TR)	Oleic	Linoleic
Plants infested (%)[1]				
Çat	37.0 a	44.8 a	0.0 b	0.0 b
Askale	—	76.7 a	7.7 b[2]	—
Number of insects per infested plant (±SE)[3]				
Çat	5.0 ± 0.8 a	3.4 ± 0.4 a	—	—
Askale	—	4.1 ± 0.5 a	1.0 ± 0.0 b	—
Number of plants sampled				
Çat	27	29	27	30
Askale	—	30	39	—

C. basicorne was reared only from *Ce. solstitialis*.
[1] Values followed by the same letter in the same row are not significantly different (chi-square test, $P < 0.01$).
[2] Three adults unidentifiable.
[3] Values followed by the same letter in the same row are not significantly different (Student's t test, $\alpha < 0.05$).

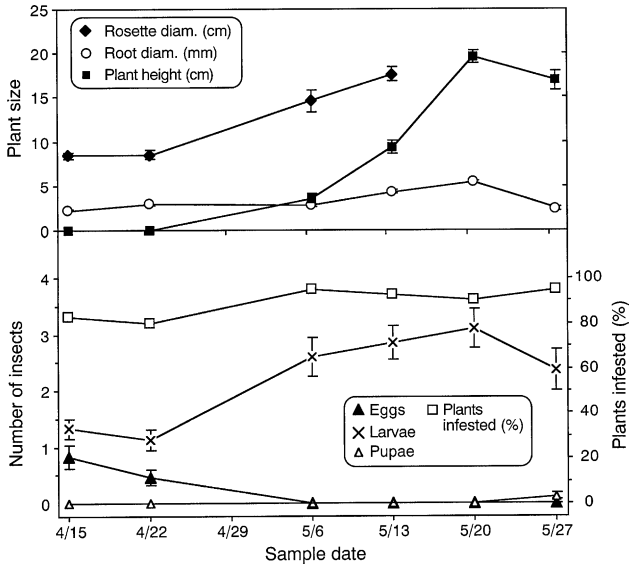

Fig. 3. Seasonal changes in infestation rate and developmental stage of *C. basicorne* on naturally growing *Ce. solstitialis* plants and size of plants at Horasan in 2004 (±95% CI; $n = 20$–40 plants).

first observed in wild *Ce. solstitialis* plants on May 27 and June 2, and test plants were collected on June 1 and 18 at Horasan and Askale, respectively. Diameter of wild *Ce. solstitialis* rosettes increased during the course of the experiment, indicating that the insect begins attacking the plant when it is still small (Figs. 3 and 4). *Ce. solstitialis* plants at Horasan began to bolt in early May while those at Askale did not bolt before the last observation on June 2, reflecting the warmer climate at the former location. Eggs of *C. basicorne* were not seen after April 22 at Horasan, whereas they were seen until May 28 at Askale. Test plants appear to have been exposed during the peak oviposition period at Askale, but were at the end of the oviposition period at Horasan Nevertheless, *Ceratapion* infestation of *Ce. solstitialis* test plants was very high at both sites (98–100%; Table 3).

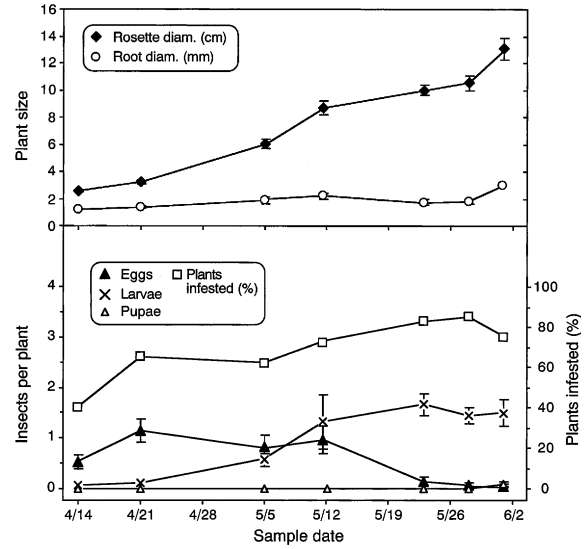

Fig. 4. Seasonal changes in infestation rate and developmental stage of *C. basicorne* on naturally growing *Ce. solstitialis* plants and size of plants at Askale in 2004. (±95% CI; $n = 20$–40 plants).

Table 3
Infestation of root crowns or lower stems of test plants by apionid weevils (including larvae, pupae, and adults) in 2004

Site	Test plant	
	Ce. solstitialis (TR)	Safflower[1]
Plants infested (%)[2]		
Horasan	98 a[3]	0 b
Askale	100 a[4]	26 b[5]
Number of insects per plant (±SE)[6]		
Horasan	1.7 ± 0.2 a	0.0 ± 0 b
Askale	2.4 ± 0.2 a	1.09 ± 0.05 b
Number of plants sampled		
Horasan	40	250
Askale	40	99

C. basicorne was reared only from *Ce. solstitialis*.
[1] Oleic (Seedtec-317).
[2] Values followed by the same letter in the same row are not significantly different (chi-square test, $P < 0.01$).
[3] Adults identified: 24 *C. basicorne*, 1 *C. orientale*.
[4] Adults identified: 67 *C. basicorne*, 2 *C. orientale*.
[5] Adults identified: 8 *C. scalptum*, 2 *C. orientale*.
[6] Values followed by the same letter in the same row are not significantly different (Student's t test, $\alpha < 0.05$).

None of 225 safflower plants were infested at Horasan, and 46 of 179 plants were infested at Askale. *Ce. solstitialis* was infested primarily by *C. basicorne* (91 adults identified), although a few *C. orientale* (3–4% of identified insects) were also reared out. Safflower was infested by *C. scalptum* (80% of insects) and *C. orientale* (20%), but not by *C. basicorne*.

3.4. Risk to safflower

During three years of field studies in eastern Turkey, we never reared *C. basicorne* from safflower. Deterioration of some of the harvested plants before insects could complete

development to provide identifiable specimens was a persistent problem that left some uncertainty about whether any of the unidentifiable insects from safflower could have been *C. basicorne* (Table 4). However, at Çat and Horasan, where *C. basicorne* was the only apionid species observed, safflower was never infested by any internal feeding insect, despite infestation rates of 48–98% of the *Ce. solstitialis* test plants and up to 100% infestation of wild *Ce. solstitialis* plants. This indicates a risk of infestation less than 0.27% (less than 1 of 365 plants sampled); i.e., we are more than 99.73% (±0.27% SE; Gottelli and Ellison, 2004) certain that *C. basicorne* does not attack safflower at these sites. At Askale, where *C. scalptum*, *C. orientale*, and *C. onopordi* were present, 8–26% of safflower plants were infested, but of 19 identifiable insects reared from safflower during 3 years, none were *C. basicorne*. *Ce. scalptum* is known to develop in stems of *Carthamus lanatus* L., *Carthamus oxyacantha* Bieb., and *Silybum marianum* (L.) Gaertner and adults have been found resting on *Carduus acanthoides* L., *Carduus pycnocephalus* L., *Cirsium arvense* (L.) Scop., *Cirsium dissectum* (L.) Hill [= *anglicum* Lob.], *Cirsium vulgare* (Savi) Ten. [= *lanceolatum* (L.) Scop.], *Cynara scolymus* L., and *Onopordum illyricum* L. (Alonso-Zarazaga, 1990; Wanat, 1994). *Ceratapion orientale* has been reared from *Cn. benedictus*, *Crupina vulgaris* Cass., and *Ce. solstitialis* (Clement et al., 1989; Smith and Drew, in press; J.K. Balciunas, unpubl data), and adults have been found resting on *Centaurea rhenana* Bor. (Alonso-Zarazaga, 1990). *Ceratapion onopordi* also has a wide host range, including *Arctium*, *Carduus*, *Centaurea*, *Cirsium*, and *Onopordum* (Wanat, 1994). In contrast, *C. basicorne* has been reared only from *Ce. solstitialis*, *Ce. cyanus*, *Ce. depressa*, and *Cn. benedictus* (Alonso-Zarazaga, 1990; Campobasso et al., 1999; Wanat, 1994). *Cnicus* appears to be a paraphyletic taxon that lies within the Jacea clade of the genus *Centaurea* (Garcia-Jacas et al., 2000). The Jacea clade also includes *Ce. solstitialis* (Wagenitz, 1975), so *Cnicus* does not appear to be a very anomalous host record. However, attempts to rear *C. basicorne* on *Cn. benedictus* under no-choice conditions in the laboratory have failed (Clement et al., 1989; L. Smith, unpublished data), suggesting that this is not a usual host.

In comparison, field surveys by Balciunas and Villegas (2001) to assess the risk to safflower of the accidentally introduced fly, *Chaetorellia succinea* (Costa) (Diptera: Tephritidae), recorded 109 adults emerging from 22,016 safflower capitula (flower heads) collected in growers' fields. This represented an overall attack rate of 0.5%, but the attack rate was as high as 5% at one site on one variety, which was not being grown for harvest. No flies were observed at 45 of 47 sites monitored. At three other sites where these authors planted safflower "trap plants" near infested *Ce. solstitialis* populations, they observed no emergence of the fly from 1060 safflower capitula sampled (less than 0.1% attack rate). To date, the fly is not considered to be a problem in safflower in California, despite the widespread abundance of this insect on *Ce. solstitialis* (Pitcairn et al., 2003). This suggests that observed attack rates on the order of 0–5% in field studies indicate insignificant risk to the nontarget crop.

Our experiments conclusively show that under field conditions *C. basicorne* does not attack safflower, even when 100% of nearby *Ce. solstitialis* is infested. With respect to this crop, the insect is safe to introduce to North America. However, it is critical to be certain that all individuals that would be released are correctly identified to avoid the accidental release of *C. orientale* or *C. onopordi*. Well illustrated

Table 4
Identification of apionid weevils infesting root crowns or lower stems of test plants at field sites in Turkey in 2002–2004

Ceratapion species	Number of individuals					
	Askale		Çat		Horasan	
	Ce. solstitialis	Safflower	*Ce. solstitialis*	Safflower	*Ce. solstitialis*	Safflower
2002						
C. basicorne	15	0	15	0	5	0
C. orientale	0	1	0	0	0	0
C. onopordi	0	2	0	0	0	0
C. scalptum clavipes	0	6	0	0	0	0
Unidentifiable specimens	118	3	86	0	56	0
2003						
C. basicorne	10	0	15	0	—	—
C. orientale	0	0	0	0	—	—
C. onopordi	0	0	0	0	—	—
C. scalptum clavipes	0	0	0	0	—	—
Unidentifiable specimens	61	3	29	0	—	—
2004						
C. basicorne	67	0	—	—	24	0
C. orientale	2	2	—	—	1	0
C. scalptum clavipes	0	8	—	—	0	0
Unidentifiable specimens	24	5	—	—	1	0

keys for these species have been developed by B. Korotyaev (unpublished). *Ceratapion scalptum* has never been reared from *Ce. solstitialis*, so there is no risk of accidentally introducing this species as long as we continue to use only insects reared from *Ce. solstitialis*.

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References

Alonso-Zarazaga, M.A., 1990. Revision of the sub-genera *Ceratapion* S. Str. and *Echinostroma* Nov. of the genus *Ceratapion* Schilsky, 1901. Fragmenta Entomol, Rome 22, 399–528.

Anon., 1963. Insects not known to occur in the United States: Safflower fruit fly (*Acanthiophilus eluta* (Meigen)). Coop. Econ. Insect Rep. 13 (49).

Avidov, Z., Kotter, E., 1966. The pests of safflower *Carthamus tinctorius* L., in Israel. *Scripta hierosolymitana*. Jerusalem 18, 9–26.

Balciunas, J.K., Villegas, B., 2001. Unintentionally released *Chaetorellia succinea* (Diptera: Tephritidae): is this natural enemy of yellow starthistle a threat to safflower growers? Environ. Entomol. 30, 953–963.

Briese, D.T., 1999. Open field host-specificity tests: is "natural" good enough for risk assessment? In: Withers, T.M., Stanley, J., Barton Browne, L. (Eds.), Host Specificity Testing in Australasia: Towards Improved Assays for Biological Control. CRC for Tropical Pest Management, Brisbane, Australia, pp. 44–59.

Bytinski-Salz, H., 1952. Safflower pests in Israel. Trans. Ninth Int. Congr. Ent. 1, 745–750.

Campobasso, G., Colonnelli, E., Knutson, L., Terragitti, G., Cristofaro, M., 1999. Wild Plants and their Associated Insects in the Palearctic Region, Primarily Europe and the Middle East. United States Department of Agriculture, Agricultural Research Service, ARS-147.

Clement, S.L., 1990. Insect natural enemies of yellow starthistle in southern Europe and the selection of candidate biological control agents. Environ. Entomol. 19, 1882–1888.

Clement, S.L., 1994. Resistance among populations of yellow starthistle to thistle-head insects: results from garden plots in Italy. BioControl 4, 149–156.

Clement, S.L., Cristofaro, M., 1995. Open-field tests in host-specificity determination of insects for biological control of weeds. Biocontrol. Sci. Techn. 5, 395–406.

Clement, S.L., Alonso-Zarazaga, M.A., Mimmocchi, T., Cristofaro, M., 1989. Life history and host range of *Ceratapion basicorne* (Coleoptera: Apionidae) with notes on other weevil associates (Apioninae) of yellow starthistle in Italy and Greece. Ann. Entomol. Soc. Am. 82, 741–747.

Cristofaro, M., Hayat, R., Gultekin, L., Tozlu, G., Zengin, H., Tronci, C., Lecce, F., Sahin, F., Smith, L., 2002. Preliminary screening of new natural enemies of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae) in Eastern Anatolia. In: Proceeding of the Fifth Turkish National Congress of Biological Control (4–7 September, 2002), Erzurum, Turkey, pp. 287–295.

Duncan, C.L., 2001. Knapweed management: another decade of change. In: Smith, L. (Ed.), Proceedings of the First International Knapweed Symposium of the Twenty-First Century, March 15–16, 2001, Coeur d'Alene, Idaho. USDA-ARS, Albany, CA, pp. 1–7.

El-Sheikh, M.A.K, Ismail, I.I., Herakly, F.A., 1990. Survey and population dynamics of insects attacking safflower, (*Carthamus tinctorius*), in Giza region, Egypt. Ann. Agric. Sci. Mosh. 28, 1755–1764.

FAO, 2005. Food and agricultural commodities production. Food and Agriculture Organization. (safflower seed production statistics for 2002, accessed 3 May 2005) <http://www.answers.com/topic/safflower/>.

Freidberg, A., 1996. The Israeli Tephritid fauna revisited. In: The 8th Conference of the Entomological Society of Israel, January 18, 1996. ARO, The Volcani Center, Bet Dagan, Israel. Phytoparasitica 24, 127–128.

Garali, B., Merz, B., Jozyan, A., 2004. The first report of *Urophora mauritanica* (Tephritidae) from Iran. J. Entomol. Soc. Iran 24, 131–132.

Garcia-Jacas, N., Susanna, A., Mozaffarian, V., Ilarsian, R., 2000. The natural delimitation of *Centaurea* (Asteraceae: Cardueae): ITS sequence analysis of the *Centaurea jacea* group. Plant Syst. Evol. 223, 185–199.

Gottelli, N.J., Ellison, A.M., 2004. A Primer of Ecological Statistics. Sinauer Associates, Sunderland, Massachusetts.

Logozzo, G., Alba, E., 1990. Cartamo. Ital. Agric. 127, 25–29.

Maddox, D.M., 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and central valley situations in California. US Department of Agriculture, Agricultural Research Service, Agricultural Research Results. ARR-W-20, July 1981, USDA-ARS, Oakland, CA.

Piper, G.L., 2001. The biological control of yellow starthistle in the Western U.S.: four decades of progress. In: Smith, L. (Ed.), Proceedings of the First International Knapweed Symposium of the Twenty-First Century, March 15–16, 2001, Coeur d'Alene, Idaho. USDA-ARS, Albany, CA, pp. 48–55.

Pitcairn, M.J., Villegas, B., Woods, D., Wilber, G., Duffy, A., El-Bawdri, M., 2003. Statewide survey of yellow starthistle biological control agents. In: Woods, D.M. (Ed.), Biological Control Program Annual Summary, 2002. California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, California, pp. 45–49.

Pitcairn, M.J., Piper, G.L., Coombs, E.M., 2004. Yellow starthistle. In: Coombs, E.M., Clark, J.K., Piper, G.L., Cofrancesco, Jr., A.F. (Eds.), Biological Control of Invasive Plants in the United States. Oregon State University Press, pp. 421–435.

Purdue University, 2005. Center for New Crops and Plant Products, Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN (accessed 13 May 2005). <http://www.hort.purdue.edu/newcrop//>.

Rosenthal, S.S., Davarci, T., Ercis, A., Platts, B., Tait, S., 1994. Turkish herbivores and pathogens associated with some knapweeds (Asteraceae: *Centaurea* and *Acroptilon*) that are weeds in the United States. Proc. Entomol. Soc. Wash. 96, 162–175.

Sheley, R.L., Larson, L.L., Jacobs, J.J., 1999. Yellow starthistle. In: Sheley, R.L., Petroff, J.K. (Eds.), Biology and Management of Noxious Rangeland Weeds. Oregon State Univ. Press, Corvallis, Oregon, pp. 408–416.

Smith, L., Drew, A.E., In press. Fecundity, development and behavior of *Ceratapion basicorne* (Coleoptera: Apionidae), a prospective biological control agent of yellow starthistle. Environ. Entomol.

Turner, C.E., Johnson, J.B., McCaffrey, J.P., 1995. Yellow starthistle. In: Nechols, J.R., Andres, L.A., Beardsley, J.W., Goeden, R.D., Jackson, C.G. (Eds.), Biological Control in the Western United States: Accomplishments and Benefits of Regional Research Project W-84, 1964–1989. University of California, Division of Agriculture and Natural Resources, Oakland, Publ. 3361, pp. 270–275.

USDA, 2005. Crop Production, 2004 Summary. United States Department of Agriculture, National Agricultural Statistics Service. Cr Pr 2–1 (05).

Uygur, S., Smith, L., Uygur, F.N., Cristofaro, M., Balciunas, J., 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, 525–541.

Wagenitz, G., 1975. 79. *Centaurea* L.. In: Davis, P.H. (Ed.), Flora of Turkey, vol. 5. Edinburgh University Press, Edinburgh, pp. 465–585.

Wanat, M., 1994. Systematics and phylogeny of the tribe Ceratapiini (Coleoptera, Curculionoidea, Apionidae), Genus, International Journal of Invertebrate Taxonomy (Suppl.).

White, I.M., Groppe, K., Sobhian, R., 1990. Tephritids of knapweeds, starthistles and safflower: results of a host choice experiment and the taxonomy of *Terellia luteola* (Wiedemann) (Diptera: Tephritidae). Bull. Entomol. Res. 80, 107–111.

Zwölfer, H., 1965. Phytophagous insect species associated with *Centaurea solstitialis* L. in south-western Europe. Report on investigations carried out in 1965. Commonw. Inst. Biol. Control.