Field assessment in land of origin of host specificity, infestation rate and impact of *Ceratapion basicorne* a prospective biological control agent of yellow starthistle

Sibel UYGUR¹, Lincoln SMITH^{2,*}, F. Nezihi UYGUR¹, Massimo CRISTOFARO³ and Joe BALCIUNAS²

¹Department of Plant Protection, Agricultural Faculty, Çukurova University, TR-01330 Adana, Turkey; ²USDA-ARS Western Regional Research Center, 800 Buchanan Street, Albany, CA 94710; ³ENEA C.R. Casaccia, BIOTEC, via Anguillarese 301, 00060 Rome, Italy *Author for correspondence; e-mail: lsmith@pw.usda.gov

Received 24 January 2004; accepted in revised form 28 October 2004

Abstract. Yellow starthistle, Centaurea solstitialis (Asteraceae), is an important invasive alien weed in the western United States. Currently established biological control agents attack only the capitula (flowerheads), and are not effectively controlling the plant in much of its range. The geographic center of diversity for the plant appears to be in Turkey, but no agents have been introduced from this country. Ceratapion basicorne (Coleoptera: Apionidae) is common in Central Turkey, attacking 25-100% of yellow starthistle plants. In a field experiment, Ceratapion spp. attacked 90% of yellow starthistle plants and 88% of milk thistle plants (Silybum marianum) but not seven other plant species, including artichoke and safflower. We suspect that a different species of insect attacked milk thistle, but they emerged before the plants were sampled. Laboratory tests showed that C. basicorne does not oviposit in milk thistle. Ceratapion basicorne appears to be more host specific than was suggested by previous studies of a population in Italy (Clement et al. 1989. Ann. Entomol. Soc. Am. 82: 741-747). The insect is gregarious, and the number of larvae per plant was positively correlated to root diameter. The level of damage to individual plants was positively correlated to the proportion of plants attacked, indicating aggregation both among plants and within plants. Field data did not show any impact of the insect on plant size or number of capitula, but germination rate of seeds produced by infested plants was 15% lower than for uninfested plants at two of three sites studied.

Key words: biological control, Centaurea solstitialis, Ceratapion basicorne, root-crown feeding insect, Turkey

Introduction

Yellow starthistle (*Centaurea solstitialis* L., Asteraceae) is an important invasive rangeland weed in the western United States that is continuing to spread (Maddox et al., 1985; Duncan, 2001). It is an alien plant that originates from the Mediterranean region, and has been the target of classical biological control (Maddox, 1981; Rosenthal et al., 1992; Sheley et al., 1999; Piper, 2001). Six species of insects that attack the flowerheads (capitula) have been approved and released (Rees et al., 1996; Balciunas, 1998). Although five of these agents established, they are generally not providing effective control, especially in California (Pitcairn et al., 1998, 2000), where the plant is most abundant. Therefore, efforts have been renewed to find and evaluate additional agents, especially those that attack vegetative parts of the plant (Balciunas, 1998; Bruckart and Eskandari, 2002; Smith, 2002).

The exceptionally high number of other *Centaurea* species recorded from Turkey, including two endemic subspecies of *C. solstitialis* (Wagenitz, 1975), makes it probable that this region is the center of origin of yellow starthistle (Uygur et al., 2004). Central and southern Turkey also have climates that are similar to the regions heavily infested in the western U.S. Thus, Turkey is a promising location to search for candidate biological control agents to introduce to the U.S. Previous exploration in Turkey has discovered interesting possibilities (Rosenthal et al., 1994), but the five approved agents that have established in the U.S. have all come from Greece (Rees et al., 1996). Therefore, the current effort to discover new agents is focused primarily in Turkey (Smith, 2002).

Previous investigators have found apionid larvae commonly infesting a large proportion of yellow starthistle plants in Turkey (up to 97%, near Corum) (Rosenthal et al., 1994). Ceratapion basicorne (Illiger) (Coleoptera: Apionidae), C. orientale (Gerstaecker), C. scalptum and Diplapion detritum (Mulsant and Rey) have been reared from yellow starthistle in central Turkey, but all of these were rare on yellow starthistle except C. basicorne (Rosenthal et al., 1994). During surveys in Turkey from 1996 to 1999, Balciunas (unpublished data) confirmed the high infestation rates of yellow starthistle plants by apionid larvae. He reared 60 adults from larvae found in yellow starthistle root crowns at eight different sites in central Turkey. These were all identified as C. basicorne (Balciunas, unpublished data). Although Balciunas reared five other species of Ceratapion from other host plants, none of them were found in yellow starthistle. In the literature, C. basicorne is primarily associated with yellow starthistle and bachelor button (Centaurea cyanus L.), but adults have also been collected from a variety of Cardueae species (Alonso-Zarazaga, 1990; Wanat, 1995). Balciunas (unpublished data) confirmed that larvae of *C. basicorne* were restricted to the above two *Centaurea* species, and possibly a third member of the Cardueae tribe. This insect can cause substantial damage to the root crown of yellow starthistle (Figure 1) and is also common in Italy and Greece (Clement, 1990).

Previous studies of a population of C. basicorne in Italy suggested that it may not be safe enough to introduce as a biological control agent (Clement et al., 1989). Their laboratory studies of 5 field-collected adult females indicated that it would oviposit on safflower in no-choice experiments, but not in choice experiments when yellow starthistle was present. When newly hatched larvae were transferred into the test plants, they were able to develop to the third instar or pupal stage on safflower, Carduus pycnocephalus L. and Galactites tomentosa (L.) Moench. However, Balciunas's field observations suggest that C. basicorne in Turkey may be more specific than Clement et al.'s study suggests. Perhaps the host specificity of the two populations differ or perhaps the ecological host range is more specific than the physiological range that was demonstrated in Clement et al.'s study (Cullen, 1990). In either case, it appears that C. basicorne in Turkey warrants further evaluation to determine if it would be suitable as a classical biological control agent. This requires determining both host specificity and impact on the target weed (Balciunas, 2004).



Figure 1. Damage to yellow starthistle root crowns in central Turkey caused by *Ceratapion basicorne*; pupa; adult female (reared from yellow starthistle and identified by B. Korotyaev).

The purpose of this study was to gather more information about the risk *C. basicorne* poses to safflower in the field and to measure its attack rate and damage to yellow starthistle in the field in central Turkey.

Methods

At all our sites, plants were exposed to ambient populations of *C. basicorne*. Adults oviposit on rosettes in early spring (March to May in Italy) and adults emerge in early summer (May to July; Clement et al., 1989), although the timing is not well known in Turkey. Yellow starthistle bolts in May to June, attaining its final height and number of capitula by the end of June or July (Maddox, 1981). It flowers from June to August, depending on location; i.e., after the adult weevils have emerged. Therefore, in order to keep plants long enough to measure the impact of *C. basicorne* infestation on the ultimate size and reproduction of the plants, we had to forego collecting insects from the study plants. However, because *C. basicorne* is by far the most common weevil attacking yellow starthistle root crowns in Turkey, we believe that virtually all the apionid damage observed in our study was caused by larvae of *C. basicorne*.

Field host range tests

An adult specimen of C. basicorne was collected on a yellow starthistle flower (10-VI-98) at Kamisli, confirming that the species occurs in this region. We established a test garden 12 km away in Pozanti (lat. 37°28'41.8" N, long. 34°54'26.6" E, elevation 1223 m), which represents a high elevation site in Turkey's Mediterranean climatic zone. In 1999, yellow starthistle and four species of nontarget test plants were planted in a small field garden. Because the species differed in development rates and season of normal germination, we planted them in different ways in order to obtain plants of similar size and developmental stage during the anticipated oviposition season (April-May). Small rosettes of commercial artichoke (Cynara scolymus L.) were transplanted in mid-March, Russian knapweed (Acroptilon repens (L.) DC.) rosettes from Göreme were transplanted on March 22, Centaurea solstitialis var solstitialis L. rosettes collected at Kamisli (12 km away) were transplanted on April 7, and milk thistle (Silybum marianum (L.) Gaertn.) and safflower (Carthamus tinctorius L., Turkish variety) seeds were planted on April 7. On May 22, all the plants were roughly similar in size and were in late rosette to early bolting stage. We examined a dozen

528

yellow starthistle plants and found that at least half contained *C. basicorne larvae*, most of which were in the third instar. We left the remaining plants undisturbed to allow the insects to develop further and returned on June 16 to harvest all the test plants, which were between bolting stage and budding stage. We also collected some nearby, naturally occurring mature plants of distaff thistle (*Carthamus lanatus* L.), yellow plumed thistle *Picnomon acarna* (L.) Cass., spring groundsel (*Senecio vernalis* Waldst. & Kit.) and hawksbeard (*Crepis* sp.). Each plant species was held in a separate container in the laboratory until July 21 to collect adult insects that might emerge. Each plant was then dissected to detect and record *C. basicorne* damage.

Larval damage and infestation rate studies

During 1999 and 2000, we examined yellow starthistle plants growing naturally at three sites in central Turkey to determine infestation rate and damage caused by C. basicorne. All sites were at uncultivated areas along the roadside. Each site was 72-78 km from the others. Descriptions of climate and weather during the course of the study are reported in Uygur et al. (2004). The sites were: Catalan, Camardi and Göreme. Catalan (latitude 37°05'21.6" N, longtitude 35°22'53.5" E, elevation 198 m, precipitation 613 mm) is near Adana, and represents Turkey's Mediterranean climate, with a hot dry summer and mild rainy winter. Camardi (latitude 37°43'36.2" N, longtitude 35°01'13.1" E, elevation 1460 m, precipitation 333 mm) is near Nigde, and represents Turkey's Central Anatolia climatic region. It has a mild summer and cold rainy winter. Göreme (latitude N 37°39'44.2", longtitude 35°49'55.8", elevation 1160 m, precipitation 401 mm) is near Nevsehir and is also in Turkey's Central Anatolia climatic region, but the site was drier than Camardi because the soil had more sand and volcanic tufa. Climatic data are from meteorological stations 10-15 km from our field sites (station no. 17351, 17906, 17835) operated by the Turkish State Meteorogical Service (see Uygur et al., 2004). An endemic subspecies of vellow starthistle occurred at the Catalan site: C. solstitialis subsp. carneola (Boiss.) Wagenitz whereas C. solstitialis subsp. solstitialis L. occurred at the other two sites.

Ten yellow starthistle plants were sampled at each site: Catalan on August 17, 1999 and August 2, 2000, Camardi and Göreme on July 27, 1999 and August 10 in 2000, when plants had completed flowering. Plants were collected at every second step along an arbitrary transect at each site. Plant height was measured from root crown to the top of the highest capitulum (flowerhead), and all capitula were counted. Plants

were dissected in the laboratory and damage caused by *C. basicorne* larvae was scored as 'light' (1–25% of root crown area damaged), 'medium' (26–50%), or 'heavy' (>50%). At the Pozanti host range test site, damage was similarly assessed, but with an additional category for 76–100% damage.

Impact on plant size and seed germination

During our visits to these three sites in 2000, we made additional collections of yellow starthistle in an attempt to assess if the damage caused by *C. basicorne* larvae had an impact on the mature size and reproduction of yellow starthistle. We collected an additional 10 infested and 10 uninfested yellow starthistle plants and recorded plant height (from root crown), root diameter (just below the crown), number of capitula and number of *Ceratapion* emergence holes for each plant.

We collected achenes (seeds) from 20 *Ceratapion*-infested and 20 uninfested plants from each of the three sites. Achenes were held under room conditions, until germination tests were done, between January 30, 2001 and February 26, 2001. Forty achenes from each plant (10 achenes × 4 Petri dish replications) were tested. We did not distinguish between pappus and non-pappus achenes because germination rate does not differ when achenes stored at room temperature for several months are exposed to light (Joley et al., 1997). For germination, achenes were placed on a double layer of moist filter paper in covered glass Petri dishes (9 cm diameter) placed in a dark incubator at 25 °C, following Uygur (1985). Petri dishes were checked daily and watered with distilled water. Achenes that produced at least 0.5 cm of growth were scored as germinated. Germinating achenes were counted and removed from petri dishes on days 1, 3, 5, 7, 14, and 21.

Statistical analysis

Analysis of variance (ANOVA) and general linear models (GLM) were performed using SuperANOVA (version 1.11, Abacus Concepts, Inc.). Count data were transformed by square root(*Y*), plant height by Log(*Y*), and proportion data by $\arcsin(\text{square root}(Y))$ to help normalize the data for ANOVA. Posthoc multiple comparisons were made using Fisher's protected LSD with $\alpha = 0.05$. All reported means and confidence intervals were back-transformed. Independence of proportion data was tested using chi-square tests.

Results and discussion

Field host range tests

Typical C. basicorne damage was observed in 90% of yellow starthistle plants, in 88% of milk thistle plants and in 0% of the remaining 7 plant species harvested at Pozanti in 1999 (Table 1). Experiments performed

Table 1. Infestation of plants at Pozanti in 1999

Plant species ¹	Plant subtribe	No. plants sampled	Proportion infested, %
Planted species			
<i>Centaurea solstitialis</i> , vellow starthistle ^b	Centaureinae	50	90
<i>Carthamus tinctorius</i> , safflower ^r	Centaureinae	50	0
<i>Cynara scolymus,</i> artichoke ^r	Carduinae	6	0
<i>Silybum marianum</i> , milk thistle ^b	Carduinae	50	88
<i>Naturally occurring species</i> Cardueae ²			
<i>Carthamus lanatus</i> , saffron thistle ^b	Centaureinae	18	0
Acroptilon repens (L.) DC., Russian knapweed ^b	Centaureinae	5	0
Picnomon (Cirsium) acarna, yellow plumed thistle ^b Senecioneae ²	Carduinae	2	0
Senecio vernalis, spring groundsel ^b Lactuceae ²	Senecioninae	25	0
<i>Crepis</i> sp., hawksbeard ^b	Crepidinae	25	0

¹Developmental stage at time of collection, on June 16:^r – large rosette, ^b – bolted and flowering. ²Plant tribe.

in quarantine laboratory in California indicate that adult *C. basicorne* do not feed or oviposit on milk thistle plants (Smith, unpublished data). This suggests that the damage observed in milk thistle was caused by a species other than *C. basicorne*. Other species of *Ceratapion* known to develop in milk thistle are *C. gibbirostre* Gyllenhal (Balciunas, unpublished data) and *C. scalptum* (Mulsant and Rey) (Alonso-Zarazaga 1990; Wanat 1994), neither of which are known to attack yellow starthistle. The absence of attack on safflower and Russian knapweed, a close relative of yellow starthistle, indicates that *C. basicorne* may be more specific than suggested by Clement et al. (1989) larval transfer and no-choice oviposition results.

Ceratapion basicorne damaged at least 50% of the root crown tissue in 34% of yellow starthistle plants (Figure 2). It is a small insect (2– 3 mm), but up to 7 larvae have been found in a yellow starthistle plant in Turkey (see below). So, the damage levels probably reflect the number of insects infesting individual plants. These results encouraged us to conduct the following study to measure the rate of attack and damage to yellow starthistle in different regions of Turkey.

Larval damage and infestation rate studies

During both 1999 and 2000, infestation rates of yellow starthistle root crowns by *C. basicorne* at our sites in Turkey were quite high, falling below 50% only once, and on two occasions all the plants were attacked (Figure 3). Rosenthal et al. (1994) found that at 13 sites in Turkey sampled during May and June, 50-97% of yellow starthistle plants were damaged. Clement et al. (1989) reported infestation rates of 12-56% in



Figure 2. Frequency distribution of level of damage to yellow starthistle caused by a natural population of *C. basicorne* at Pozanti, Turkey in 1999.



Figure 3. Natural infestation rates of yellow starthistle plants by *C. basicorne* at three sites in central Turkey.

Italy during late April to late June. High attack rates over a broad geographic area are encouraging for the potential impact of this insect as a biological control agent.

In 1999, a lower proportion of yellow starthistle plants was infested by *C. basicorne* at Göreme than at the other two sites ($\chi^2 = 12.0$, df = 2, p < 0.005). This may be due to the unusually high density of yellow starthistle plants at this site during that year (24.1 m^{-2} at Göreme vs. 5.8 m⁻² at Catalan and 5.4 m⁻² at Camardi); however, all sites had similar densities in 2000 (4.4, 5.4, and 6.6 m^{-2} , respectively) (Uygur et al., 2004). We suspect that the unusually high density of plants at Göreme in 1999 may have been caused by physical disturbance of the site. In 2000, when the yellow starthistle density at Göreme decreased to 4.4 m⁻², the proportion of plants infested rose to 70% ($\chi^2 = 4.6$, df = 1, p < 0.05). The drastic changes in infestation rate and plant population density at Göreme are consistent with the hypothesis that the plant population increased after a disturbance, followed by an increase in attack by natural enemies of the plant, and subsequent decline of the plant population. However, Catalan did not fit this pattern. There, the yellow starthistle population remained the same (5.8 and 5.4 m^{-2}), but the infestation rate decreased from 100% in 1999 to 30% in 2000 $(\chi^2 = 10.8, df = 1, p < 0.005)$. Precipitation (September to August) in 2000 was lower (505 mm) than in 1999 (700 mm) at Catalan, but this pattern was similar at the other two sites, where the infestation rate did not decrease. So, the change in precipitation does not explain the decrease in infestation rate at Catalan.

533



Figure 4. Frequency distribution of level of damage to yellow starthistle plants by natural infestations of *C. basicorne* larvae (light, 1–25%; medium, 26–50%, heavy, >50% of root crown area damaged).

The frequency distribution of level of *C. basicorne* larval damage to yellow starthistle plants varied among the three sites in 1999 ($\chi^2 = 14.9$, df = 6, $p \chi^2 = 8.6$, df = 6, p > 0.1), and it changed between years only at Catalan ($\chi^2 = 14.3$, df = 3, p < 0.005)(Figure 4). However, in both years, the sites that had the highest percentage of plants infested also had the highest proportion of plants with medium to heavy damage (Figure 5; linear regression: $Y = 0.021 (\pm 0.001 \text{ SE})^* X$, $F_{(1.5)} = 316.6$, p = 0.0001; $R^2 = 0.984$). This suggests that when the insect is abundant, it infests a higher proportion of plants, and more larvae occur in each infested plant. This gregarious behavior is a favorable characteristic for a prospective biological control agent (Smith, 2004).

Impact on plant size

The number of yellow starthistle capitula per plant at the three sites was lower in 2000 (43 [95% CI: 30–58]) than in 1999 (131 [103–162]) (2-way



Figure 5. Relationship of the mean level of damage of yellow starthistle root crowns to the proportion of plants infested by *C. basicorne* (damage level: 0 = none, 1 = light, 2 = medium, 3 = heavy).

ANOVA $F_{(1.54)} = 39.0$, p = 0.0001), and it was lower at Göreme (52 [30–79]) than at Catalan (97 [61–141]) or Camardi (98 [67–135]) ($F_{(2.54)} = 47.9$, p = 0.009; Figure 6). These differences appear to be related to differences in precipitation, which decreased in 2000 at all the sites (Uygur et al., 2004), and Göreme was the most xeric site. In any



Figure 6. Relationship of number of capitula and plant height to *C. basicorne* infestation (mean $\pm 95\%$ CI).

case, the number of capitula per plant did not differ between infested and uninfested plants (ANOVA of 'infestation' nested within 'site-year', omitting Camardi-1999 and Catalan-1999 because they had only infested plants: $F_{_{(4,32)}} = 0.60$, p = 0.66).

The number of capitula per plant was a function of root diameter, and this relationship was different at Catalan than at Camardi and Göreme, which may reflect differences between the two subspecies of yellow starthistle (GLM: root diameter, $F_{_{(1,56)}} = 40.6$, p = 0.0001; site, $F_{_{(3,56)}} = 8.9$, p = 0.0001; Catalan: Y = 101.4 [±13.3 SE] * X; Camardi and Göreme: Y = 46.5 [±3.0] * X). However infestation by *Ceratapion* did not significantly affect either the slopes or the intercepts at any site.

Yellow starthistle plant height was lower in 2000 (68 cm [95% CI: 62–76]) than in 1999 (84 cm [75–95]) (2-way ANOVA $F_{(1.54)} = 13.3$, p = 0.006), and it was higher at Catalan (99 cm [87–113]) than at Camardi (63 cm [58–69]) or Göreme (70 cm [62–79]) ($F_{(2.54)} = 22.9$, p = 0.0001). Infested plants were taller (73 cm [64–83]) than uninfested ones (67 cm [59–75]) (ANOVA of 'infestation' nested within 'site-year', omitting Camardi-1999 and Catalan-1999 because they had only infested plants: $F_{(4, 32)} = 3.17$, p = 0.026) (Figure 6). This suggests that C. basicorne may prefer to attack larger plants, which would be evident at the time of oviposition by larger rosette size. But, in any case, these data show no obvious deleterious effect of C. basicorne infestation on either



Figure 7. Relationship of *C. basicorne* infestation to size of yellow starthistle plants in 2000.

capitula production or plant height. Perhaps if experimental conditions are more controlled then we could better measure impact.

In general, the height of yellow starthistle plants was a function of root diameter and site (GLM: root diameter, $F_{_{(1,56)}} = 7.0$, p = 0.011; site, $F_{_{(2,56)}} = 30.7$, p = 0.0001). However, the linear regression model was significant only at Camardi: $Y = 20.2 \ [\pm 4.6 \ \text{SE}) * X + 29.5 \ [\pm 7.5]$, $F_{_{(1,18)}} = 19.1$, p = 0.0004). For a given root diameter, plants at Göreme were tallest and those at Camardi were shortest (least square means: $103 \pm 4.2 \ \text{SE}$ cm, $84 \pm 4.6 \ \text{cm}$ and $58 \pm 4.2 \ \text{cm}$). Infestation did not significantly affect either the slopes or the intercepts at any site.

The number of *C. basicorne* larvae attacking a plant increased as a function of the root diameter at all three sites (Figure 7; GLM: root diameter, $F_{_{(1,27)}} = 101.1$, p = 0.0001; site-by-root diameter interaction, $F_{_{(2,27)}} = 4.9$, p = 0.039). Linear regression models for each site were: Catalan: Y = 0.94 [+0.11 SE] * X; Camardi Y = 1.62 [+0.22] * X; and Göreme: Y = 1.99 [+0.31] * X. So, large plants are able to support a larger number of insects. Whether cannibalism occurs is not known, but up to 7 exit holes were found in a plant, and the mean was 2.2. Clement et al. (1989) found up to 5 larvae per plant and reported that H. Zwölfer had found up to 25 larvae per plant.

Impact on seed germination

Germination rate of yellow starthistle seeds was affected by site (twoway ANOVA, $F_{_{(2,114)}} = 19.9$, p = 0.0001) and infestation ($F_{_{(1,114)}} = 5.5$, p = 0.020), and there was a site-by-infestation interaction ($F_{_{(2,114)}} = 4.9$, p = 0.009; Figure 8). Germination rates were highest for seeds collected



Figure 8. Effect of *C. basicorne* infestation on germination of yellow starthistle seeds (mean \pm 95% CI).

at Camardi and lowest for seeds from Göreme. Catalan seed germination rate was not affected by infestation of parent plants. However, for Camardi and Göreme, germination of seeds from infested plants was 15% less than for uninfested plants (ANOVA, $F_{(1,38)} = 5.3$, p = 0.027and $F_{(1.38)} = 6.9$, p = 0.012, respectively). Göreme plants also had the fewest capitula per plant, suggesting that plants at this site were more stressed, presumably by drought, than at the other two sites. Stressing mature yellow starthistle plants by herbicides is known to reduce germinability of seeds (Carrithers et al., 1997), perhaps drought and insect root damage have similar effects. Among all the plants tested, 8 of the 10 plants with the lowest germination rate (0-38%) were infested, whereas 8 of 10 plants with the highest germination rate (97–100%) were not. The absence of an impact of weevil infestation on seed germination at Catalan may be related to the higher precipitation at this site, thus reducing drought stress. Also, a different yellow starthistle subspecies, C. solstitialis subsp. carneola, occurred at this site. Presumably weevil attack consumes root reserves and/or damages translocation, which ultimately reduces the resources available to develop seeds. No data were taken on number of seeds produced per plant because the capitula can begin to release them as soon as they senesce (Maddox, 1981), but it is possible that this is also affected by the weevil.

Prospects as a biological control agent

Clement et al. (1989) reported that *C. basicorne* was very abundant in Italy, but concluded that it was not host specific enough to be considered for use as a classical biological control agent (Clement, 1990). They tested only 5 adult females for ovipositional specificity: 2 under nochoice conditions and 5 under choice conditions. They found that *C. basicorne* did not oviposit on safflower when yellow starthistle was present, but that under no-choice conditions it oviposited on safflower. Our field results on the host specificity of the population at Pozanti, Turkey suggest that this insect may be safer than previously thought. Clement et al.'s (1989) observation of no oviposition on safflower under choice conditions is consistent with our field results of no larval attack on safflower or its close relative, distaff thistle. Although oviposition on safflower under no-choice laboratory conditions is a concern, the artificial conditions may be producing results that do not occur in the field (e.g., Cullen, 1990; Marohasy, 1998; Hill, 1999).

Clement et al. (1989) tested larval survival by transferring larvae from yellow starthistle into incisions in test plants and observed some subsequent survival and development in safflower, *Galactites tomentosa* (L.) and *Carduus pycnocephalus* L. (Italian thistle). This is a highly unnatural method that may greatly overestimate the ability of the insect to attack and develop on such plants in the field (Marohasy, 1998). Considering the abundance of the insect in Italy, Greece and Turkey, it warrants further study to determine if it is safe to introduce to North America.

We failed to measure any impact of C. basicorne on the size of yellow starthistle in the field; however, our sample sizes were very small and the study was completely uncontrolled. We did not measure the possible effects of other natural enemies on the plants, which could have masked impact caused by C. basicorne. Furthermore, tightly controlled laboratory studies conducted on root feeding insects of spotted knapweed (Centaurea maculosa Lam.) show how difficult it is to measure impact on plant size and fitness (Müller, 1989; Müller-Schärer, 1991). In addition, our study did not attempt to measure mortality of yellow starthistle plants, which might be affected by C. basicorne infestation. However, several scientists have observed serious damage to individual rosettes, even to the point of death of the plant (Clement et al., 1989). More sophisticated laboratory or garden experiments should be done to determine the amount of such impact, particularly in the presence of other stresses such as drought and plant competition. The impact that we observed on seed germination suggests that there may be an interaction with drought stress, which should be investigated further.

Acknowledgements

This study was financially supported by Biotechnology and Biological Control Agency, Rome, Italy and the USDA-ARS European Biological Control Laboratory, Montferrier sur Lez, France. Identifications of weevils were made with the assistance of B.A. Korotyaev, Zoological Institute, St. Petersburg, Russia.

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. Roma 22: 399–528.
- Balciunas, J., 1998. The future of biological control for yellow starthistle. In: M.S. Hoddle (ed), *Proceedings, California Conference on Biological Control: Inno*vations in Biological Control Research, 10–11 June 1998, University of California, Berkeley, CA. pp. 93–95.

- Balciunas, J., 2004. Are mono-specific agents necessarily safe? The need for pre-release assessment of probable impact of candidate biological control agents, with some examples. In: J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds), *Proceedings of the XI International Symposium on Biological Control of Weeds*, Canberra, Australia. CSIRO Entomology, Canberra, Australia. pp. 130–136.
- Bruckart, W.L. and F. Eskandari, 2002. Factors affecting germination of *Puccinia jaceae* var. solstitialis teliospores from yellow starthistle. *Phytopathology* 92: 355–360.
- Carrithers, V.F., D.R. Gaiser, C. Duncan and D. Horton, 1997. Seed germination of yellow starthistle and spotted knapweed after treatment with picloram or clopyralid. In: *Proceedings of the Western Society of Weed Science*. 50: 39–40.
- 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., M.A. Alonso-Zarazaga, T. Mimmocchi and M. Cristofaro, 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.
- Cullen, J.M., 1990. Current problems in host-specificity screening. In: E.S. Delfosse (ed), *Proceedings of the VII International Symposium of Biological Control of Weeds*, 6–11 March 1988, Rome, Italy. Ist. Sper. Patol. Veg. (MAF). pp. 27–36.
- Duncan, C.L., 2001. Knapweed management: another decade of change. In: L. Smith (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.
- Hill, R.L., 1999. Minimising uncertainty in support of no-choice tests. In: T.M. Withers, L. Barton Browne and J. Stanley (eds), *Host Specificity Testing in Australasia: Towards Improved Assays for Biological Control*. Papers from the Workshop on Introduction of Exotic Biocontrol Agents – Recommendations on Host Specificity Testing Procedures in Australasia, Brisbane, October 1998. Scientific Publishing, Indooroopilly, Queensland, Australia. pp. 1–10.
- Joley, D.B., D.M. Maddox, B.E. Mackey, S.E. Schoenig and K.A. Casanave, 1997. Effect of light and temperature on germination of dimorphic achenes of *Centaurea solstitialis* in California. *Can. J. Botany* 75: 2131–2139.
- Maddox, D.M., 1981. Introduction, phenology, and density of yellow starthistle in coastal, intercoastal, and central valley situations in California. U.S. Department of Agriculture, Agricultural Research Service, Agricultural Research Results. ARR-W-20, July 1981, USDA-ARS, Oakland, CA. 33 p.
- Maddox, D.M., A. Mayfield and N.H. Poritz, 1985. Distribution of yellow starthistle (*Centaurea solstitialis*) and Russian knapweed (*Centaurea repens*). Weed Sci. 33: 315–327.
- Marohasy, J., 1998. The design and interpretation of host-specificity tests for weed biological control with particular reference to insect behaviour. *Biocontr. News Inform.* 19(1): 12–20.
- Müller, H., 1989. Growth pattern of diploid and tetraploid spotted knapweed, *Centaurea maculosa* Lam. (Compositae), and effects of the root-mining moth *Agapeta zoegana* (L.) (Lep.: Cochylidae). *Weed Res.* 29: 103–111.
- Müller-Schärer, H., 1991. The impact of root herbivory as a function of plant density and competition: survival, growth and fecundity of *Centaurea maculosa* in field plots. *J. Appl. Ecol.* 28: 759–776.

- Piper, G.L., 2001. The biological control of Yellow Starthistle in the Western U.S.: four decades of progress. In: L. Smith (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., D.B. Joley and D.M. Woods, 1998. Impact of introduced insects for biological control of yellow starthistle. In: M.S. Hoddle (ed), *Innovation in Biological Control Research. California Conference on Biological Control*, June 10–11, 1998, University of California, Berkeley. pp. 88–92.
- Pitcairn, M.J., J.M. DiTomaso and V. Popescu, 2000. Integrating chemical and biological control methods for control of yellow starthistle. In, D.M. Woods (ed), *Biological Control Program Annual Summary, 1999.* California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, CA. pp. 58–61.
- Rees, N.E., P.C. Quimby, G.L. Piper, E.M. Coombs, C.E. Turner, N.R. Spencer and L.V. Knutson (eds), 1996. *Biological Control of Weeds in the West*. Western Society of Weed Science, USDA-ARS, Montana State University, Bozeman, MT. World Color Printers, Bozeman, MT.
- Rosenthal, S.S., G. Campobasso, L. Fornasari, R. Sobhian and C.E. Turner, 1992. Biological control of *Centaurea* spp. Chap. 29. In: L.F. James, J.O. Evans, M.H. Ralphs and R.D. Child (eds), *Noxious Range Weeds*. Westview Press, Boulder, Colorado. pp. 292–302.
- Rosenthal, S.S., T. Davarci, A. Ercis, B. Platts and S. Tait, 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., L.L. Larson and J.J. Jacobs, 1999. Yellow starthistle. In: R.L. Sheley and J.K. Petroff (eds), *Biology and management of noxious rangeland weeds*. Oregon State University Press, Corvallis. pp. 408–416.
- Smith, L., 2002. New Developments in the Biological Control of Invasive Weeds. In: Proceedings of the California Weed Science Society. 54: 159–165.
- Smith, L., 2004. Avoiding and exploiting trophic cascading: its role in the selection of weed biological control agents. In: J.M. Cullen, D.T. Briese, D.J. Kriticos, W.M. Lonsdale, L. Morin and J.K. Scott (eds), *Proceedings of the XI International Symposium on Biological Control of Weeds*, Canberra, Australia. CSIRO Entomology, Canberra, Australia. pp. 175–179.
- Uygur, F.N., 1985. Untersuchungen zu Art und Bedeutung der Verunkrautung in der Çukurova unterbesonderer Berücksichtingung von Cynodon dactylon (L.) Pers. und Sorghum halepense (L.) Pers. Plant Protection in Tropics and Subtropics, vol. 3(5), Josef Margraf Verlag, Aichtal.
- Uygur, S., L. Smith, F.N. Uygur, M. Cristofaro and J. Balciunas, 2004. Population densities of yellow starthistle (*Centaurea solstitialis*) in Turkey. *Weed Sci.* 52: 746–753.
- Wagenitz, G., 1975. 79. Centaurea L. In: P.H. Davis (ed), Flora of Turkey, vol. 5. Edinburgh University Press, Edinburgh. pp. 465–585.
- Wanat, M., 1995. Systematics and phylogeny of the tribe Ceratapiini (Coleoptera, Curculionoidea, Apionidae). *Genus, Int. J. Invertebrate Taxon.* (Suppl.).