Refining methods to improve pre-release risk assessment of prospective agents: the case of *Ceratapion basicorne*

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**Summary**

*Ceratapion basicorne* (Illiger) (Coleoptera: Apionidae) is a univoltine weevil native to Eurasia whose larvae develop in root crowns of yellow starthistle, *Centaurea solstitialis* L. (Asteraceae). This insect was ‘rejected’ as a prospective biological control agent about 15 years ago after preliminary evaluation of its host plant specificity showed that it could develop on safflower (Clement et al., 1989). However, it is known to attack very few plant species in the field and has never been reported from safflower. We conducted a series of no-choice, choice and field experiments to measure the risk that this insect would pose to non-target plants. Larval development occurred on nine species, including *Carthamus tinctorius* (safflower) and *Centaurea cyanus* (bachelor’s button and cornflower). These host plants are within a small monophyletic clade within the Centaureinae. Three years of field studies conducted in eastern Turkey, at three sites with natural populations of the insect, demonstrated that the weevil does not damage safflower plants despite attack rates of 48–98% on *C. solstitialis*. A combination of taxonomic analyses and a hierarchy of host-specificity experiments were required to determine that this insect would be safe to introduce.

**Keywords:** host plant specificity, risk assessment, field experiments, phylogeny.

**Introduction**

Determination of the host plant specificity of a prospective biological control agent plays a key role in the process of selecting and approving new biological control agents of weeds. Today, many consider host-specificity evaluation to be so routine that some professional journals refuse to publish such work. However, there still remains a great deal of ‘art’ to this work, which suggests room for improving the science. The goal of host-specificity evaluation is to predict the behaviour of agents after they are released into a new environment. To do this accurately is no small feat. Such predictions are usually based on the results of highly artificial laboratory experiments conducted under the constraints of working inside quarantine space. Furthermore, the tolerance of the public and regulatory agencies for risk of injury to non-target species has been decreasing. The combined effect is to reduce the probability of finding agents that can be approved for release. Can improving our methods help counter this trend?

There is a substantial literature reviewing methods of host-specificity evaluation, e.g. Clement and Cristofaro (1995), Withers et al. (1999), van Driesche (2000), Spafford Jacob and Briese (2005) and Sheppard et al. (2005). These reviews discuss many of the parameters that should be considered in the design of experiments. However, the choice of design should always reflect knowledge of the life history, ecology and behaviour of the agent. Thus, accurate testing requires customizing the experimental design for a particular agent on a particular target weed. A flow chart (Fig. 1) developed by Sheppard (1999) provides a starting point for choosing the most appropriate types of host-specificity experiments for a particular agent. However, given the multitude of experimental parameters that must be chosen for any experiment, these guidelines are only a beginning (Marohasy, 1998).

In this paper, we present an example of a prospective biological control agent that was once rejected based on preliminary experimental observations. However, because natural history observations suggested that the agent was more specific than what was indicated...
by the experimental results, we conducted more thorough experiments, which have shown that the insect is indeed suitable for introduction. Nevertheless, preliminary studies are a critical early step in the process of focusing research efforts to efficiently develop new biological control agents. We will discuss what kinds of experiments are most likely to improve the reliability of such preliminary studies.

Yellow starthistle, *Centaurea solstitialis* L. (Asteraceae: Cardueae), is an herbaceous winter annual that is adapted to a Mediterranean climate: mild wet winters and dry hot summers (Keil and Turner, 1993; Roché and Roché, 2000). It is native to Eurasia and was introduced to the west coast of the USA over 100 years ago (Maddox, 1981). Seeds usually germinate in the autumn after the onset of winter precipitation. Rosettes grow during winter and spring, bolt in May to June and flower continually until frost or lack of moisture kills the plant. The plant has been the target of classical biological control in the USA since the late 1960s, but despite the introduction of six seed-head-attacking insects, it is not yet under control over most of its range (Turner et al., 1995; Piper, 2001; Pitcairn et al., 2004, 2006). This suggests the need for agents that attack vegetative parts of the plant and *Ceratapion basicorne* (Il-liger) (Coleoptera: Apionidae) was considered a likely prospect (Zwölfer, 1965; Rosenthal et al., 1994).

*C. basicorne* is distributed throughout Europe and southwestern Asia, from Spain to Azerbaijan (Alonso-Zarazaga, 1990; Wanat, 1994). It commonly infests yellow starthistle in Turkey and Greece (Rosenthal et al., 1994). This species has primarily been reared from yellow starthistle, but there are also reports of rearing it from *Centaurea cyanus* L. (bachelor’s button and cornflower), *Centaurea depressa* Bieb. (which is very similar to *C. cyanus*), and in one case, *Cnicus benedictus* L. *C. benedictus* has recently been placed in the Jacea group of *Centaurea* (which includes yellow starthistle), based on phylogenetic analysis of DNA (Garcia-Jacas et al., 2000). Thus, the insect has only been reared from a few species of plants in the Jacea and Cyanus groups, within the genus *Centaurea*. Adults have been found resting on plants only in the Cardueae tribe, which includes *Centaurea*.

*C. basicorne* adults emerge from hibernation in the early spring and feed on yellow starthistle leaves (Clement et al., 1989; Smith and Drew, 2006). Females lay eggs in the leaves of rosettes from late March to early May (as observed in central Italy). Eggs hatch after about 10 days at room temperature, and first instar larvae mine in the leaf blade and down the petiole to the root crown. Larvae feed primarily in the root crown, complete development in about 2 months and pupate inside the plant. Adults emerge in June, feed on yellow...
starthistle leaves for a few days and then disappear. They are thought to aestivate and hibernate in secluded places, and adults have been found under tree bark in July (Hayat et al., 2002). Newly emerged females are in reproductive diapause, and although they mate, they are not able to lay eggs until completion of hibernation. In the spring, after feeding for 1–2 weeks, females lay a few eggs per day for 1–2 months before dying (Smith and Drew, 2006).

Clement et al. (1989) studied a population of C. basicorne in Italy. Because the species is univoltine and newly emerging adults do not oviposit, they used adults collected in the field on wild yellow starthistle plants. This greatly limited the number of adults that they could test (nine females). These adults were used in a few choice and no-choice feeding and oviposition experiments. Adults fed and oviposited on safflower under no-choice conditions but preferred yellow starthistle under choice conditions. Neonate larvae from these females were transferred to holes made in the central meristem of test plants. Larval development was observed on three non-target plants, including safflower. Because of these results, further evaluation of the insect was abandoned.

Considering that C. basicorne has never been reported as a pest of safflower and that field records indicate that it develops in only a few species of Centaurea, we decided to conduct a more thorough study of its host specificity.

Methods

No-choice oviposition

Individual mated females that had completed reproductive diapause were placed in a clear plastic tube (3.5 × 11 cm) mounted on an intact rosette leaf of a non-target plant species for 4 to 5 days (Smith, 2007). Each trial was preceded and followed by a positive control: placing the female with a cut yellow starthistle leaf for 2 to 3 days to determine if she could still oviposit. For each valid trial, we recorded adult feeding damage, oviposition and larval development. In general, we tested eight replicates per plant species in the tribe Cardueae and four in the more distantly related taxa. We doubled the number of replicates if there were any signs of larval development.

Lab choice

An ovipositing female was placed inside a wooden sleeve box (73 × 43 × 43 cm; length, width and height) containing cut leaves of four to five species of test plants for 5 days. Each species was represented by a cluster of two cut leaves held in a vial of water with a foam stopper. Yellow starthistle leaves were included as a positive control in each trial. Adult feeding damage and oviposition were recorded. The number of valid replicates ranged from four to 18 for each of six non-target species tested.

Field choice

We conducted experiments during 3 years (2002–2004) at three sites in eastern Turkey (Askale, Horasan, Çat) where C. basicorne was naturally abundant (Smith et al., 2006). We tested two accessions of yellow starthistle: ‘US’ (seed collected in Davis, California) and ‘Turkey’ (seed collected at the three Turkish sites) and two commercial safflower, C. tinctorius L., varieties CW1221 (linoleic, CalWest) and S317 (oleic, SeedTec). All test plants were transplanted into the field sites as soon as C. basicorne feeding damage was observed on wild yellow starthistle plants. Plants were harvested as soon as C. basicorne pupae were observed in wild yellow starthistle plants and were either dissected or individually bagged to allow adults to emerge. Adult insects were identified by either Dr. Enzo Colonnelli (University of Rome ‘La Sapienza’, Italy) or Dr. Boris Korotyaev (Russian Academy of Sciences, St. Petersburg). Larvae were preserved in 99% ethanol for DNA extraction.

Results and discussion

No-choice oviposition

In no-choice oviposition tests, C. basicorne females oviposited on 94% of plant species in the subtribe Centaureinae (Smith, 2007), including safflower and the native species Centaurea americana Nutt. and Centaurea rothrockii Greenm. (results for Centaureinae are in Fig. 2). There was no larval damage to plants outside the tribe Cardueae. The highest occurrence of insect larval development was observed on C. solstitialis and C. cyanus, but there was significant development on C. melitensis L. (localote), C. benedictus (blessed thistle), safflower and Crupina vulgaris Cass. (common crupina). These results corroborate the previous observation that C. basicorne can develop in safflower (Clement et al., 1989). However, neither safflower nor bachelor’s button appears to be normal hosts for C. basicorne because they do not form a rosette. Thus, when young larvae tunnel down a leaf on either of these plants, they cannot reach the root crown. Such larvae develop in the woody outer portion of the stem, not in the central pith. The relatively thin cortex provides a limited space for the insect and as the plant continues to grow, it sometimes crushes the pupa.

Lab choice

In the sleeve-box choice experiment, adult feeding and oviposition was significantly greater on yellow starthistle than on any of the six other non-target
species tested. About 72% of eggs were deposited on yellow starthistle, 20% on bachelor’s button, 5% on *C. melitensis*, 1% on *C. americana* and 1% on safflower (Fig. 3). These results indicate that *C. basicorne* females are more attracted to yellow starthistle than to bachelor’s button or any of these other non-target test plants. However, bachelor’s button and, to a lesser degree, safflower appear to be at risk of some attack, at least under these confined laboratory conditions.

**Field choice**

The infestation of the yellow starthistle test plants was between 48% and 92% (US and Turkish plants
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combined) at the three sites, indicating that there was a substantial infestation rate to challenge the safflower plants (Table 1). We reared 87, 145 and 297 individuals of *Ceratapion basicorne* from yellow starthistle at Horasan, Çat and Askale, respectively, but many were immature and others were in poor condition for taxonomic determination. All the adults that could be identified were *C. basicorne* (29, 30 and 92 from Horasan, Çat and Askale, respectively), except for two *C. orientale* (Gerstaecker). No safflower plants were infested by internal feeding insects at either Horasan or Çat. Thirty safflower plants were infested at Askale, but none of the insects reared from these plants were *C. basicorne*. We reared only *C. scalptum* (Mulsant and Rey), *C. orientale* and *C. onopordi* (Kirby) from these safflower plants. Subsequent identification of preserved larvae using molecular genetics has confirmed these results (Antonini *et al.*, 2008, this proceedings).

![Figure 3. Oviposition and adult feeding by *Ceratapion basicorne* during choice oviposition experiments in sleeve boxes (one female for 5 days exposed to cut leaves of four to five plant species at a time, always including yellow starthistle). Number of eggs was multiplied by ten for visibility on the same scale; FH Number of adult feeding holes, each approximately 1 mm²; error bars = SE.](image)

### Table 1. Infestation of root crowns or lower stems of test plants by apionid weevils (including larvae, pupae and adults) during 3 years at three field sites in eastern Turkey. *Ceratapion basicorne* was reared from yellow starthistle but not from safflower (Smith *et al.*, 2006).

<table>
<thead>
<tr>
<th>Site</th>
<th>Test plant</th>
<th>Proportion of plants infested (%)</th>
<th>No. safflower plants sampled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(US) (Turkey)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Yellow starthistle</td>
<td>Oleic</td>
<td>Linoleic</td>
</tr>
<tr>
<td>Horasan</td>
<td>83 b 100 a 0 c 0 c 45</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Çat</td>
<td>28 b 67 a 0 c 0 c 38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Askale</td>
<td>59 b 87 a 19 c 16 c 40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Çat</td>
<td>37 a 45 a 0 b 0 b 57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Askale</td>
<td>77 a 8 b 39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2004</td>
<td>98 a 0 b 250</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horasan</td>
<td>100 a 26 b 99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Values followed by the same letter in the same row are not significantly different (chi-square test, *P* < 0.01)

* Adults identified: 4 *C. scalptum*, 1 *C. orientale*, 2 *C. onopordi*

* Adults identified: 2 *C. scalptum*

* Three unidentifiable adults

* Adults identified: 8 *C. scalptum*, 2 *C. orientale*

### Conclusions

Although *C. basicorne* can develop on safflower in laboratory experiments, we never found evidence of attack during 3 years of field studies. The results of our no-choice, choice and field experiments concur with current theory that no-choice experiments tend to overestimate risk to non-target plants under field conditions (physiological vs ecological host range). To avoid rejecting prospective agents that might actually be suitably host-specific, more emphasis should be placed on observations in the natural environment (i.e.
natural history). This includes both examining non-target plants during foreign exploration and conducting relatively simple field experiments. Preliminary studies should focus on the most critical results for the particular agent. In this example, the critical issue was the attack on *C. americana*, *C. rothrockii* and safflower because they are the closest relatives to yellow starthistle deemed beneficial in North America. Preliminary experiments should focus on the developmental stage and conditions under which the agent chooses the target; in this case, females ovipositing on rosette leaves in early spring. Sheppard’s (1999) flowchart provides a good guide, but with the caveat that for species in which the female chooses the host plant, adult oviposition should be tested regardless of whether the agent is easy to rear in the lab. The modified flowchart (Fig. 4) shifts the emphasis away from whether or not the agent is easy to rear towards which stage or stages choose the host plant. Conducting larval no-choice experiments for a species whose larvae are not capable of choosing their host plant is more likely to mistakenly eliminate it than if the adults were tested.

![Type of Test Flowchart](image)

**Figure 4.** Revised decision tree for choosing the general type of host-specificity test. The emphasis is on what stages are capable of selecting the host. When suitable adults cannot be obtained by rearing or field collection, then field experiments should be used rather than larval transfer. Use of choice or no-choice experiments could be appropriate for any of these three types of tests.

References


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