Non-target impacts of *Aphthona nigriscutis*, a biological control agent for *Euphorbia esula* (leafy spurge), on a native plant *Euphorbia robusta*

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**Summary**

*Aphthona nigriscutis* Foudras, a biological control agent for *Euphorbia esula* L. (leafy spurge), has been established in Fremont County, Wyoming since 1992. Near one *A. nigriscutis* release site, a mixed stand of *E. esula* and a native plant, *Euphorbia robusta* Engelm., was discovered in 1998. During July of 1999, *A. nigriscutis* was observed feeding on both *E. esula* and *E. robusta*. A total of 31 *E. robusta* plants were located and marked on about 1.5 ha of land that had an *E. esula* ground-cover of over 50%. Eighty-seven percent of the *E. robusta* plants showed adult feeding damage. There was 36% mortality for plants with heavy feeding, 12% mortality for plants with light feeding, and no mortality for plants with no feeding. By August of 2002, the *E. esula* ground-cover had declined to less than 6% and the *E. robusta* had increased to 542 plants of which only 14 plants (2.6%) showed any feeding damage. For the four-year period, the *E. esula* ground-cover was inversely correlated to *E. robusta* density and positively correlated to *A. nigriscutis* feeding damage, showing that as *E. esula* density declines so does *Aphthona nigriscutis* feeding on *E. robusta*.

**Keywords**: *Aphthona*, density, *Euphorbia*, mortality, non-target impacts.

**Introduction**

A parcel of land 4.8 km (3 miles) south-west of Lander, Fremont County, Wyoming has been infested with *Euphorbia esula* (leafy spurge) for over 30 years. Owned for many years by the Majdic family, it was used mainly for livestock grazing during the summer. In 1995, the land was subdivided and today is only grazed occasionally by antelope and deer. In the late 1970s, the land was treated with herbicides on a regular basis, but a groundwater contamination in the area stopped the use of herbicides and the *E. esula* reestablished at the site and spread into new areas. *Aphthona nigriscutis* was released on the Christiansen property, just west of the site, in 1990 and the insects established well. Many redistribution releases were made between 1993 and 1996 on the Majdic land from those earlier-established populations. These sites were monitored annually to assess the establishment of the bioagents. There was a strong contrast between the Majdic land and the Christiansen properties where the insects were prospering and impacting the spurge in a dramatic way. *Euphorbia esula* ground-cover fell from the 50–70% range to 5–10% by 1998 at Christiansen’s, but at Majdic’s the spurge continued to spread. At Christiansen’s, it was possible to sweep over 100 beetles in one swing of the net in hot spots, while just 0.8 km (0.5 miles) away, we seldom averaged one beetle per sweep. It was while monitoring *A. nigriscutis* on the Majdic property that we observed a small colony of a native spurge, *Euphorbia robusta* Engelm.

Early in the *E. esula* biocontrol effort, *E. robusta* had been identified as a species of interest because it is closely related to *E. esula*, both belonging to the subgenus *Esula*, is a perennial which could support the long life cycle of *Aphthona* beetles and is sympatric with *E. esula* (Pemberton 1985). Since the late 1980s, Fremont County Weed and Pest had supplied the
United States Department of Agriculture–Agricultural Research Service with E. robusta plant material. Typically, E. robusta is found sparsely growing on rocky, wind-swept ridges where the tools of extraction were pry bars rather than shovels. We had to force the rock layers apart to follow the roots and it took hours to find and collect just a few plants. The Majdic site was refreshingly different with the E. robusta growing in deep soil and the digging was easy. We returned often to this site to get more plant material.

In 1997 and 1998, we observed E. robusta plants with feeding scars on the leaves and occasionally saw A. nigriscutis feeding on the plants. A few of these E. robusta plants were photographed and marked with pin flags for future reference. The next year, the marked plants were gone. Actually, this feeding activity by A. nigriscutis could be anticipated. An examination of the petitions to introduce Aphthona flava (Pemberton & Rees 1990), A. cyparissiae (Pemberton 1986) and A. czwalinae (Pemberton 1987) into the United States showed that acceptance of E. robusta was almost as large as for E. esula. Euphorbia robusta was not used for host-testing A. nigriscutis (Pemberton 1989).

Early host-plant testing was designed to demonstrate that new biocontrol of weeds agents would not attack economically valuable crop species. In recent years, concern has shifted toward the impacts, both direct and indirect, that biological agents might cause to native species. Rhinocyllus conicus, a biological control agent for Carduus nutans L. (musk thistle) has been found to impact a wide variety of native thistles, some endangered (Gassmann & Louda 2001). Increased concern has stimulated a call for greater scrutiny of new biological control agents, more thorough study of the target species before release, and post-release tracking of host range under field conditions (Waage 2001). It is in the spirit of post release evaluation that these data are offered.

Dr Peter Harris (Ag Canada, Lethbridge, Alberta) suggested that the feeding we were seeing on E. robusta was incidental and would probably be inversely related to distance from the host. We set up an experiment to evaluate the impact of Aphthona nigriscutis feeding on E. robusta at a location where there was a gradient of E. esula density and distribution associated with E. robusta.

**Materials and methods**

Between May and August of 1999, the Majdic site was visited several times and E. robusta plants were located, marked and photographed. The soils are red loam, 50 to 150 cm deep. The site slopes 10 to 20 degrees to the north-east. Average annual precipitation is 33 cm, although over the last five years the rainfall has been 50 to 75 percent of normal.

Each plant was marked with a numbered wooden stake driven into the ground 60 cm north of the plant to avoid shading the plant or injuring the root. The latitude and longitude of each plant was determined with a Garmin IIIa global positioning system (GPS) device. This device is not capable of differential correction for atmospheric errors, but is generally accurate to within 3 m most of the time since selective availability was switched off in the United States.

A study site boundary was established using three Aphthona nigriscutis release locations marked with steel posts on the west and a road on the east with parallel north and south lines to enclose a rectangle of about 2 ha where 36 E. robusta plants had been marked. Euphorbia esula was heaviest along the western boundary. The E. robusta was roughly distributed in two groups toward either side of the site, plants 1–20 on the west where the E. esula was heaviest, and 26 to 36 on the east where the E. esula was lighter.

The degree of adult feeding was determined by visual examination of the Euphorbia robusta plants. If more than half of the leaves on more than half of the stems showed feeding activity, it was categorized as “heavy”. Feeding on less than half of the stems and leaves down to 25% was “medium” damage. If the feeding was on less than 25% of the stems and leaves, the damage was “light”. If there was no discernable feeding injury zero damage was recorded.

The distance from each E. robusta plant to the nearest E. esula plant was measured up to 3 m in 1 m increments. Experience suggested that the beetles, although capable of flight, simply did not move very far if host plants were abundant. A few years after release, there would often be distinct “craters” in the E. esula, centered on the point of release. The craters were seldom more than 3 m across until the E. esula really began to die out at the point of release. Additionally, there were few E. robusta plants that were not within 10 m of at least one E. esula plant. At other E. robusta sites in the area, where the closest E. esula was hundreds of metres away, there was no feeding damage to the plants from A. nigriscutis. Preliminary observations indicated that the E. robusta plants with feeding damage were growing within jumping distance of the E. esula.

In 2000, the E. esula population was in dramatic decline and there were many new E. robusta plants which were marked and evaluated. In addition to assessing feeding damage on E. robusta and measuring the distance to the nearest E. esula, we counted the number of E. esula plants inside a 1 m square frame centred on each E. robusta plant. Euphorbia esula density for the whole site was taken by walking in a roughly grid pattern back and forth across the site guided by the GPS unit. The grid consisted of a series of transects roughly 15 m apart with waypoints along them every 15 m. A 1 m square frame was dropped at each waypoint and E. esula and E. robusta stems were counted. The locations for all E. robusta were plotted using geographical information system (GIS) software and compared to the population density map developed from the grid data.
In 2001, we re-sampled the marked *E. robusta* locations and identified and marked new plants. We also established a permanent grid across the site on 15 m intervals. At each intersection, we measured *E. esula* and *E. robusta* density with a 1 m square frame and took ground-cover readings inside the frame with a point frame (Levy & Madden 1933) recording the first contact only for each wire in the frame. As a result of mapping the density of *E. esula*, the boundaries of the study site were altered to eliminate most of the north-western quadrats where no *E. esula* was present. This reduced the site to 1.5 ha and ensured that *E. esula* was present within 15 m of each grid point. Five *Euphorbia robusta* plants were now outside the study area, reducing the number to 31 plants marked during 1999. On 25 May 2001, 12 *E. robusta* plants, 8 from within the study area, were dug up to examine the roots for presence of *A. nigriscutis* larvae and feeding damage. In 2002, the same data were collected as in 2001.

**Results and discussion**

Upon returning to the site on 7 June 2000, we observed a dramatic change. The *E. esula* that was so dominant the year before was nearly gone. Visually, the Majdic site resembled other sites where the ground-cover had been reduced to less than 10% by *A. nigriscutis* feeding. This was unexpected as the *A. nigriscutis* numbers were always low across the site. There had never been craters at the points of release, and the *E. esula* had been expanding and becoming denser every year. Yet, the *E. esula* was no longer a major component of the site. Four of the 31 marked *E. robusta* plants were gone, but many new plants were observed. Several trips were made to the site to locate new plants, resulting in a total of 163 new *E. robusta* plants marked during 2000. A real increase of *E. robusta* had taken place at the same time the *E. esula* had declined.

It is not possible to explain adult feeding damage to *E. robusta* as a function of distance to *E. esula* plants or *A. nigriscutis* population centres (Table 1): depending on the year, the correlation coefficient was positive or negative.

In 2000, the correlation coefficient between *E. esula* and *E. robusta* densities was 0.01, –0.25 in 2001, and –0.51 in 2002, perhaps suggesting that, over time, feeding damage on *E. robusta* became more common where *E. esula* densities were lower. Moreover, the density data do not reflect the decrease in size of individual *E. esula* stems. In 1999, they were large, 25 to 50 cm tall and heavily branched, while in later years they were mostly less than 20 cm tall, unbranched and non-flowering. The decline in *E. esula* ground-cover was a result of reduced stem size and vigour rather than a reduction in stem density.

*Aphthona nigriscutis* adult feeding does appear to have a have a relationship to *E. robusta* mortality. Mortality in 2002 for each level of feeding is listed by year in Table 2. The data reflect a higher mortality rate for plants with heavy and medium feeding compared to plants with light or no feeding.

**Table 1.** *Euphorbia robusta* adult feeding damage correlation.

<table>
<thead>
<tr>
<th>Variable</th>
<th>1999</th>
<th>2000</th>
<th>2001</th>
<th>2002</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distance to 1994</td>
<td>–0.56</td>
<td>–0.18</td>
<td>–0.11</td>
<td>+0.06</td>
</tr>
<tr>
<td><em>Aphthona nigriscutis</em> release from <em>E. robusta</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distance to nearest</td>
<td>–0.43</td>
<td>–0.01</td>
<td>+0.21</td>
<td>+0.20</td>
</tr>
<tr>
<td><em>Euphorbia esula</em> plant from <em>E. robusta</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density of <em>E. esula</em> at each <em>E. robusta</em></td>
<td>No data</td>
<td>0.01</td>
<td>–0.25</td>
<td>–0.51</td>
</tr>
</tbody>
</table>

**Table 2.** *Euphorbia robusta* 2002 mortality as a function of feeding damage by adult *Aphthona nigriscutis*.

<table>
<thead>
<tr>
<th>Year</th>
<th>Feeding</th>
<th>No. of plants</th>
<th>Mortality</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1999</td>
<td>Heavy</td>
<td>11</td>
<td>4</td>
<td>36</td>
</tr>
<tr>
<td>Light</td>
<td></td>
<td>16</td>
<td>2</td>
<td>13</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2000</td>
<td>Medium</td>
<td>8</td>
<td>3</td>
<td>38</td>
</tr>
<tr>
<td>Light</td>
<td></td>
<td>85</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>59</td>
<td>8</td>
<td>14</td>
</tr>
<tr>
<td>2001</td>
<td>Light</td>
<td>19</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>221</td>
<td>20</td>
<td>9</td>
</tr>
</tbody>
</table>

In retrospect, it is questionable that adult feeding could actually kill these perennial plants. Heavier adult feeding might have been an indicator of oviposition and larvae attacking the roots. Since our focus in 1999 was on tracking the long-term impacts of *A. nigriscutis* on individual *E. robusta* plants, no attempt was made at the time to dig up the attacked plants to determine if *A. nigriscutis* larvae were present on the roots. Twelve plants each of *E. robusta* and *E. esula* were dug up on 25 May 2001 and the roots were examined for the presence of *Aphthona nigriscutis* larvae. None was found and the roots looked healthy and intact. Larvae were found on *E. esula* roots along the western edge of the research site in the fall of 2000, but none was found on *E. esula* inside the site boundaries at that time either.

In the host-specificity testing, the host range of *A. nigriscutis* was found to be the subgenus *Esula* in the genus *Euphorbia*. *Euphorbia robusta* is in the subgenus *Esula* (Pemberton 1985) and was thought to be an acceptable host even though, as indicated above, it was not tested. The *E. robusta* plants in culture were consumed during tests of the three *Aphthona* (*A. flava*, *A. cyparissiae* and *A. czwalinae*), all of which accepted
E. robusta as a laboratory host. Pemberton’s conclusion in the petition to release A. nigriscutis was that its level of specificity was similar to and perhaps somewhat narrower than the other tested Aphytisina species. The decision to petition for release of A. nigriscutis and other Aphytisina species in the western United States was made because research indicated that they could use only two native Euphorbia species (E. robusta and E. incisa) that were perennial and partly sympatric with leafy spurge. This level of risk was considered to be modest when one considers that there are 112 native Euphorbia (sensu lato) in the US that could be potential hosts of Euphorbia feeding insects (Pemberton 1985), and the leafy spurge problem was severe in the United States. Because A. nigriscutis larvae were not sampled when the population of the beetle was high, it is not clear whether the observed impact of A. nigriscutis on E. robusta was due to adult feeding alone. Adult feeding might be correlated with oviposition and thus larval damage to the plant’s root system (R.W. Pemberton 2003, pers. comm.).

No ground-cover measurements were made in 1999 or 2000, but the appearance of the Majdic site was very similar to other sites in the area where ground-cover and density data have been collected for many years. Euphorbia esula at the Majdic site was estimated to contribute between 10 and 90% to the ground-cover east to west across the site, and conservatively would have averaged 50% in 1999, dropping to less than 10% in 2000. Ground-cover of E. esula was 6.3% in 2001 and 5.7% 2002.

As the E. robusta population increased, the number of plants with feeding damage first increased, then decreased in real numbers. Percentage of plants fed upon declined annually over the period (Fig. 1). When compared to the change in E. esula ground-cover over the same period, it appears that there is a competitive impact from the E. esula at the site and as the E. esula declines the E. robusta population takes advantage of the open space. The feeding damage lags a year behind the ground-cover decline (Fig. 1) suggesting that the adult feeding by Aphytisina nigriscutis in 2000 is more closely related to the E. esula ground-cover in 1999 than in 2000.

Even though host-specificity testing predicts that E. robusta should be a good host for the Aphytisina beetles (Pemberton 1986, 1987, 1989), observations at the Majdic site indicate that Aphytisina nigriscutis only fed heavily on E. robusta when its primary host E. esula was plentiful and able to support the biological control agent in large numbers. When the E. esula ground-cover declined, feeding by A. nigriscutis on E. robusta declined as well while E. robusta plant numbers increased (Fig. 1). Even with a 17-fold increase, E. robusta still did not show up in the ground-cover measurements and did not replace the habitat and food source that the Aphytisina nigriscutis had enjoyed when the E. esula ground-cover component averaged 50%.

While it is not known if A. nigriscutis can complete its life cycle on E. robusta in the field, the strong correlation between the decline in E. esula with the decline in beetle damage to E. robusta suggests that we observed an adult feeding effect. If E. robusta was a good developmental host for the beetle, then it would have been unlikely for the adult feeding to decline and the density of the E. robusta plants to increase with E. esula decline.

This is in keeping with observations made in 1998 and 2001 at Camel’s Hump, west of Medora, North Dakota. In 1998, this site was heavily infested with E. esula which was supporting an epidemic population of Aphytisina nigriscutis and A. lacertosa. The insects were super-abundant and millions were collected for redistribution in just a few hours. Every blade of grass had notches in the leaves and the insects could be observed feeding on every plant species present. Upon returning to the site in 2001, E. esula was nearly gone. Although a number of people attempted to collect Aphytisina for redistribution, the populations were too low. At that time, we observed no Aphytisina sp. feeding activity on any species other than E. esula. Waage (2001) reports two parallel occurrences where weed biocontrol agents attacked non-target species during the epidemic period of agent development when the host plants were abundant. A lace bug, Teleonea scrupulosa, released against Lantana camara in sesame crops in Uganda attacked the crop at peak populations (Davies & Greathead 1967), and a leaf beetle, Zygogrymma bicolorata, released against Parthenium hysterothorum, attacked sunflowers in India during population explosions (Jayanth et al. 1993). In both cases, a decline in host-plant numbers resulted in a decline in the biological control agent and the non-target feeding stopped (Davies & Greathead 1967, Jayanth et al. 1993).

The Aphytisina beetles are proving to be excellent biological control agents that severely impact their
target weed, *Euphorbia esula*, in the United States (Nowierski & Pemberton 2002). Their reputation can only be enhanced by these recently observed modest transient effects on their most likely non-target host, *Euphorbia robusta* (R.W. Pemberton 2003, pers. comm.).

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**References**


