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SHORT COMMUNICATION

Non-target plant use by a weed biocontrol agent in idaho: host expansion or opportunistic behavior?

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Larinus curtus Hochhuth (Coleoptera: Curculionidae) was first introduced into the western United States from Greece for the biological control of yellow starthistle (YST), *Centaurea solstitialis* L., in 1992. The discovery of *L. curtus* adults in the open flowerheads of safflower (SF), *Carthamus tinctorius* L., near Lewiston, Idaho in 2007 suggested this weevil might be expanding its host range to include a non-target crop species closely related to YST. In 2008 field plots near the 2007 observation site, 92 *L. curtus* adults fed in open SF flowerheads (pollen feeding and minor feeding on corolla tubes). No eggs were found in the ovarioles of 19 pollen-feeding females. No eggs, larvae, or evidence of larval feeding were detected in 39 tagged SF capitula, and no adults emerged from approximately 7,135 post-flowering SF capitula. These collective results are not indicative of an expanding developmental host-range of *L. curtus*. Also, they are consistent with pre-release host-specificity test results.

Keywords: *Larinus curtus*; yellow starthistle; weed biocontrol; safflower; non-target host plant; host-specificity

The release of exotic biological weed control agents into new regions is fraught with potential hazards, not the least of which is non-target host-usage by released agents. Although predicting agent host-usage in a new region is never easy, pre-release host-specificity data has accurately predicted the host use patterns of the great majority of released agents (Pemberton 2000; Willis, Kilby, McMaster, Cullen, and Groves 2003). A variety of non-target test plants (related crop, ornamental, native, and endangered species) are included in pre-release testing. If any of these plants are found to be acceptable (feeding and oviposition preference of adults) and/or suitable (growth, survival, reproduction of immatures and adults) hosts for a candidate agent during testing (laboratory no-choice and multiple-choice tests, field cage and open-field tests), an agent's release into a new region may be delayed or canceled (e.g., Clement and Cristofaro 1995; Harris and McEvoy 1995; Briese 2005). However, despite host-range testing, there have been 'surprises in host-usage by released biocontrol agents' (Briese 2005 and references therein).

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Yellow starthistle (YST), *Centaurea solstitialis* L., is an invasive Cardueae thistle from the eastern Mediterranean Basin and Western Asia that infests several million hectares in the US, mostly in California, Oregon, Washington, and Idaho (Wilson, Jette, Connett, and McCaffrey 2003). This annual species is a target for biological control because chemical control is not feasible or environmentally sound. By the late 20th century, six seed-feeding insect species had become established as biocontrol agents in the US, including *Larinus curtus* Hochhuth (Coleoptera: Curculionidae) (Pitcairn, Joley, and Woods 1998). YST is the only recorded host of *L. curtus* in Palearctic environments (Zwölfer, Frick, and Andres 1971; Zwölfer and Brandl 1989). This weevil is univoltine and was first released in the US in 1992 to complement seed destruction by established agents. Adults are good flyers and overwinter among debris on the soil surface (Sobhian and Fornasari 1994). Adult females must feed on the pollen of YST to develop ovaries and produce eggs, which are oviposited within and between the florets of YST flowerheads (Sobhian and Fornasari 1994; Fornasari and Turner 1995).

The feeding of *L. curtus* adults on the flowers of test plants, including safflower (SF), *Carthamus tinctorius* L., in pre-release no-choice laboratory tests was documented by Sobhian and Zwölfer (1985), Fornasari and Turner (1995), and Clement and Mimmochi (unpublished, 1985 Annual Report, USDA-ARS, BCWL-Europe, Rome, Italy). These researchers used phrases like 'very little', 'only light', 'slight, moderate, and extensive', and 'damage to the stamens of safflower heads' to describe the type and extent of adult feeding on SF flower parts. Only Fornasari and Turner (1995) provided a quantitative assessment of adult feeding on SF flowers in host-specificity tests, with recorded damage ratings of 0 (no feeding) and 1 (very little feeding, no effect on flowerhead development) on a 0 to 5 scale. Importantly, Fornasari and Turner (1995) and Clement and Mimmochi (unpublished) found no weevil eggs and larvae and no larval feeding damage on SF capitula (flowerhead and seed formation stages) in their laboratory no-choice tests. They also did not find weevil eggs in the ovarioles of dissected females that had fed on SF flowers. These laboratory results, plus data from open-field tests in Greece (Groppe, Sobhian, and Kashefi 1990; Clement and Sobhian 1991), confirmed the weevil's high host-specificity to YST and hence no risk of SF being used as a developmental host plant in the US. Any indication of a potential agent's capacity to develop in SF, a crop plant in the tribe Cardueae that is related to YST and commercially important in California (CASS 2000), would likely have disqualified it for introduction into the US.

When adult weevils were discovered in open SF flowerheads in a research plot near Mann Lake, ~3 km east of Lewiston, Idaho, USA (46°22'9"N, 116°51'41"W) in July 2007 (L.J. Smith, unpublished observations), and were identified as *L. curtus* (Figure 1), we asked: 'Is this host-plant expansion by a released host-specific biocontrol agent and, if so, is the field colonization of SF by the YST agent something that could have been foreseen from the results of pre-release host-range tests?' Pinned specimens of *L. curtus* from SF flowerheads have been deposited in the William F. Barr Entomology Museum, University of Idaho, Moscow, and the United States National Museum at the Smithsonian, Washington, D.C.

Additionally, we considered the possibility that *L. curtus* might pose a risk to SF production as a new seed pest in eastern Washington and northern Idaho. In this region and other semiarid areas in the western US, winter-hardy SF germplasm is



Figure 1. Two *Larinus curtus* adults in the open flowerhead of a safflower plant.

being evaluated as a potential new crop (Johnson and Li 2008). This crop is mainly grown as a spring crop for its high seed oil content (Li and Mündel 1996), with major US production areas in the inter-mountain west, eastern Montana, and the Sacramento Valley in California.

In this communication we report on: counts of *L. curtus* adults in SF flowerheads in research plots; the sex ratio of adults in flowerheads; dissections of females in flowerheads for evidence of pollen feeding and egg development; dissections of post-flowering SF capitula for hatched eggs and larvae of *L. curtus*; and emergence of new generation adults from all harvested SF capitula (seed formation stage) in research plots.

This extemporaneous study spanned the entire 2008 flowering period of SF plants in experimental plots (covering 0.2 ha) within a commercial wheat field. These plots were established in September 2007 by agronomists for the sole purpose of evaluating crop species and cultivars for possible commercial production in Washington and Idaho. There were eight crop entries in small plots (each 1.5×6.1 m) within this study area: spring barley, *Hordeum vulgare* L.; hard red spring wheat and winter wheat, *Triticum aestivum* L.; winter lentils, *Lens culinaris* Medikus; chickpea, *Cicer arietinum* L.; and three SF accessions (WSRC01 and KN-144 [winter types]; Gila [spring type]). Each entry was replicated four times in randomized complete blocks. The spacing between plots was 2 m. YST plants were not found within a 2-km radius of the plots during surveys on 2 and 10 July 2008, although flowering YST plants were within the city limits of Lewiston, Idaho and fairly abundant 9–12 km south of the plots on 10 July 2008. A distance of 0.4 km separated the 2008 plots from the 2007 site where *L. curtus* adults were observed in SF flowerheads.

Nine censuses coincided with the 1–30 July 2008 flowering period (Figure 2). On each date, 1–2 people counted the number of adults in all flowerheads on all SF plants in the plots. Because 1–2 h censuses were done at different times (between 09:00 and 13:00 h each day) and under variable temperatures (21–31°C), the counts reflect general colonization patterns and densities. Thirty-nine flowerheads with adults were tagged (8, 11, 8, 11 tagged on 10, 15, 16, and 18 July, respectively, and one tagged on 21 July), then collected as capitula in the seed formation stage on

12 August. On 16 and 17 August, these capitula were examined for evidence of adult flower feeding and dissected to record the presence of hatched eggs and larvae, or evidence that larvae had fed in the capitula and damaged seed. In addition, 10 flowerheads were collected on two dates (15 and 21 July) and taken to a laboratory where they were immediately examined for eggs and adult flower feeding. Thirty adults were collected from flowerheads on 21 July and dissected on the same date under a stereomicroscope to record their sex and any evidence of pollen feeding and egg development by females. Finally, all capitula ($\sim 7,135$) in the seed formation stage were harvested on 12 August and placed in clear thermoplastic cages ($51 \times 51 \times 51$ cm) in a glasshouse (natural light, $20\text{--}34^\circ\text{C}$) until 29 September to record any emergence of adult *L. curtus*. Since the egg to adult developmental period for *L. curtus* in YST is ~ 28 days at 23°C (Sobhian and Fornasari 1994) and few adults hibernate in mature YST capitula (Groppe et al. 1990), we reasoned that any adults from eggs laid between mid and late July would emerge from post-flowering SF capitula before late September. Indeed, previous dissections of harvested YST capitula in the seed formation stage yielded *L. curtus* larvae and pupae, with adults also emerging from capitula in this stage (Clement and Sobhian 1991; Sobhian and Fornasari 1994; Clement and Mimmocchi, unpublished).

Ninety-two *L. curtus* adults were found in 79 yellow and yellow/orange colored SF flowerheads, with 51% these individuals counted on 18 and 21 July (Figures 1 and 2). One adult was found in 67 of the flowerheads and 11 harbored two adults (including a mating pair). Additionally, three adults were found in a flowerhead. Adults fed on pollen and nibbled on corolla tubes of the flowers (damage rating of 1 on the 0–5 scale of Fornasari and Turner [1995]) of 10 flowerheads collected and carefully examined on 15 and 21 July. The sex ratio of adults collected on 21 July was $19\text{♀}:11\text{♂}$. These 19 dissected females had fed on SF pollen but their ovarioles were devoid of eggs.

Eggs, larvae, or evidence of larval feeding were not detected in the 39 tagged and dissected SF capitula. Of these capitula, 36 contained full complements of mature seed, whereas three were devoid of seed but exhibited no evidence of larval feeding.

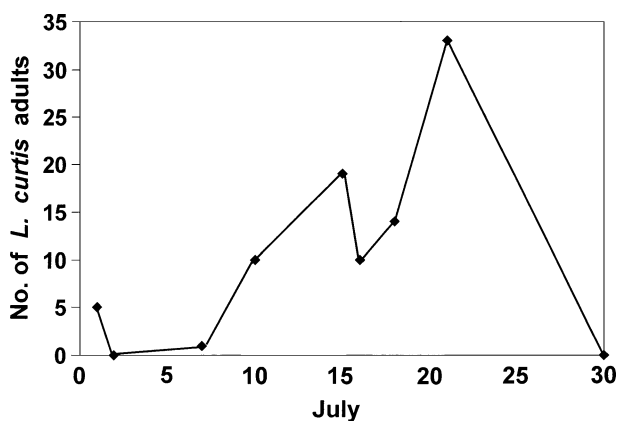


Figure 2. Counts and seasonal occurrence of *Larinus curtus* adults in the flowerheads of safflower plants, northern Idaho, July 2008.

Because there was evidence of adult flower feeding (presence of fecal material) on the apex of these seed-free capitula, we cannot rule out the possibility that adult feeding interfered with pollination and subsequent seed development of these capitula. However, the production of seed-free capitula by SF plants has been observed in areas of eastern Washington where *L. curtus* has not been found in flowerheads (R.C. Johnson, unpublished observations).

We found no evidence that the field occupancy of SF flowerheads by adult *L. curtus* in northern Idaho portends an expanding developmental host-range of a weed biocontrol agent in a new region. Our dissections and close examinations revealed no egg development by pollen-feeding females and no egg laying in SF flowerheads. In addition, no adults emerged from an estimated 7,135 SF capitula. Thus, SF flowerheads are not suitable substrates for *L. curtus* oviposition and development, as predicted by pre-release no-choice laboratory tests. The collective results indicate that *L. curtus* is unlikely to become a new seed pest of cultivated SF in northern Idaho and eastern Washington. However, one must consider the possibility that adult *L. curtus* could alight in SF flowerheads in California and feed on flowers. Low numbers of another YST biocontrol agent (*Chaetorellia succinea* Hering (Diptera: Tephritidae)) have been associated with SF capitula in California (Balciunas and Villegas 2001).

We suggest that results from pre-release no-choice laboratory tests (Sobhian and Zwölfer 1985; Fornasari and Turner 1995; Clement and Mimmocchi, unpublished) and an open-field test in 1988 in Greece (Groppe et al. 1990) are consistent with our field results of adult *L. curtus* finding SF plants in the wild and feeding on the flowers (pollen and corolla tubes) of SF flowerheads. Although only two adults located and settled in SF flowerheads in a 1988 open-field test in Greece, which was set up as a choice test, this observation provided evidence of the strong sensory and host selection capacities of adult *L. curtus*. Therefore, *L. curtus* adults are behaviorally equipped to locate SF in the wild. Our study could be viewed as an extemporaneous no-choice open-field test involving a biocontrol agent released into the invaded range of its host plant. Historically, open-field tests have not been conducted as no-choice tests (Van Driesche and Murray 2004), although a 'no-choice' component is part of the 'two-phase open-field test method' to evaluate the host-specificity of candidate agents (Briese 1999; Briese, Zapater, Andorno, and Perez-Camargo 2002).

Given our findings, we are left wondering why high numbers of adult *L. curtus* dispersed at least 2 km and settled in flowerheads on SF plants surrounded by wheat. Perhaps the adults were induced to disperse by a reduction of suitable oviposition sites (flowerheads) in nearby YST infestations. As suggested by Winston (2007), a reduction of *L. curtus* oviposition sites in northern Idaho could be due to the attack of pre-flowering capitula (mature buds) by *Eustenopus villosus* Boheman (Coleoptera: Curculionidae) and other biocontrol agents. In northern Idaho, *E. villosus* is the dominant YST biocontrol agent and *L. curtus* occurs in low numbers (Winston 2007 and references therein). We suggest that SF plants in northern Idaho were found by dispersing *L. curtus* adults that opportunistically settled in the flowerheads of these plants and fed on the flowers.

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