Frequency-dependent host species use by a candidate biological control insect within its native European range

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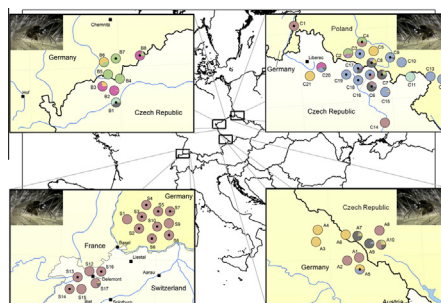[b] Agriculture and Agri-Food Canada, Lethbridge Research Centre, 5403 1 Ave South, Lethbridge, Alberta T1J 4B1, Canada
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HIGHLIGHTS

- Broad, native range surveys of candidate agent hosts can inform biocontrol programs.
- We surveyed host use on 16 potential host plant species in four geographic regions.
- We found 5 of 16 species to be ecological hosts, but host use to differ regionally.
- Even when hosts co-occurred, only one host species was used, often the most abundant.
- Frequency-dependent host use may facilitate control of invasive species complexes.

GRAPHICAL ABSTRACT

ABSTRACT

When a classical biological control program targets multiple congeneric invasive species, comprehensive native range host-use surveys may be effective in accurately predicting which species would most likely be controlled by a candidate agent in its introduced range. At least fifteen species of *Pilosella* hawkweeds invade North America, where they aggressively out-compete native vegetation in areas of economic and ecological importance, and continue to spread. A promising candidate agent for multiple *Pilosella* hawkweeds is the leaf-gall wasp *Aulacidea pilosellae*. Previous surveys revealing inconclusive patterns of ecological host-use across the native range led us to hypothesize that *A. pilosellae* host species use may follow a rank-preference order. An extensive survey conducted across the native range of *A. pilosellae*, assessing all potential host species in four distinct geographic regions, found that host species use varied dramatically in different parts of the survey area. The hypothesized pattern of fixed host-preference ranking by the gall wasp within mixed host-species patches was not supported; instead, when multiple host species co-occurred, a frequency-dependent pattern of host-species use among sites was revealed. Specifically, we found that when multiple putative host species co-occurred, the most abundant host species present within mixed species stands was the only species used at a site significantly more often than expected by chance. We further found that only one species was ever used as a host at a given site, regardless of the number of potential host species available. This first known instance of frequency-dependent host-species use reported for an insect weed biological control agent suggests that similar native range assessments for other multiple congeneric host – insect systems may be universally valuable.

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1. Introduction

Accurate assessments of the host range of candidate biological control agents are pivotal to the continued success of classical biological control, especially in light of recent concerns and controversy regarding the potential ecological risks of foreign agent introductions (Louda et al., 2003; Thomas and Reid, 2007). To assess the potential risk of candidate agents to non-target species ahead of release outside of the native range, the agents undergo extensive host specificity testing to determine both the acceptability (i.e. agent's preference for) and suitability (i.e. ability to develop on) of potential host species (McFadyen, 1998; van Klinken, 2000). The results from these standard tests can then be used to predict the 'fundamental host range' (i.e. the complete set of host species which the agent can accept and complete development on), and of ultimate concern to any classical biological control program, the 'realized host range', which is the actual field (ecological) expression of the fundamental host range (van Klinken, 2000). Various controlled experimental methods are used in host specificity testing today (McFadyen, 1998; Schaffner, 2001). These include no-choice laboratory or field experiments, increasingly together with molecular (phylogenetic) analyses of insects and/or plants (Briese, 2005; Gaskin et al., 2011) to predict fundamental host range, and multiple-choice laboratory or natural/semi-natural field experiments to better predict the realized host range. Continual improvements in both the methodology (Heard and van Klinken, 1998) and interpretation of test results (Schaffner, 2001); however, are ongoing in the quest for improved accuracy in predicting attack and potential unintended impacts on native, endangered or economically important species post field release of agents (Schaffner, 2001; Sheppard et al., 2005).

Although it is understood that factors both internal (e.g. ability to find and both physiologically and physically use hosts) and external (e.g. availability, quality and distribution of hosts in the field) to a released biological control insect can affect the expression of its fundamental host range once in a new field setting (van Klinken, 2000), ascertaining the role of environmental heterogeneity pre-release is challenging. As is well-recognized, controlled (laboratory, greenhouse or field-cage) assessments of the fundamental host range often suggest significantly broader host use compared to assessments of the realized host range (Balciunas et al., 1996; Cullen, 1989; Haye et al., 2005; Morehead and Feener, 2000; Schaffner, 2001; Zwölfer and Harris, 1971). However, even when conducted in an open-field setting, multiple-choice tests may exclude the full range of potential ecological and environmental variability that a released agent may encounter and that may affect realized host-use (van Klinken, 2000). Thus, there has been a reassessment of the value of initial native range field studies as a means to better quantify and predict patterns of host use in natural settings (Goolsby et al., 2006; Haye et al., 2005; Schaffner, 2001). The results of such studies can be considered in addition to the experimental assessments of host specificity for a more comprehensive understanding of the role of anticipated new environments on field host range, and thus, the risks to non-target species; similar to the marrying of "description of innate host specificity" and "description of release environment" for better prediction of field host range and relative host attack proposed by van Klinken (2000).

Surveys of host use by candidate agents in their native range, specifically where multiple, related host species co-occur, also can be highly useful in setting the course for a biological control program (Schaffner, 2001). Assessments of the ecological host range in disparate regions of the candidate agent's distribution are especially desirable, as they allow a fuller expression of an candidate agents potential host choice and use patterns under different climatic and/or habitat conditions, and thus allow a more informed selection of the most host-specific and effective candidates for further study (Haye et al., 2005). Native range assessments of host use also would be useful in situations where multiple, congeneric, and potentially sympatrically occurring, target host species are involved. Many weed biological control programs attempt to maximize return for effort by employing one control agent for two or more congeneric invasive species (an extreme example being Cactoblastis cactorum Bergroth on Opuntia spp., Dodd, 1933; but see Julien and Griffiths, 1998 for several examples). However, few programs have assessed under what environmental conditions, such as the relative availability of different ecological hosts, different congeneric species are used and to what extent. Thus, defining the ecological host range and further assessing patterns of host-species use in the native range could be of great predictive power in determining which members of a complex of closely related invasive species would be controlled by a candidate agent. In the current study, we conducted an assessment of ecological host use of a candidate weed biological control agent considered for multiple species of hawkweeds in the genus *Pilosella* (invasive in North America), across disparate regions of its native European range.

The *Pilosella* hawkweeds are native to Central Europe and known invaders of both the Americas and Oceania (Cipriotti et al., 2009; Duncan et al., 1997; Wilson et al., 1997, 2006; Williams and Holland, 2007; Wallace et al., 2010). There are at least fifteen species in the genus *Pilosella* Vaill. (syn. *Hieracium* subgenus *Pilosella* L., Asteraceae: Lactuceae) that have been introduced into North America (Gaskin and Wilson, 2007), a number of which have become particularly invasive. While sometimes considered a sub-genus of *Hieracium*, current convention among European taxonomists is for *Pilosella* to be treated as a distinct genus (Tyler, 2001; Bräutigam and Greuter, 2007b); we follow the taxonomy of Bräutigam and Greuter (2007b). Where they are introduced, the *Pilosella* hawkweeds aggressively out-compete native and cultivated plant species on disturbed sites, forming either dense, mono-specific patches or multi-*Pilosella* species stands (Makepeace, 1985; Lass and Callihan, 1997; Wilson et al., 1997). In the United States alone, an estimated $58 million annually is lost to reduced resource revenues and expenses related to hawkweed control (Wilson, 2002). Invasive hawkweeds are predicted to have eventual economic impacts in the tens of millions of dollars in the province of British Columbia, Canada, if they continue to spread at current rates (Frid et al., 2009).

A biological control program targeting invasive *Pilosella* hawkweeds in North America was initiated in 2000, following, and investigating many of the same agents as an earlier-initiated program against *Pilosella* species invasive in New Zealand (Syrett et al., 1999). Five insects were released as agents for invasive *Pilosella* in New Zealand (Grosskopf et al., 2001; Syrett et al., 1999) where no native hawkweeds are present. In contrast to New Zealand, there are numerous native hawkweeds in the genus *Hieracium* present in North America, although no native *Pilosella* species occur (Beaman, 1990; Wilson et al., 2006). Only the stolon-gall wasp *Aulacidea subterminalis* Niblett (Hymenoptera: Cynipidae) has so far been determined sufficiently host-specific for release in North America (Cordat et al., 2012). While *A. subterminalis* has been shown in laboratory and field cage tests of its fundamental host range to develop on several stoloniferous hawkweeds (i.e., *P. flagellaris, P. aurantiaca* and *P. floribunda*) (Grosskopf et al., 2008), in its native range *A. subterminalis* reportedly only uses *P. officinarum* as an ecological host (Littlefield et al., 2008). At present, there are no approved biological control agents available for the remaining *Pilosella* hawkweed species that invade North America, including *P. caespitosa, P. glomerata, P. piloselloides*,

and depending on the establishment success of *A. subterminalis*, this list may also still include *P. flagellaris*, *P. aurantiaca* and *P. floribunda*. Thought to be the most widespread invasive *Pilosella* hawkweed in both western Canada and the neighboring US states (Gaskin and Wilson, 2007), the original target of the North American hawkweed biocontrol program, *P. caespitosa*, remains without a biocontrol agent.

Field surveys for phytophages attacking *P. caespitosa* conducted for the North American hawkweed biocontrol program identified the leaf-gall wasp *Aulacidea pilosellae* Kieffer (Hymenoptera: Cynipidae) as a potential candidate agent (Grosskopf et al., 2002, 2003, 2004). Believed to be the sister species to *A. subterminalis* (Nieves-Aldrey, 1994, 2001), *A. pilosellae* is a small (1.0–1.5 mm) uni- to bi- (tri-) voltine cynipid, known to induce small (2–4 mm) uni-locular galls on the abaxial rosette leaf midribs, and occasionally on the stems and the stolons of multiple *Pilosella* species. The host range of *A. pilosellae* reported in the European literature includes *Pilosella officinarum* (syn. *Hieracium pilosella*; Dalla Torre and Kieffer, 1910; Eady and Quinlan, 1963; Ionescu, 1957); *P. cymosa*, *P. echioides*, *P. flagellaris*, *P. floribunda* (Buhr, 1964); and also possibly all or some members of the *P. piloselloides* complex (Houard, 1913) (for *Hieracium* synonyms of these species, see Table 1). However, the complex and often overlapping morphology of *Pilosella* hawkweeds, the ambiguous taxonomy of the genus (e.g. Nägeli and Peter, 1885; Zahn, 1923; Šingliarová et al., 2011), and the fact that most of the authors who evaluated the host range of *A. pilosellae* were not botanists, suggests that the reported host range needs re-examination. Interestingly, survey records (for the North American hawkweed biocontrol program) indicated that while multiple species of *Pilosella* were used as hosts across the Northern extent of *A. pilosellae*'s range, often only one species was mentioned as being galled at a particular site, despite multiple host species being present (Grosskopf et al., 2004). We hypothesized that this pattern indicated that host-species use by *A. pilosellae* may follow a rank-preference order, as has been proposed by Singer (1971; 1983).

Having an accurate and complete characterization of *A. pilosellae*'s ecological host range and further determining if *A. pilosellae* exhibits a preference for any species of *Pilosella* could be critical to predicting which species of *Pilosella* would be effectively controlled if *A. pilosellae* were introduced to North America. We there-fore investigated patterns of ecological host use of *A. pilosellae* in its native central European range. Our primary objective was to determine if *A. pilosellae* showed evidence of host-preference among species of *Pilosella* hawkweeds within its ecological host range, and if so, if host-use patterns were consistent among our four delineated regions. Our null hypothesis was that each *Pilosella* species within the host range of *A. pilosellae* was being used in proportion to its relative abundance or availability within a surveyed habitat or field site (i.e. a proportional null model). We would identify hosts as "preferred" by *A. pilosellae* if those *Pilosella* species were galled significantly more frequently than predicted by our null model. Our secondary objective was to quantify the ecological host range of *A. pilosellae* in its native distribution, by not only documenting positive host-use records but also recording true absences. As the majority of *Pilosella* species invasive in western North America are abundant and common in central Europe, especially in the Czech Republic and surrounding areas, we focused our surveys in this area. We conducted our surveys in three ecologically distant regions in this area, and also sampled a fourth even more distant region in the German Black Forest and Swiss Jura, in order to ensure we assessed patterns of host use in disparate parts of the candidate agent's native range.

2. Materials and methods

2.1. Study area

This study was conducted within the broad geographic distribution of *A. pilosellae* in central Europe (Fig. 1). The study area comprised four ecologically distinct sampling regions: (S) the Jura Canton (*Canton du Jura*) of northwest Switzerland and the Black Forest (*Schwarzwald*) area in the *Baden-Württemberg* region of southwest Germany; (A) the Bavarian-Bohemian (*Bayerischer Wald/Zadní Bavorský les – Böhmerwald/Šumava*) forest region bordering eastern Germany and the southwestern Czech Republic; (B) the Ore Mountain range (*Erzgebirge/Krušné hory*), bordering eastern Germany and the western Czech Republic; and (C) the Sudetes mountain range (*Sudeten/Sudety*) in the tri-border area of Germany, Poland and the Czech Republic (Fig. 1). Region S is

Table 1

Basic, sub- and stabilized hybrid species of *Pilosella* present at the 55 field sites surveyed in central Europe, based on the classification of Bräutigam and Greuter (2007b) and the corresponding *Hieracium* synonym obtained from the Euro + Med Plantbase Project (Bräutigam and Greuter, 2007–09), ‡ denotes species invasive in North America (Gaskin and Wilson, 2007; Wilson, 2006 *revised* 2007).

Pilosella species	Homotypic synonym/Basionym	Species designation	Postulated parental species
‡*Pilosella aurantiaca* (L.) F. W. Schultz & Sch. Bip.	*Hieracium aurantiacum* L.	basic	–
‡*Pilosella caespitosa* (Dumort.) P. D. Sell & C. West	*Hieracium caespitosum* Dumort.	basic	–
‡*Pilosella floribunda* (Wimm. & Grab.) Fr.	*Hieracium floribundum* Wimm. & Grab.	hybrid	*Pilosella caespitosa<>lactucella*
‡*Pilosella fuscoatra* (Nägeli & Peter) Soják	*Hieracium fuscoatrum* Nägeli & Peter	hybrid	*Pilosella aurantiaca<>caespitosa*
‡*Pilosella glomerata* (Froel.) Fr.	*Hieracium glomeratum* Froel.	hybrid	*Pilosella caespitosa<>cymosa*
Pilosella iserana (R. Uechtr.) Soják	*Hieracium iseranum* (R. Uechtr.) Fiek*	hybrid	*Pilosella caespitosa<>lactucella<>officinarum*
‡*Pilosella lactucella* (Wallr.) P. D. Sell & C. West	*Hieracium lactucella* Wallr.	basic	–
Pilosella melinomelas (Peter) Holub	*Hieracium melinomelas* Peter	hybrid	*Pilosella caespitosa<>officinarum<>piloselloides*
‡*Pilosella officinarum* Vaill.	*Hieracium pilosella* L.	basic	–
‡*Pilosella piloselliflora* (Nägeli & Peter) Soják	*Hieracium piloselliflorum* Nägeli & Peter	hybrid	*Pilosella caespitosa<>lactucella<>officinarum*
‡*Pilosella piloselloides* (Vill.) Soják	*Hieracium piloselloides* Vill.	basic	–
‡*Pilosella piloselloides* subsp. *bauhini* (Schult.) S. Bräut. & Greuter	*Hieracium bauhini* Schult.	basic sub-species	–
Pilosella polymastix (Peter) Holub	*Hieracium polymastix* Peter	hybrid	*Pilosella caespitosa<>piloselloides*
Pilosella rubra (Peter) Soják	*Hieracium rubrum* Peter	hybrid	*Pilosella aurantiaca<>officinarum*
Pilosella scandinavica (Dahlst.) Schljakov	*Hieracium scandinavicum* Dahlst.	hybrid	*Pilosella caespitosa<>cymosa<>lactucella*
‡*Pilosella stoloniflora* (Waldst. & Kit.) F. W. Schultz & Sch. Bip.	*Hieracium stoloniflorum* Waldst. & Kit.	hybrid	*Pilosella aurantiaca<>hoppeana*
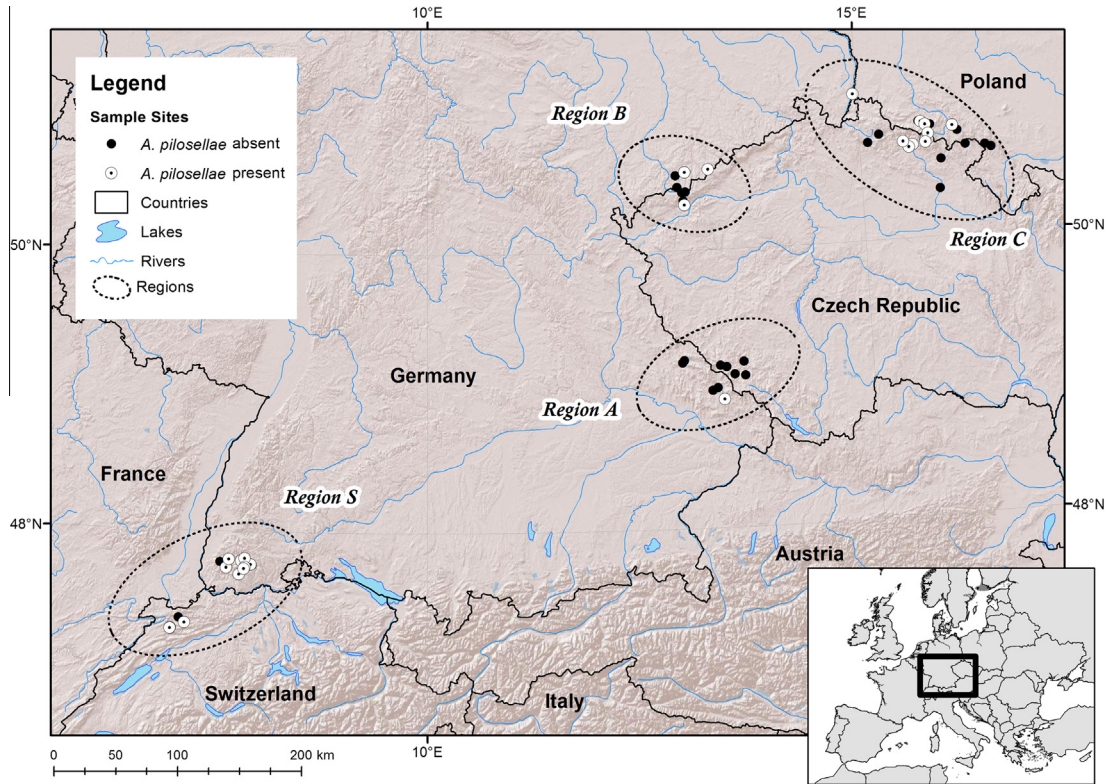
Fig. 1. Study area comprising the central European distribution of *Aulacidea pilosellae*, divided into Regions A, B, and C (Northern Range) and Region S (Southern Range), consisting of 55 sites of *Pilosella* species, of which *A. pilosellae* was present at 26 sites. Map generated using ArcGIS 10.0 (ESRI 2011), using the Europe Albers Equal Area Conic projection based on European Datum 1950; scale is approximate due to slight distance distortion. Inset indicates the location of the study region within Europe.
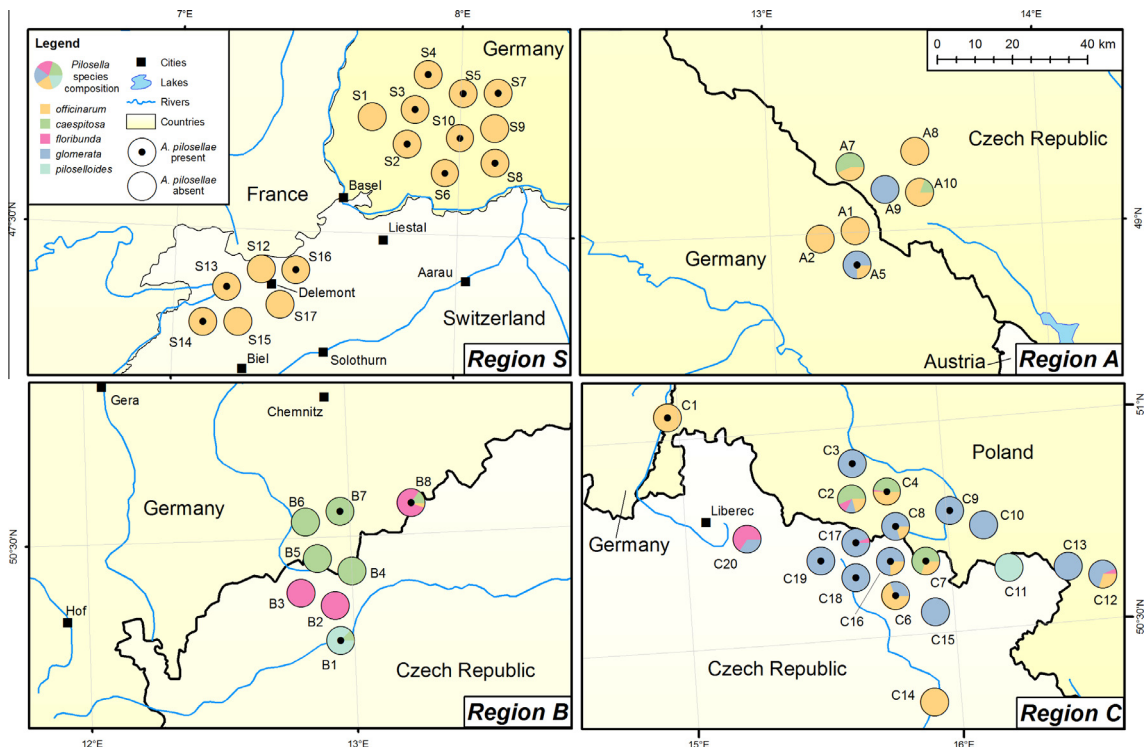
Fig. 2. Each region of the study area (S, A, B, C) showing sites where at least one of five putative host *Pilosella* species of *Aulacidea pilosellae* was found. Pie graphs show the putative host species composition at each site (*P. caespitosa*, *P. glomerata*, *P. floribunda*, *P. piloselloides* and *P. officinarum*), as well as if *A. pilosellae* is present or absent. Site S11 was omitted subsequent to surveying and is not included on this map or in any analyses. Map generated using ArcGIS 10.0 (ESRI 2011), using the Europe Albers Equal Area Conic projection based on European Datum 1950; scale is consistent across panels and approximate due to slight distance distortion.

considered as being in the 'Southern Range' of *A. pilosellae*, and A, B and C are all considered as being in the 'Northern Range'.

2.2. Site selection and characteristics

A total of 55 field sites were visited (Fig. 1) during the sampling season, which occurred during 5–30 June 2010 in Regions A–C and 28 June-2 July and 23–24 July 2010 in Region S. In the Northern Range, 10 sites were visited in region A (A1–A10), 8 in region B (B1–B8), and 21 in region C (C1–21), for a total of 39 sites (Fig. 2). In the Southern Range, 17 sites were visited in region S (S1–17; S11 was omitted subsequent to surveying), the first ten of which were located in the Black Forest and the latter seven in the Swiss Jura (Fig. 2). Sites were selected using locations identified in previous studies that confirmed the presence of *Pilosella* species (G. Grosskopf-Lachat, *unpublished data*; F. Krahulec, *pers. comm.*; Suda et al., 2007) as well as sites located opportunistically. Our sites included localities where *A. pilosellae* had been observed in the past and sites where its presence or absence was undocumented. All field sites were disturbed herbaceous plant communities; with the majority being managed, semi-disturbed to disturbed open-pasture montane-meadows, characteristic of the regions surveyed, and the remaining sites heavily disturbed roadside mixed-herbaceous plant communities, often near forest edges. Site coordinates and elevation were estimated and recorded in the field, using a Garmin GPSMap 60CSx global positioning system, along with other cursory abiotic data (Appendix 1).

2.3. Estimation of host plant availability

Throughout our study, all *Pilosella* species encountered were considered as possible hosts for *A. pilosellae* and surveyed without bias as to those previously reported or unreported as hosts. In addition, we examined any true *Hieracium* species encountered for the presence of *A. pilosellae*. Post-survey, we defined 'putative host *Pilosella* species' as being only those species utilized by *A. pilosellae* in this or other published studies. We did this as to distinguish between the dozens to hundreds of species (depending on taxonomic treatment) in the genus *Pilosella*, and only those reported as host species.

To estimate *Pilosella* host plant availability we followed the methodology of Daubenmire (1959), which includes classifying plant cover using Daubenmire's 0.1 m² (20 × 50 cm) quadrat into one of six cover categories (0–5%, 5–25%, 25–50%, 50–75%, 75–95%, 95–100%) and then converting these cover categories to cover mid-points (2.5%, 15%, 37.5%, 62.5%, 85%, 97.5%) post survey (Daubenmire, 1959). At all sites our methodology of assessing plant cover was consistent within quadrats, but our method of laying these quadrats at a site differed out of necessity, based on the density and distribution of *Pilosella* plants at a site. While we attempted at all sites to delimit patches of *Pilosella* and sub-sample these patches, at some sites (B3, B4, C1, C6, C11, C15, S2) we encountered rather low-density and more or less evenly spaced plants, thus we could not delimit patches at these sites. We modified our design to allow for sub-sampling of these low-density, continuous distribution sites, by laying the quadrats along transects, instead of within patches.

Where density of *Pilosella* plants at a site fell in our lowest cover category (0–5% cover), a 'transect method' of laying quadrats was employed. At these low density sites, we oriented transects by determining the centroid of the site by measuring two axes of the site and then ran three 30 m transects, oriented at a random heading between 1 and 360°, from the centroid of the site radiating outwards towards the site edge. If any transect reached the site edge prior to reaching 30 m in length, the remaining length was added to one of the remaining transects. We then placed a single

Daubenmire quadrat systematically every 3 m along each transect and assessed *Pilosella* identity and density, using Daubenmire's cover categories (Daubenmire, 1959). We considered *Pilosella* plant distribution at a site to be 'patchy' if we observed a density of at least our second cover category (5–25%) over a discrete area of at least 5 m², together with other patches at a site of the same (or greater) density that covered at least 0.1 m² in area each; however, patches at sites were often much larger and more numerous than this. For this patch method of laying quadrats, we first calculated patch size by measuring the axes of the patch and estimating its area in m². In general, one quadrat was used as a sub-sample for each m² of patch area, with a maximum of 20 quadrats being placed in the largest patches. Quadrats were placed using randomly generated *x,y* locations in the patch. Other haphazard searches were made in small roadside patches or in other areas or patches of a site not selected by either random method; however, these searches did not yield any additional plants galled by *A. pilosellae*. All *Pilosella* species surveyed exhibited variable foliar cover/canopy architecture on a per ramet basis, often appearing to depend on the height and density of surrounding vegetation.

We follow the taxonomy of Bräutigam and Greuter (2007b), which uses a framework of 'basic' species (species in the traditional sense), sub-species (of 'basic' species), and 'collective' species, which we refer to as stabilized hybrids – that is, species which have been postulated or shown to have arisen from an hybridization event between two basic species, but which now self-propagate. Some species of *Pilosella* also actively produce infertile hybrids, commonly termed 'in situ hybrids'. Within quadrats, all basic species, sub-species, stabilized hybrid and in situ hybrid species of *Pilosella* were identified in the field using Sell and West (1976) and species descriptions compiled by G. G.-L. from S. Bräutigam and from the Hieracium Study Group at the Czech Institute of Botany (CIB). Any plants unidentifiable in the field were assigned a morpho-species identification, collected and pressed. Both unidentified *Pilosella* plants and representative samples of field-identified plants were collected and their identification (as basic, sub-, stabilized or in situ hybrid species) verified by *Pilosella* experts F. Krahulec and J. Chrtek Jr. at the CIB. Voucher specimens of these pressed *Pilosella* specimens are stored at CABI Europe-Switzerland in Delémont, Switzerland.

2.4. Estimation of host-species use

Pilosella leaves, stems and stolons were examined for galls of *A. pilosellae* on all *Pilosella* species within a quadrat, and the number of galls on each leaf and stem recorded (no stolon galls were found). The method of counting leaves and stems allowed for an assessment of the density of galls per species at a given site. The number of leaves and stems per species per site was not intended as a measure of the relative abundance of *Pilosella* species, as there is high variability in leaf size both within and among *Pilosella* species. Due to the clonal nature of some *Pilosella* hawkweeds, no attempt was made to discriminate individual plants.

Once quadrat surveys were completed, galled plants were excised from the soil along with approximately 15–20 square centimeters of surrounding soil, potted, and watered as needed. All live galled *Pilosella* plants were transported to the CIB where their identification was also verified by experts (except plants from site B1 as these rotted; the galls however, remained intact). Galled plants were then transported to CABI Europe-Switzerland where they were re-potted in larger pots and kept in mesh Aerarium® (http://www.wins.ch/aerarium.html) 60 × 60 × 180 cm field cages, allowing *A. pilosellae* larvae to mature, over-winter and be used in host-specificity and other trials in subsequent studies.

2.5. Analysis of host preference

As per the null hypothesis being tested, lack of preference would result in patterns of host-use among *Pilosella* populations (field sites) that would only differ based on the relative availability of hosts present at sites. Our results (below) precluded the use of such a proportional null model, which would have required multiple host species being used at a site, which never occurred. Instead, we employed Fisher's Exact test (Fisher, 1922, 1954) in order to assess the patterns of host use (see Results). In this analysis, we only included sites where multiple putative host species were present, which precluded us from using any sites in the Southern Range (S) as these were dominated by a single *Pilosella* species, *P. officinarum*, which was the only species ever galled in the Southern Range, as well as select sites in the Northern Range where only one putative host species was present (Table 2). Our analyses were conducted in R Version 2.12.2 (R Development Core Team, 2011).

2.6. Distribution of host species

For each site where at least one putative host species was found, the relative abundance of each putative *Pilosella* species is shown as a pie chart representing a site on the maps in Fig. 2 (data shown in Table 2). All maps were generated in ArcGIS 10.0 (ESRI 2011), all data were projected using the Europe Albers Equal Area Conic with European Datum 1950 (ED50), and the countries layer converted from World Geodetic System 1984 (WGS 1984) to ED50. All map scales are approximate as distance is slightly distorted in equal area projections.

3. Results

3.1. Distribution of host-species availability

A total of 16 basic, sub- and stabilized hybrid species of *Pilosella* were surveyed for the presence of *A. pilosellae* across the survey area (Table 1); in situ hybrids of the recorded species were occasionally encountered and also surveyed, and are mentioned in the text where relevant. In the Northern Range, all 16 of the encountered species were present at one or more sites (Table 3a), whereas in the Southern Range, only four of the 16 species were present (Table 3b). Within the Northern Range, the *Pilosella* species composition was variable among both regions and sites within regions. Region A was characterized by *P. aurantiaca* and *P. officinarum*, with *P. caespitosa* and *P. glomerata* both often present. Region B was characterized by *P. caespitosa*, with *P. aurantiaca* and *P. floribunda* also often present. Region C was dominated by *P. glomerata* but many other species were also common. The Southern Range/Region S was heavily dominated by *P. officinarum*, which was present at all sites; other species were rare.

3.2. Host-species use

Aulacidea pilosellae was only found to occur on species of *Pilosella*, despite *Hieracium* species being present in sympatry with *A. pilosellae* at nine field sites. Across the four regions surveyed, *A. pilosellae* was detected at a minimum of one location in each sampling region. A total of 15 of 39 sites in the Northern Range and 11 of 16 sites in the Southern Range (Table 2) had the gall wasp present. In the Northern Range, *A. pilosellae* was found to commonly gall *P. glomerata* and *P. caespitosa* (multiple sites) and rarely gall *P. floribunda* and *P. piloselloides* (one site each), while *P. officinarum* was galled in both ranges. We thus defined the 'putative host *Pilosella* species' to include *P. glomerata*, *P. caespitosa*, *P. floribunda*, *P. piloselloides* and *P. officinarum*. In the Southern Range, *A. pilosellae* was found to gall *P. officinarum* at 11 of 16 sites where *P. officinarum* was present, as compared to 1 of 16 sites in the Northern Range where *P. officinarum* was present (seven of which where *A. pilosellae* was also present).

At any site where *A. pilosellae* was present, only one species of *Pilosella* was ever galled (Tables 2, 3a and 3b). The exceptions to this general pattern occurred at sites C3 and C4 where the "species" attacked was determined to be an hybrid swarm. Plants at

Table 2
Relative abundance of each putative host *Pilosella* species at each site where *Aulacidea pilosellae* was found. Only sites (in bold) with multiple putative host species were included in the frequency-dependence analysis, which found that the most abundant putative host species (also in bold) was galled significantly more often than expected (exceptions were sites C4 and C6).

Site	Putative host *Pilosella* species relative abundance					
	caespitosa	*floribunda*	*glomerata*	*officinarum*	*piloselloides*	Species Galled
A5	0	0	**75**	25	0	*glomerata*
B1	13	0	0	0	**87**	*piloselloides*
B7	100	0	0	0	0	*caespitosa*
B8	16	**79**	0	5	0	*floribunda*
C1	0	0	0	100	0	*officinarum*
C3	0	0	100	0	0	*cae x glo*
C4	48	2	0	**51**	0	*caespitosa*
C6	0	0	30	**70**	0	*glomerata*
C7	**70**	0	0	30	0	*caespitosa*
C8	0	0	**80**	20	0	*glomerata*
C9	0	0	100	0	0	*glomerata*
C16	0	0	**75**	25	0	*glomerata*
C17	0	6	**94**	0	0	*glomerata*
C18	0	0	100	0	0	*glomerata*
C19	0	0	100	0	0	*glomerata*
S2	0	0	0	100	0	*officinarum*
S3	0	0	0	100	0	*officinarum*
S4	0	0	0	100	0	*officinarum*
S5	0	0	0	100	0	*officinarum*
S6	0	0	0	100	0	*officinarum*
S7	0	0	0	100	0	*officinarum*
S8	0	0	0	100	0	*officinarum*
S10	0	0	0	100	0	*officinarum*
S13	0	0	0	100	0	*officinarum*
S14	0	0	0	100	0	*officinarum*
S16	0	0	0	100	0	*officinarum*

Table 3a

Basic, sub- and stabilized hybrid species of *Pilosella* present at each site in the Northern Range. For each site (A1-A10, B1-B8, C1-C21), species present are denoted with an X, and those present and galled by *Aulacidea pilosellae* are denoted by an O. *Pilosella* species names are abbreviated: *P. aurantiaca* = aur, *P. caespitosa* = cae, *P. caespitosa* x *P. glomerata* = cae x glo, *P. floribunda* = flo, *P. fuscoatra* = fus, *P. glomerata* = glo, *P. iserana* = ise, *P. lactucealla* = lac, *P. melinomelas* = mel, *P. officinarum* = off, *P. pilosselliflora* = pilf, *P. piloselloides* = pil, *P. piloselloides* subsp. *baunini* = bau, *P. polymastix* = pol, *P. rubra* = rub, *P. scandinavica* = sca, *P. stoloniflora* = sto.

	Region A										Region B								Region C																					
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	B1	B2	B3	B4	B5	B6	B7	B8	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21
aur	X	X	X	X	X	X	X			X	X	X	X	X	X	X				X			X			X						X					X		X
cae		X	X	X		X	X			X	X		X	X	X	X	O	O	X	X			X	X	O	X						X						X	X
cae x glo																					O																		
flo													X	X				O		X		X								X					X		X	X	
fus					X																	X																	
glo					O															X				O	X	O	O	X		X	X	X	X	O	O	O	O		
ise									X																X	X	X					X						X	
lac																																						X	
mel																						X																	
off	X	X			X		X	X		X	X	X					X		X	O	X	X	X	X	X	X	X	X		X	X	X	X	X	X				
pilf							X																	X						X		X			X				
pil											O																		X										
bau											X											X																	
pol																										X													
rub										X																													
sca								X	X																		X												
sto																										X													

site C3 were determined as in situ hybrids of *P. caespitosa* x *P. glomerata*, but more similar to *P. glomerata* (F. Krahulec, *pers. comm.*). At site C4 the majority of galled plants were *P. caespitosa* (72%) while the remaining galled plants were described as an in situ hybrid of *P. caespitosa* x *P. piloselloides*, but more closely resembling *P. caespitosa* (F. Krahulec, *pers. comm.*). At site C3, attacked plants included only the in situ hybrids (the plants were a remnant patch of a mowed field and neither parental species was present) while at site C4 both in situ hybrids and one of the putative parents (*P. caespitosa*) were attacked and the other putative parent was absent.

Where multiple putative host *Pilosella* species were present (i.e. where *A. pilosellae* had a 'choice' of multiple hosts), either the most abundant or second most abundant putative host *Pilosella* species present was the host species used by *A. pilosellae* (Fig. 3). These findings precluded the use of the proportional null model; all sites included only a single attacked host species, and the composition of putative host species varied considerably among sites. Our findings show no evidence that *A. pilosellae* demonstrates a clear preference for any particular *Pilosella* species, but rather that *A. pilosellae* tends to choose the most locally abundant species within its ecological host range regardless of identity. An evaluation of the latter proposition is provided by a Fisher's Exact test (Fisher, 1922, 1954), wherein the observational unit is the site (n = 9 sites with more than one putative species available, all in the Northern Range), and putative host species are categorized as being the "most abundant" or "subordinate" species at a site, and are cross-classified against categories "attacked" or "not attacked". If abundance, ranked using these two simplified categories, does not matter to host choice (i.e. host species will be used proportionally, the null hypothesis), then the most abundant and subordinate species should be attacked with frequencies equal to their relative abundance. This was not the case: the most abundant species was attacked seven of nine times, whereas the subordinate species was attacked only in the other two instances (Fisher's Exact test, P = 0.028, odds ratio = 10.24). In both cases of the subordinate species being attacked, the most abundant species was *P. officinarum*. Our results clearly show that when there were multiple putative host *Pilosella* species present at a site, the most abundant putative host *Pilosella* species was galled by *A. pilosellae* significantly more frequently than expected by chance (Fig. 3).

4. Discussion

4.1. Frequency-dependent host species use

We have demonstrated clear differences in patterns of host use of *A. pilosellae* across different regional localities. Specifically, we found *A. pilosellae* in the Southern Range to only use *P. officinarum* (Table 3b), the dominant species of *Pilosella* in this region. In contrast, we found *A. pilosellae* in the Northern Range to attack multiple species of *Pilosella*: *P. glomerata*, *P. caespitosa*, *P. floribunda*, *P. piloselloides* and *P. officinarum* (Table 3a, Figs. 2 and 3). Importantly, our study did not provide any direct evidence that *A. pilosellae* exhibits any preference for given *Pilosella* species in its ecological host range. Instead, we found *A. pilosellae* to only select a single *Pilosella* species at any given site across the study area, and wherever there were multiple putative host species present in the Northern Range, the most abundant host species was used significantly more frequently than expected by chance (Fig. 3).

The contrasting pattern of usage of *P. officinarum* between *A. pilosellae* from the Southern and Northern Ranges suggests that use of this particular host species does not fit the pattern of frequency-dependent attack observed in all other populations and on other host species. While we found no evidence of host-preference

Table 3b

Basic, sub- and stabilized hybrid species of *Pilosella* present at each site (S1–10, S12–17) in the Southern Range. For each site, species present are denoted with an X, and those present and galled by *Aulacidea pilosellae* are denoted by an O. *Pilosella* species names are abbreviated: *P. aurantiaca* = aur, *P. lactucealla* = lac, *P. officinarum* = off, *P. rubra* = rub.

| | Region S | | | | | | | | | | | | | | | | |
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S12	S13	S14	S15	S16	S17
aur																X
lac					X											
off	X	O	O	O	O	O	O	O	X	O	X	O	O	X	O	X
rub																X

of *A. pilosellae* per se, the *P. officinarum* usage pattern may be interpreted as *A. pilosellae* having a low relative preference (or reduced host acceptance) for *P. officinarum*. In the Northern Range, we encountered *P. officinarum* at seven sites in sympatry with *A. pilosellae*, yet it was galled at only one of these sites, where it was the only species of *Pilosella* present, this despite *P. officinarum* being

the most abundant species at two sites (C4 and C6) where another *Pilosella* species was galled. In the Southern Range, *P. officinarum* was the only suitable host that we encountered, thus wherever we also found *A. pilosellae* it had galled *P. officinarum*. This suggests the exclusive use of *P. officinarum* by *A. pilosellae* in the Southern Range may simply be due to the absence of other more preferred
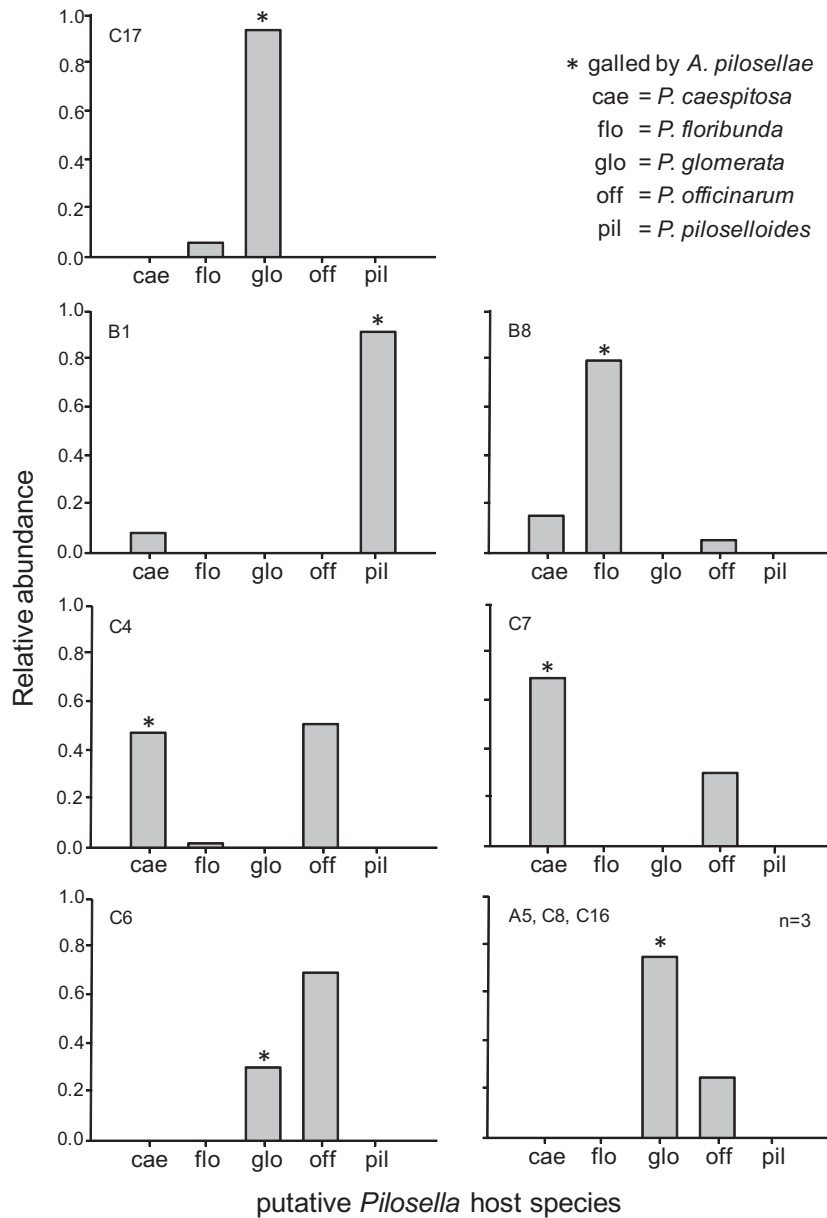
Fig. 3. Distribution of putative *Pilosella* host species relative abundance showing which species were galled by *Aulacidea pilosellae* (*), for sites in the Northern Range of the survey area where more than one putative *Pilosella* host species was present. Plots representing all nine sites in the Northern Range where *A. pilosellae* occurred with multiple putative host species are shown; site identity is shown in the top left corner (e.g. C17) of each plot, the bottom right plot represents three sites. At seven of nine sites, the most abundant *Pilosella* species was the species galled.

species, such as *P. caespitosa* and *P. glomerata*, possibly coupled with some selection on the gall wasp populations in this Range to enhance either preference or performance. We encourage further work to investigate if *A. pilosellae* has a reduced preference for *P. officinarum*. Furthermore, we are currently assessing the potential for genetic differentiation of *A. pilosellae* from the Southern and Northern Ranges to investigate any biological explanations for patterns of *P. officinarum* use. Early results suggest genetic structure between *A. pilosellae* found on *P. officinarum* and those found on other hosts (Moffat et al., *in prep*). We thus repeated our host use analyses, with the exclusion of sites where *P. officinarum* was the most abundant species. Importantly, our finding that the most abundant putative host *Pilosella* species was galled by *A. pilosellae* significantly more often than expected by chance ($P = 0.014$, odds ratio $= \infty$) holds under this conservative approach.

4.2. Ecological host range

Our results confirm that *P. officinarum* is a common (and thus far the only) host of *A. pilosellae* in the Southern Range and a rare host of *A. pilosellae* in the Northern Range, despite *P. officinarum* being a common species in the Northern Range. Furthermore, we found *P. glomerata* and *P. caespitosa* to be common hosts of *A. pilosellae* in the Northern Range, and that *P. floribunda* and *P. piloselloides* are also acceptable, but not common, hosts. These results are consistent with those of, (Grosskopf et al. 2002, 2003, 2004), who similarly found *A. pilosellae* in the Northern Range to commonly gall both *P. glomerata* and *P. caespitosa*, and was the first to document these species as hosts of *A. pilosellae*. We found *P. floribunda* and *P. piloselloides* to be used only once each and only when they were the most abundant species. Grosskopf et al. (2003) also detected *A. pilosellae* on *P. floribunda* and it is mentioned as a host by Buhr (1964), so our record is not the first. We suspect that *P. floribunda* is only an occasional host for *A. pilosellae*, but consider that it could be a common host if *P. floribunda* were more locally abundant. Grosskopf et al. (2003) also found *P. cymosa* to be a host species, an association that we could not evaluate as we did not locate this species at any of our study sites. Similarly, both *P. echioides* and *P. flagellaris* (*P. caespitosa<>P. officinarum*) have been reported as hosts of *A. pilosellae* in previous literature (Buhr, 1964), but neither we nor any of Grosskopf et al. (2002, 2003, 2004) surveys encountered these species, thus we cannot confirm if these species are within the ecological host range of *A. pilosellae*.

While *P. piloselloides* has been a known invasive in British Columbia, Canada for several years (Wilson et al., 1997), the extent of this invasion has only recently been documented (Moffat and Ensing, *unpublished data.*). The *P. piloselloides* species aggregation is among the most difficult of *Pilosella* species complexes to resolve and has been subjected to many taxonomic revisions (Bräutigam and Greuter, 2007b). *Pilosella piloselloides* is now treated as a basic species aggregation which includes sub-species recognized as distinct species in other treatments, including *P. piloselloides* subsp. *bauhini* (*H. bauhini*) and *P. piloselloides* subsp. *praealta* (*H. praealtum*) (Bräutigam and Greuter, 2007b). Wilson et al. (2006) lists *H. bauhini* as invasive in North America and as synonymous with *H. praealtum*, and lists *H. piloselloides* separately from *H. bauhini*. It is currently unclear if other members of the *P. piloselloides* complex have invaded North America, however a rigorous molecular-morphometric investigation of invasive *Pilosella* hawkweeds, including *P. piloselloides*, is currently underway (De Clerck-Floate, Ensing, Gaskin, Moffat, and Pither, *in prep.*). While we found *A. pilosellae* galls on only a single plant clearly identified to be in the *P. piloselloides* aggregation (which we assigned to the basic species *P. piloselloides*), this plant rotted prior to having our identification confirmed. Records from Grosskopf et al. (2003, 2004) also show collections of *A. pilosellae* from *P. piloselloides* subsp. *bauhini* (then

H. bauhini). Furthermore, Houard (1913) mentions *H. florentinum* (syn. *P. piloselloides*) as a host of *A. pilosellae*. Thus, we are confident in our assessment that *A. pilosellae* does use some members of the *P. piloselloides* aggregation, but which members remains to be definitively established, as does the frequency of use of this species.

Pilosella aurantiaca is an aggressive invader that is a high priority for the North American hawkweed biological control program. There has been some optimism that the *Aulacidea* species currently being screened would accept *P. aurantiaca* under natural conditions. Grosskopf et al. (2004) found one wild individual *P. aurantiaca* plant attacked by *A. pilosellae* within Region A of our survey area. However, in the present study, we did not find *P. aurantiaca* to be attacked despite surveying 16 sites where *P. aurantiaca* was present in the Northern Range. At two of these sites, *P. aurantiaca* was the most abundant species and *A. pilosellae* was also present, but the less abundant *P. glomerata* was galled. *Pilosella aurantiaca* was not documented as a host in the remainder of Grosskopf et al. (2001, 2002, 2003, 2004) studies, nor is it mentioned elsewhere in the literature as a host species, therefore due to the lack of records, we do not consider *P. aurantiaca* to be a putative host species. Under no-choice tests of its fundamental host range, Grosskopf et al. (2002, 2003, 2004) found that *A. pilosellae* originating from both the Southern and Northern Ranges would accept *P. aurantiaca* and that it was a viable host. Similarly, *A. subterminalis*, which has only one known ecological host, *P. officinarum*, also successfully develops on *P. aurantiaca* under cage and greenhouse conditions. These results suggest that *P. aurantiaca* does not fall within the normal ecological host range of either gall wasp species despite functioning as a viable fundamental host. Due to the lack of ecological host use, we do not recommend *A. pilosellae* be considered as a candidate agent for *P. aurantiaca*.

4.3. Implications for biological control of invasive hawkweeds in North America

Based on our findings of the frequency of host-species use, *A. pilosellae* from the Northern Range should be considered as a candidate biological control agent primarily for *P. caespitosa* and *P. glomerata*, and possibly for *P. piloselloides* and *P. floribunda*. Our findings, and those of Grosskopf et al. (2003), indicate that *A. pilosellae* collected only from the Southern Range should be considered for *P. officinarum*. We also surveyed a number of other *Pilosella* species (Table 2, 3a and 3b), some of which are also invasive in North America, but did not find *A. pilosellae* to gall these species. Thus, we do not recommend *A. pilosellae* be considered as a candidate agent for any of the *Pilosella* species that were not found to be used as ecological hosts in our study.

Our finding of the pattern of frequency-dependent host use by *A. pilosellae* may have important implications for the *Pilosella* biological control program, depending on the mechanism regulating this pattern. Local adaptation (or host tracking) to the most common host (Kaltz and Shykoff, 1998; Lively and Dybdahl, 2000), 'predator' switching (Cornell, 1976; also known as apostatic selection (Clarke, 1962) or prey switching (Murdoch, 1969)), and cryptic host associations (e.g. Burns et al., 2008; Smith et al., 2008; Li et al., 2010; Toševski et al., 2011) represent plausible explanations for this pattern of frequency dependent attack, and warrant further investigation.

5. Conclusions

Recent literature (e.g. Goolsby et al., 2006; Haye et al., 2005; Schaffner, 2001) in the field of biological control has emphasized that an increasing level of prominence should be placed on pre-re-

lease assessments of candidate agents. While a number of avenues of research may improve the success of a biological control program, by better predicting efficacy, non-target risk, or climatic suitability, many authors agree that more comprehensive native range studies offer under-exploited predictive power for biological control programs (Goolsby et al., 2006; Schaffner, 2001). When multiple congeneric species invade a common area, as is the case with several invasive plant groups, including the hawkweeds (Wilson et al., 1997, 2006), obtaining an agent that could effectively control all members of such a group would have many benefits. By being able to only release one agent to target multiple invading congeners, the potential for non-target risks would be lower than by introducing multiple agents (Louda et al., 2003; McClay and Balciunas, 2005), and the costs associated with both pre-release and post-release assessments would likely be markedly lower. However, being able to predict which species within the fundamental host range of a candidate agent are most likely to be used in the introduced range could maximize the efficiency of a biological control program in a new way, by better matching candidate agents to the species they are most likely to control.

Our study is one of the first to attempt to systematically assess both patterns of host species use and the ecological host range of a candidate weed biological control agent in its native range prior to introduction. This approach has yielded a number of findings relevant to increasing the understanding of the *A. pilosellae – Pilosella* study system and underscores the predictive power of conducting comprehensive, native range studies. We found that in its native range, the candidate agent, *A. pilosellae*, commonly uses a sub-set of available *Pilosella* species as hosts, but where multiple species recorded as hosts co-occur, only one species was ever used at a given site. We believe this is the first instance of frequency-dependent host species use being reported in a weed biological control insect. We suggest that patterns of host-species use in the native range be quantified for all candidate agents that are considered for multiple congeneric invasive species. Specifically, we urge that all putative host species be surveyed without bias, not only those of interest to the biological control program. Revealing patterns of discriminating host species use, such as frequency-dependence, can likely only be done by examining host-use across all putative hosts in natural environments. We further advocate sampling multiple, disparate populations across a broad geographic range (as did Haye et al., 2005), as we found strong regional patterns to host species use by *A. pilosellae*. In particular, our main finding was that different putative host species were used, and at different frequencies, in different parts of the geographic distribution. Had studies been conducted in only one of the four regions we surveyed, very different patterns of native host species use would have emerged, which further emphasizes why only the tested population(s) of a biological control agent should be released, as other populations may exhibit different host-use patterns. Our finding of frequency-dependent host species use is indicative of the complex life-histories of the insects used in biological control programs, some of which are only revealed by careful, systematic studies such as demonstrated here.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biocontrol.2 013. 10.007.

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