# Host-range investigations of potential biological control agents of alien invasive hawkweeds (*Hieracium* spp.) in the USA and Canada: an overview

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*Summary*

Several European *Hieracium* species, e.g. *Hieracium caespitosum* Dumort. and *Hieracium aurantiacum* L., are noxious weeds in North America. A project for the biological control of alien invasive hawkweeds has therefore been initiated in 2000. Five European insect species investigated before their release in New Zealand and two additional gall wasps have been tested on North American test plants. The stolon-tip galling cynipid, *Aulacidea subterminalis* Niblett (Hym., Cynipidae) proved to be the most specific candidate attacking four *Hieracium* spp. in the subgenus *Pilosella*. *Aulacidea hieracii* (L.), a gall wasp reared from *Hieracium procerum* Fries (subgenus *Pilosella*) and *Hieracium robustum* Fries (subgenus *Hieracium*), which severely galls the flower stalks, did not attack any of the target weeds. Another gall wasp, *Aulacidea pilosellae* (Kieffer), galling the midrib of leaves, stolons and flower stalks, attacked two native North American hawkweed species under no-choice conditions but none of the natives exposed in open-field tests. As a negative effect on the target weeds has not yet been shown, host-range investigations are postponed. Preliminary tests with *Oxyptilus pilosellae* Zeller (Lep., Pterophoridae) were stopped due to attack of non-target species. *Macrolabis pilosellae* (Binnie) (Dipt., Cecidomyiidae), a multivoltine gall midge galling the rosette centre, flower heads and stolon tips, can develop on most native North American *Hieracium* spp. As attack occurred also in field cages and in the field, this agent was removed from the list of potential agents. The root-feeding hoverfly, *Cheilosia urbana* (Meigen) (Dipt., Syrphidae), and the rosette-feeding hoverfly, *Cheilosia psilophthalma* (Becker), develop on seven and at least two native hawkweed species, respectively, in no-choice larval transfer tests. However, under open-field conditions, attack rates of *C. urbana* on native *Hieracium* spp. are much lower than on *H. caespitosum*. Further experiments are planned to explore the level of *C. urbana* attack in the field. Neither test nor control plants were attacked by *C. psilophthalma* in open-field tests in 2005 and 2006. Therefore, host-range tests with *C. psilophthalma* are progressing slowly.

***Keywords***: host specificity, non-target feeding, *Cheilosia urbana*, *Cheilosia psilophthalma*, *Aulacidea pilosellae*, *Aulacidea hieracii*, *Aulacidea subterminalis*, *Macrolabis pilosellae*.

## Introduction

Several *Hieracium* spp. (Asteraceae, Lactuceae) of Eurasian origin have become troublesome weeds in pastures, nature reserves, roadsides, and in deforested areas in New Zealand (Syrett and Smith, 1998) and North America (Wilson *et al.*, 1997). The most invasive hawkweed species in North America are species within the subgenus *Pilosella*, which have a high rate of reproduction and dispersal due to high seed output and asexual propagation, e.g. *Hieracium caespitosum* Dumort. and *Hieracium aurantiacum* L. (Wilson and Callihan, 1999). Traditional management efforts, e.g. fertilizer and herbicide application, are not only costly but are also problematic in remote areas and nature reserves or other sensitive sites. A biological control project was therefore initiated in New Zealand in 1993

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(Syrett and Smith, 1998). Based on a literature review and initial field surveys, five European insect species attacking different plant parts of mouse-ear hawkweed, *Hieracium pilosella* L., were chosen for further investigation for New Zealand: the plume moth, *Oxyptilus pilosellae* Zeller, the gall midge, *Macrolabis pilosellae* (Binnie), the gall wasp, *Aulacidea subterminalis* Niblett, and the hoverfly species, *Cheilosia urbana* (Meigen) and *Cheilosia psilophthalma* (Becker) (Syrett *et al.*, 1999; Grosskopf, 2006). Host-specificity tests indicated that these five insect species are at least genus-specific and therefore sufficiently host-specific for release in New Zealand (Syrett *et al.*, 1999; Grosskopf, 2006) where all *Hieracium* spp. are naturalized (Webb *et al.*, 1988). In contrast, approximately 29 *Hieracium* spp. in the subgenera *Chionoracium* and *Hieracium* are indigenous to North America (Strother, 2006). Therefore, to predict the potential host range of these biological control agents, native North American *Hieracium* spp. were tested with all of the above-mentioned insect species. *O. pilosellae* was removed from the list of potential biological control agents at an early stage due to difficulties in rearing the moth and its lack of specificity, and the results are therefore not presented. A second gall wasp, *Aulacidea pilosellae* (Kieffer), was collected from several target weeds in Germany, Poland and the Czech Republic, e.g. *H. aurantiacum* and *H. caespitosum,* and thus included in the list of potential agents (Grosskopf *et al.*, 2004a). A third cynipid, *Aulacidea hieracii* (L.), was collected from *Hieracium procerum* Fries (subgenus *Pilosella*) and *Hieracium robustum* Fries plants (subgenus *Hieracium*) in the Ukraine but repeatedly failed to produce galls on any of the target weeds (Grosskopf *et al.*, 2004a,b). This paper provides an overview of the host specificity and the current status of five potential biological control agents of invasive hawkweeds in North America, i.e. *A. subterminalis*, *A. pilosellae*, *M. pilosellae*, *C. urbana* and *C. psilophthalma*.

## Potential biological control agents

### *C. urbana* (Diptera, Syrphidae)

Females of *C. urbana* oviposit into the leaf axils of *Hieracium* spp., and the neonate larvae move into the soil to feed externally on the roots, creating small holes resulting in reduced above-ground biomass (Grosskopf, 2005). In Europe, mature larvae pupate in late September/October and overwinter within the soil, very close to the surface.

### *C. psilophthalma* (Diptera, Syrphidae)

Adults of this univoltine hoverfly emerge in April and May. Females oviposit into the leaf axils of hawkweed plants and the larvae feed on the above-ground plant parts, i.e. rosette centre, leaf axils and stolon tips (Grosskopf, 2005). The larvae are mobile and can migrate among plants as host quality decreases. Mature larvae pupate on the soil surface in late September and October. *C. psilophthalma* and the root-feeding hoverfly, *C. urbana*, often co-occur and have a very similar phenology (Grosskopf, 2005).

### *M. pilosellae* (Diptera, Cecidomyiidae)

This multivoltine gall midge deforms stolon tips, flower heads and rosette centres. Females oviposit in leaf axils close to the meristematic tissue. Larval feeding prevents the unfolding of the leaves, and *M. pilosellae* larvae live gregariously in-between them. Mature larvae move into the soil where they spin a cocoon in which they pupate. The gall midge has three generations at Delémont, Switzerland, and overwinters in the larval stage. Galled plants have shorter stolons and fewer flower heads than uninfested plants (Grosskopf, 2006). According to the literature, the host range of this gall midge is restricted to *Hieracium* spp. in the subgenus *Pilosella* (Buhr, 1964).

### *A. subterminalis* (Hymenoptera, Cynipidae)

This univoltine gall wasp induces multi-chambered galls in the tips of elongating stolons. The larvae overwinter within the galls, pupate in spring and adults emerge in May and June. Adults exhibit thelytoky. In host-range tests carried out for New Zealand, only two *Hieracium* spp. out of nine, i.e. *H. aurantiacum* and *H. pilosella*, were attacked (Syrett *et al.*, 1999), indicating a narrow host range.

### *A. pilosellae* (Hymenoptera, Cynipidae)

*A. pilosellae* is a small, uni- to bivoltine gall wasp, which induces galls on stolons, midrib of leaves and flower stalks (Buhr, 1964). Thus far, we have not been able to demonstrate significant impact of this insect on plant growth in garden studies (Grosskopf *et al.*, 2007).

## Materials and methods

### Test plant list

A test plant list was compiled by L. Wilson and J. Birdsall (2001, unpublished results) based on the phylogenetic approach proposed by Wapshere (1974, 1989). Emphasis was placed on native North American *Hieracium* species in the subgenera *Hieracium* and *Chionoracium* and target weeds in the subgenus *Pilosella*. Other plant species belonging to the closely related subtribes Crepidinae, Lactucinae, Microseridinae and Stephanomeriinae were also tested but were not attacked.

## Host-range tests

All tests were carried out at the CABI Europe-Switzerland Centre at Delémont, with the exception of *A. subterminalis* testing, which was conducted in quarantine facilities at Montana State University, Bozeman, MT, USA. As all insects are of Central European origin and known to have a narrow host range, open-field and field-cage tests could be carried out without restrictions at Delémont.

## No-choice tests

No-choice larval transfer tests were carried out with *C. urbana* and *C. psilophthalma*. Seven neonate larvae were transferred into the leaf axils of potted test plants. All plants were individually covered with gauze bags and embedded in sawdust in a garden bed. All pots were checked for immature stages from the middle of September onwards. In the case of *C. urbana*, the soil was sieved and checked for larvae and puparia. In tests carried out with *C. psilophthalma*, only the upper 5 cm of the soil and the above-ground plant parts were checked for immature stages.

As *M. pilosellae* adults are short-lived and hard to relocate, three males and three females were released onto potted test plants covered with gauze bags and were left on the plants during their entire life span. *A. subterminalis* was tested in sequential no-choice tests. Three females were transferred onto a caged test plant for 3 days, and transferred onto a different test plant afterwards. In contrast, due to their small size, *A. pilosellae* adults were not retrieved from the plants. Two females and two males of *A. pilosellae* were placed onto potted test plants covered with gauze bags. The number of galls was recorded on each test plant, at the earliest 4 to 6 weeks after exposure to the gall midge or gall wasps.

## Multiple-choice tests

Potted *Hieracium* plants, i.e. target weeds and test plants, were exposed to naturally occurring *Cheilosia* females in garden beds at Delémont in 2005 and 2006. In 2005, control plants (*H. caespitosum*) and test plants were exposed simultaneously. All plants were checked at the end of the summer for mature hoverfly larvae of *C. urbana* and *C. psilophthalma*. However, as numerous test plants died before evaluation of the tests in 2005, a two-phase open-field oviposition test was carried out in 2006. In the first phase, test and control plants (*H. caespitosum* and *H. aurantiacum*) were exposed simultaneously, while in the second phase, only native North American hawkweed species were exposed. The number of eggs on the different plants was recorded. As *C. urbana* and *C. psilophthalma* eggs cannot be distinguished, eggs were kept in separate Petri dishes for hatch, and freshly hatched larvae were transferred onto *H. aurantiacum* plants to determine the syrphid species at the pupal stage (Grosskopf *et al.*, 2007). Larvae of *C. urbana* and *C. psilophthalma* have high survival rates on *H. aurantiacum*. The gall midge *M. pilosellae* was tested in three field cages measuring 2 · 2 · 1.6 m. In two cages, *H. caespitosum* and test plants were exposed simultaneously to the gall midges and, in the third one, test plants only. *M. pilosellae* adults emerged from rearing pots placed into the field cages. *A. pilosellae* adults were released onto open-field plots previously used for host-range testing of *Cheilosia* (see above). Plants were checked for galls 6 to 8 weeks after release of the adults.

# Results and discussion

As none of the insects tested developed on any plant species outside the genus *Hieracium*, we only focus on the presentation of the hawkweeds tested. The testing programme was impeded by the difficulty in rearing *Hieracium* spp. in the subgenus *Chionoracium* resulting in numerous invalid replicates. Therefore, not all *Hieracium* spp. could be tested in sufficient replicates.

Of the five insect species presented in this paper, the cynipid, *A. subterminalis*, is by far the most specific potential biological control agent attacking four *Hieracium* spp. in the subgenus *Pilosella*, i.e. *H. pilosella*, *Hieracium flagellare* Willd., *H. aurantiacum* and *Hieracium floribundum* Wimmer & Grab. (Table 1). Its narrow host range is due to the fact that *A. subterminalis* females oviposit into the stolon tips, which are exclusively produced by *Hieracium* spp. in the subgenus *Pilosella*, whereas native North American hawkweed species never produce stolons for vegetative propagation. Insects that exclusively or mainly attack stolons are therefore given priority and should result in a lower risk of non-target effects. Although *H. caespitosum* plants from Idaho also produce stolons, they were not galled by *A. subterminalis*. This gall wasp appears to be sufficiently host specific for introduction into North America.

*Hieracium scouleri* and *Hieracium bolanderi*, both native North American hawkweed species, were utilized as hosts in no-choice tests carried out with *A. pilosellae* and adults emerged from *H. scouleri* galls. However, the gall wasp has not galled any of the indigenous *Hieracium* spp. exposed in open-field tests (Table 2) conducted to date. Although *A. pilosellae* seems to have a narrow field host range, screening tests are postponed since a negative impact on the target weeds has not yet been shown (Grosskopf *et al.*, 2007). Further impact experiments with this cynipid are being carried out in 2007.

Although gall-inducing insects are generally known to have a restricted host range (Shorthouse and Watson, 1976), the gall midge *M. pilosellae* does not show a sufficient level of specificity for field release in North America. *M. pilosellae* galls 12 indigenous

**Table 1.** No-choice tests with five potential biological control candidates.

| Insect species | *Cheilosia urbana* | *Cheilosia psilophthalma* | *Macrolabis pilosellae* | *Aulacidea subterminalis* | *Aulacidea pilosellae* |
|---|---|---|---|---|---|
| | % larvae retrieved | | % plants galled | | |
| Subgenus *Pilosella* | | | | | |
| *Hieracium aurantiacum* L. | 60.7 (*n* = 8) | 52.4 (*n* = 15) | 50.0 (*n* = 6) | 38.6 (*n* = 44) | 55.6 (*n* = 9) |
| *Hieracium caespitosum* Dumort. | 25.6 (*n* = 73) | 24.9 (*n* = 59) | 83.9 (*n* = 62) | 0 (*n* = 3) | 100 (*n* = 21) |
| *Hieracium flagellare* Willd. | | | | 50.0 (*n* = 16) | |
| *Hieracium floribundum* Wimm. et Grab. | 25.4 (*n* = 9) | 10.0 (*n* = 10) | 60.0 (*n* = 15) | 52.9 (*n* = 17) | |
| *Hieracium glomeratum* Froel. | 58.7 (*n* = 9) | 52.9 (*n* = 10) | 44.4 (*n* = 9) | 0 (*n* = 7) | 100 (*n* = 4) |
| *Hieracium pilosella* L. | 27.4 (*n* = 12) | 15.9 (*n* = 9) | 83.3 (*n* = 12) | 54.3 (*n* = 46) | |
| *Hieracium piloselloides* Vill. | 44.4 (*n* = 9) | 3.2 (*n* = 9) | 53.3 (*n* = 15) | 0 (*n* = 11) | 83.3 (*n* = 6) |
| Subgenus *Hieracium* | | | | | |
| *Hieracium canadense* Michx.[a] | 16.7 (*n* = 12) | 0 (*n* = 12) | 14.3 (*n* = 21) | 0 (*n* = 15) | 0 (*n* = 14) |
| *Hieracium umbellatum* L.[a] | 4.8 (*n* = 12) | 0 (*n* = 9) | 20.0 (*n* = 30) | 0 (*n* = 13) | 0 (*n* = 16) |
| Subgenus *Chionoracium* | | | | | |
| *Hieracium albiflorum* Hook.[a] | 0 (*n* = 3) | 9.5 (*n* = 3) | 5.9 (*n* = 17) | 0 (*n* = 18) | 0 (*n* = 5) |
| *Hieracium argutum* Nutt.[a] | 0 (*n* = 3) | 0 (*n* = 1) | 0 (*n* = 1) | 0 (*n* = 18) | |
| *Hieracium bolanderi* Gray[a] | 0 (*n* = 5) | | 66.7 (*n* = 6) | 0 (*n* = 15) | 33.3 (*n* = 3) |
| *Hieracium carneum* Greene[a] | 0.8 (*n* = 18) | 0 (*n* = 15) | 47.1 (*n* = 17) | 0 (*n* = 15) | 0 (*n* = 9) |
| *Hieracium fendleri* Schultz-Bip.[a] | 0 (*n* = 12) | 0 (*n* = 1) | 14.3 (*n* = 7) | 0 (*n* = 10) | |
| *Hieracium gracile* Hook.[a] | 0 (*n* = 1) | | 25.0 (*n* = 12) | 0 (*n* = 7) | 0 (*n* = 3) |
| *Hieracium greenei* Porter et Britt.[a] | 0 (*n* = 7) | | 10.0 (*n* = 10) | 0 (*n* = 14) | |
| *Hieracium gronovii* L.[a] | 3.9 (*n* = 11) | 0 (*n* = 4) | 0 (*n* = 17) | 0 (*n* = 17) | 0 (*n* = 10) |
| *Hieracium horridum* Fries[a] | 0 (*n* = 5) | 3.6 (*n* = 4) | | 0 (*n* = 3) | |
| *Hieracium longiberbe* T. J. Howell[a] | 0 (*n* = 2) | | 16.7 (*n* = 6) | 0 (*n* = 8) | |
| *Hieracium longipilum* Torr.[a] | 6.1 (*n* = 7) | 0 (*n* = 5) | 0 (*n* = 3) | 0 (*n* = 15) | 0 (*n* = 2) |
| *Hieracium parryi* Zahn[a] | 0 (*n* = 6) | 0 (*n* = 2) | 27.3 (*n* = 11) | 0 (*n* = 11) | |
| *Hieracium scabrum* Michx.[a] | 6.1 (*n* = 7) | 0 (*n* = 9) | 37.5 (*n*=16) | 0 (*n* = 15) | 0 (*n* = 2) |
| *Hieracium scouleri* Hook.[a] | 0 (*n* = 14) | 0 (*n* = 8) | 30.8 (*n* = 13) | 0 (*n* = 11) | 80.0 (*n* = 5) |
| *Hieracium venosum* L.[a] | 3.6 (*n* = 4) | | 0 (*n* = 3) | 0 (*n* = 5) | |

Values in brackets indicate the number of replicates

[a] Hawkweed species indigenous to North America.

**Table 2.** Multiple-choice host-range tests

| | *Cheilosia urbana* | | *Macrolabis pilosellae* | | *Aulacidea pilosellae* |
|---|---|---|---|---|---|
| | *Hieracium caespitosum* present | *Hieracium caespitosum* absent | *Hieracium caespitosum* present | *Hieracium caespitosum* absent | |
| Subgenus *Pilosella* | | | | | |
| *Hieracium aurantiacum* | + | | | | + |
| *Hieracium caespitosum* | + | | + | | + |
| Subgenus *Hieracium* | | | | | |
| *Hieracium canadense*[a] | + | + | | | – |
| *Hieracium umbellatum*[a] | – | + | | | – |
| Subgenus *Chionoracium* | | | | | |
| *Hieracium albiflorum*[a] | + | + | – | | – |
| *Hieracium argutum*[a] | | | – | – | |
| *Hieracium bolanderi*[a] | – | | | | |
| *Hieracium carneum*[a] | – | + | + | + | – |
| *Hieracium fendleri*[a] | | | | | |
| *Hieracium gracile*[a] | – | – | – | | – |
| *Hieracium gronovii*[a] | + | – | + | + | – |
| *Hieracium longipilum*[a] | | | | + | – |
| *Hieracium scabrum*[a] | – | – | – | – | – |
| *Hieracium scouleri*[a] | + | + | – | – | – |
| *Hieracium venosum*[a] | + | | – | | |

*C. urbana* and *A. pilosellae* were tested in the field, whereas the gall midge *M. pilosellae* was tested in $2 \cdot 2 \cdot 1.6$ m field cages. *H. caespitosum* present: test plants and *H. caespitosum* plants were exposed simultaneously, *H. caespitosum* absent: test plants were exposed in the absence of *H. caespitosum*.

[a] Hawkweed species indigenous to North America; +, attack; –, no attack

North American *Hieracium* species, including *Hieracium carneum* and *Hieracium gronovii* (Table 1), which were also attacked in field cage tests in the presence of the target weed *H. caespitosum*. Adult midges were reared from eight native North American hawkweed species. In addition, in open-field tests carried out in 2004, the indigenous North American hawkweed species *H. carneum* and *H. scouleri* were attacked in the presence of the target weed *H. caespitosum* (Grosskopf *et al.*, 2004b). However, alien invasive hawkweeds are of increasing concern in North America (Wilson and Callihan, 1999). Recently, the European species, *Hieracium glomeratum* Froel., was recorded to also become invasive in North America (Wilson *et al.*, 2006). If *M. pilosellae* proves to be a successful biological control agent of North American target *Hieracium* species in New Zealand, a reconsideration of the risk–benefit assessment for this agent might be worthwhile.

In no-choice tests, *C. urbana* and *C. psilophthalma* develop on seven and at least two native hawkweed species, respectively. However, under open-field conditions, attack rates of *C. urbana* are much lower on the native North American *Hieracium* spp. than on the target weeds, i.e. in open-field tests, 5.6 larvae were recorded on average on *H. caespitosum* in comparison to 0.1 on *H. scouleri* and 0.3 on *H. gronovii* and *H. venosum*, respectively (Grosskopf *et al.*, 2006). Further experiments are planned to explore the level of *C. urbana* attack in the field. The low number of native *Hieracium* spp. attacked by *C. psilophthalma* is probably due to the low number of valid replicates and due to the fact that not all species have been tested. No immature stages of *C. psilophthalma* were retrieved from plants exposed in open-field tests. Due to repeated failure of open-field tests with *C. psilophthalma*, host-range investigations with this potential agent will require more time than needed for *C. urbana*.

## Conclusions

The selection of potential biological control agents for use in North America against alien invasive hawkweeds has proven difficult. We contend with a complex of target weeds and must consider potential non-target impacts on numerous native species. The most host specific of the agents tested, *A. subterminalis*, only attacks a portion of the target species, whereas less specific agents, such as *M. pilosellae*, attack most or all of the target weeds but will also infest several native species. Therefore, for a majority of our agents, we may have to balance possible non-target impacts with benefits obtained by effectively controlling the invasive species. Additional field surveys in Russia, Romania and Ukraine for stolon-attacking and gall-inducing insects are planned in the near future.

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