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Investigations on three species of Diptera associated with hawkweeds in Europe and their potential for biological control of alien invasive *Hieracium* spp. in New Zealand and North America

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Der Dekan

Table of contents

1 General introduction 1

1.1 The taxonomy and distribution of *Hieracium* 1

1.2 *Hieracium pilosella* and other hawkweeds as introduced invasive plants in New Zealand and North America 2

1.3 Plant characters facilitating invasiveness: the morphology and ecology of mouse-ear hawkweed, *H. pilosella* 3

1.4 Control methods for invasive hawkweeds 4

1.5 Classical biological weed control 5

1.6 The project for the biological control of hawkweeds in New Zealand and North America 8

1.7 Outline of this thesis 10

2 Biology and life history of *Cheilosia urbana* (Meigen) and *Cheilosia psilophthalma* (Becker), two sympatric hoverflies approved for the biological control of hawkweeds (*Hieracium* spp.) in New Zealand 12

2.1 Introduction 12

2.2 Materials and Methods 13
- 2.2.1 Adult emergence 13
- 2.2.2 Longevity 14
- 2.2.3 Experiments to obtain mating in captivity 14
- 2.2.4 Release of marked females and field collection of gravid females 14
- 2.2.5 Life span and fecundity of field-collected females 15
- 2.2.6 Egg morphology and development 16
- 2.2.7 Phenology of *C. urbana* and *C. psilophthalma* 16
- 2.2.8 Impact on *H. pilosella* 17
- 2.2.9 Parasitoids 18

2.3 Results 19
- 2.3.1 Adult emergence 19
- 2.3.2 Longevity 19
- 2.3.3 Experiments to obtain mating in captivity 20
- 2.3.4 Release of marked females and field collection of gravid females 20
- 2.3.5 Life span and fecundity of field-collected females 24
- 2.3.6 Egg morphology and development 28
- 2.3.7 Phenology of *C. urbana* and *C. psilophthalma* 29
- 2.3.8 Impact on *H. pilosella* 30
- 2.3.9 Parasitoids 33

2.4 Discussion 33

3 Host range of *Cheilosia urbana* and *Cheilosia psilophthalma* (Diptera: Syrphidae), candidates for the biological control of invasive alien hawkweeds in New Zealand 37

3.1 Introduction 37

3.2 Materials and Methods 38
- 3.2.1 General 38
- 3.2.2 No-choice larval transfer tests in Switzerland 41
- 3.2.3 No-choice larval transfer tests in New Zealand 42
- 3.2.4 Single-choice oviposition tests in Switzerland 42
- 3.2.5 Open-field tests in Switzerland 43

3.3 Results 44
- 3.3.1 No-choice larval transfer tests in Switzerland 44

3.3.2 No-choice larval transfer tests in New Zealand ... 45
3.3.3 Single-choice oviposition tests in Switzerland ... 45
3.3.4 Open-field tests in Switzerland ... 50
3.4 Discussion ... 51
3.4.1 Estimating physiological host range ... 51
3.4.2 Predicting field host range and conclusions ... 51
4 Life history and host-specificity of *Macrolabis pilosellae*, a biological control agent of hawkweeds in the subgenus *Pilosella* in New Zealand ... 55
4.1 Introduction ... 55
4.2 Materials and Methods ... 58
4.2.1 Life cycle and phenology ... 58
4.2.2 Dissection of field-collected galls ... 59
4.2.3 Longevity and fecundity of egg-laying females ... 59
4.2.4 Mortality factors ... 60
4.2.5 Host range investigations ... 60
4.3 Results ... 62
4.3.1 Life cycle and phenology ... 62
4.3.2 Dissection of field-collected galls ... 66
4.3.3 Longevity and fecundity of egg-laying females ... 68
4.3.4 Mortality factors ... 69
4.3.5 Host range investigations ... 70
4.4 Discussion ... 75
5 The impact of *Macrolabis pilosellae* herbivory on plant parameters of mouse-ear hawkweed ... 78
5.1 Introduction ... 78
5.2 Materials and Methods ... 79
5.2.1 Experiment 1 ... 79
5.2.2 Experiment 2 ... 79
5.3 Results ... 80
5.3.1 Experiment 1 ... 80
5.3.2 Experiment 2 ... 82
5.4 Discussion ... 85
6 Suitability of *Macrolabis pilosellae* for the biological control of invasive hawkweeds (*Hieracium* spp.) in North America with special regard to potential non-target effects ... 87
6.1 Introduction ... 87
6.2 Materials and Methods ... 87
6.2.1 No-choice gall formation tests with potted plants ... 90
6.2.2 Single-choice gall formation tests with potted plants ... 90
6.2.3 Single-choice oviposition tests in cups with cut plant material ... 90
6.2.4 Multiple-choice gall formation tests in field cages ... 91
6.2.5 Open-field gall formation tests ... 92
6.3 Results ... 93
6.3.1 No-choice gall formation tests with potted plants ... 93
6.3.2 Single-choice gall formation tests with potted plants ... 93
6.3.3 Single-choice oviposition tests in cups with cut plant material ... 97
6.3.4 Multiple-choice gall formation tests in field cages ... 97
6.3.5 Open-field gall formation tests ... 101

6.4 Discussion 102
7 General discussion 105
7.1 Factors facilitating the invasiveness of non-indigenous plants 105
7.2 Benefits and challenges of biological control.. 106
7.2.1 Successes in biological control... 106
7.2.2 Direct and indirect non-target effects of biological control agents 107
7.3 Suitability and potential effectiveness of the herbivores investigated................................. 108
7.3.1 Host-specificity 108
7.3.2 Life cycle and biology 109
7.3.3 Impact... 110
8 Summary.. 112
9 Zusammenfassung.. 115
10 Acknowledgements 118
11 References.. 120
Annex.. 139

1 General introduction[1]

Hieracium spp. (Asteraceae) of Eurasian origin have become weeds in New Zealand, Australia and North and South America. They are particularly a problem in low production areas, nature reserves, and forest margins. Since traditional management efforts are either uneconomic or impractical, a project for biological control was initiated. In this thesis, the biology and host-specificity of the phytophagous hoverflies *Cheilosia urbana* (Meigen) and *Cheilosia psilophthalma* (Becker), and of the gall midge *Macrolabis pilosellae* (Binnie) were investigated. The aim of this study was to evaluate the suitability and potential effectiveness of these selected herbivores as biological control agents of alien invasive hawkweeds.

1.1 The taxonomy and distribution of *Hieracium*

Hawkweeds, *Hieracium* spp., are perennial rhizomatous herbs in the Lactuceae tribe comprising a total of 850 to 1,000 species worldwide (Gottschlich, 1996). The genus is divided into the three subgenera *Hieracium* L., *Pilosella* (Hill) S. F. Gray, and *Chionoracium* Dum., syn. *Stenotheca* (Monn.) Torr. et Gray (Bräutigam, 1992). Most *Hieracium* species occur in the mountainous regions of Western Eurasia (Gottschlich, 1996), belonging exclusively to the subgenera *Hieracium* and *Pilosella*. The 120 species in the subgenus *Chionoracium* are restricted to Asia (i.e. Japan and Kamtchatka) and North, Central and South America with most species occurring in the Andes (Bräutigam, 1992; Gottschlich, 1996). Several European *Hieracium* species of the subgenera *Pilosella* and *Hieracium* have been introduced into other parts of the world, e.g. North and South America (Wilson and Callihan, 1999), New Zealand (Hunter, 1991; Rose et al., 1998), Australia (Hnatiuk, 1990), and Japan (Suzuki and Narayama, 1977) where some of them have become troublesome weeds as discussed in the following section.

[1] Parts of this chapter were the basis for the following tool:

CAB International, 2004. *Hieracium aurantiacum, Hieracium caespitosum* and *Hieracium pilosella* [original text by Grosskopf, G.]. In: Crop Protection Compendium 2004 Edition. Wallingford, UK: CAB International.

1.2 *Hieracium pilosella* and other hawkweeds as introduced invasive plants in New Zealand and North America

All ten *Hieracium* spp. naturalized in New Zealand were accidentally introduced, presumably as contaminants of agricultural seed: *H. aurantiacum* L., *H. caespitosum* Dumort., *H. pilosella* L., *H. praealtum* Vill. ex Gochnat and *H.* × *stoloniflorum* Waldst. et Kit. (subgenus *Pilosella*) and *H. argillaceum* Jordan, *H. lepidulum* (Stenström) Omang, *H. murorum* L., *H. pollichiae* Schultz-Bip. and *H. sabaudum* L. (subgenus *Hieracium*) (Makepeace, 1985a; Webb et al., 1988). Four of them, i.e. *H. pilosella, H. caespitosum, H. praealtum* and *H. lepidulum*, are considered weeds (Hunter, 1991). In their new environment, hawkweeds are particularly successful in establishing and becoming dominant in degraded grasslands with thin soil, stressed by burning, heavy grazing, and climatic events, especially drought (Hunter, 1991). In terms of overall geographic extent and the abundance of cover within affected areas, *H. pilosella* is the most significant species (Hunter, 1991). It was first recorded in New Zealand in 1878 (Webb et al., 1988).

Invasive hawkweed species have a significant economic impact in New Zealand. It is estimated that *Hieracium* spp. reduce the value of high country agricultural production by between $1.1 and $4.4 million annually (Grundy, 1989). However, unlike these direct economic costs, the environmental and aesthetic costs of hawkweeds are difficult to estimate. Negative effects of hawkweeds are (i) loss of production, (ii) loss of scenic values in national parks and other reserves, (iii) threat to native plants, and (iv) loss of conservation values and species within agricultural areas (Grundy, 1989). The benefits of *Hieracium* in New Zealand such as (i) source of umbelliferone, (ii) food source for stock, (iii) soil conservation, (iv) horticultural plants, (v) pollen source for honey production, (vi) suppression of other weeds, and (vii) seed for herbal purposes are considered negligible with regard to the negative impacts (Grundy, 1989).

Eurasian *Hieracium* spp. have also been introduced and naturalized in North America (Fernald, 1950; Gleason and Cronquist, 1991; Scoggan, 1979). Amongst these, meadow hawkweed, *H. caespitosum*, and orange hawkweed, *H. aurantiacum*, are highly invasive and are targets for biological control (Birdsall and Quimby, 1996a; Birdsall and Quimby, 1996b; Grosskopf et al. 2001; Wilson and Callihan, 1999). In North America, *H. caespitosum* is widely known under its synonym *Hieracium pratense* Tausch. An inventory

of the hawkweed species introduced from Europe is being undertaken, as it is possible that other European species of *Hieracium* may have been introduced into North America, but remain unreported or misidentified. For example, *Hieracium glomeratum* Froel., a Eurasian hawkweed recently recorded in the Pacific Northwest, was initially mistaken for *H. caespitosum* (Wilson et al., 2006). In North America, hawkweeds are primarily weeds of moist pastures, forest meadows, abandoned fields, clear-cuts, and roadsides and have shown a tendency to invade mid- to high-elevation meadows and abandoned farmland (Wilson and Callihan, 1999). Like in New Zealand, there is serious concern with the loss of native plant biodiversity and forage species in pastures in North America (Wilson and Callihan, 1999).

1.3 Plant characters facilitating invasiveness: the morphology and ecology of mouse-ear hawkweed, *H. pilosella*

Hieracium pilosella is a prostrate, monocarpic herb with a rosette of small, setose, oblanceolate, entire leaves and a single terminal shoot apex. Mouse-ear hawkweed has a single flower head per stem. Florets are sulfur-yellow, often with a red stripe on the outer face and flowering occurs mainly in May and June. Seeds are produced either sexually or by apomixis and are wind-dispersed. The basic definition of apomixis is the ability to produce a seed without fertilisation of the egg cell. Apomixis allows the rapid production of a large number of genetically uniform offspring. This strategy can be advantageous when environmental conditions are favourable for this biotype (Strasburger et al., 1991). The inevitable consequence of floral evocation is the development of one or more axillary buds into stolons that bear further apical meristems at their tips and further dormant buds in the axils of their scale-leaves. In the Northern Hemisphere, *H. pilosella* rosettes start producing stolons in April. They can reach a final length of about 10 to 30 cm, occasionally with a terminal capitulum. Under certain conditions, stolon axillary buds may break dormancy and produce branching stolons. Each branch is potentially capable of developing into a new rosette. These daughter rosettes root adventitiously, their stolon connections atrophy and the parent will die. Daughter rosettes may also develop in situ from the axillary buds of the parent rosette, without a stolon (Bishop and Davy, 1985; Gottschlich, 1996). Vegetative propagation of stoloniferous hawkweeds results in a mat-forming growth.

Because rosettes are monocarpic, i.e. dying after setting seed, there can be a high turnover of rosettes within mouse-ear hawkweed populations. Makepeace (1985a) recorded a large

difference in turnover of *H. pilosella* plants between different field sites in New Zealand ranging from five new rosettes per 100 existing ones to 173 new rosettes per 100 existing *H. pilosella* rosettes. Makepeace (1985a) found that within existing *H. pilosella* populations, spread occurs mainly by vegetative means, i.e. stolon production, whereas rosettes originating from seed accounted for only 1 % of total new plants in field plots in the Mackenzie Basin, New Zealand. Similar results were obtained for *H. floribundum* Wimm. & Grab., an invasive hawkweed of Eurasian origin in North America, where only 1 % of new plants within a population were derived from seedlings (Thomas and Dale, 1975). Nonetheless, seeds remain important for long distance spread. Moreover, a mixed strategy of clonal growth and reproduction by seeds in *H. pilosella* may be necessary to maintain populations of this species in the presence of high interspecific competition and a shortage of open space (Winkler and Stöcklin, 2002). Hybridization of *H. pilosella* is possible with numerous other *Hieracium* spp. from the subgenus *Pilosella* (Zahn, 1987). For example, there is strong evidence that within New Zealand, hybridization of *H. pilosella* with a related taxon (probably *H. praealtum*) has occurred at least three times (Trewick et al., 2004).

Mouse-ear hawkweed in its native range is a plant of sunny sand and semi-dry grasslands (e.g. sheep-grazed grasslands) but it commonly occurs also in ruderal habitats, disturbed areas, along roadsides, and in bright forests (Gottschlich, 1996; Zahn, 1987) being an indicator plant of dry, nutrient-poor sites (Caputa, 1984).

Hieracium caespitosum and *H. aurantiacum* have the same main characteristics as mouse-ear hawkweed: rosettes either originate from seeds, which are produced sexually or apomictically, or rosettes are produced vegetatively by stolons or rhizomes. However, unlike *H. pilosella*, *H. caespitosum* can also produce adventitious root-buds. In their native range, *H. caespitosum* and *H. aurantiacum* are plants of humid, nutrient-poor pastures and of disturbed areas (e.g. roadsides) but are not regarded as weeds (Gottschlich, 1996). However, in Europe the abundance of many *Hieracium* species, e.g. *H. caespitosum* or *H. cymosum* L., has decreased over recent decades due to intensive land use (e.g. agriculture) and nitrogen input through rain (Gottschlich, 1996).

1.4 Control methods for invasive hawkweeds

Meeklah (1980) found that 2.4-D ester and mecoprop/MCPA/dicamba formulations are the most efficient herbicides against mouse-ear hawkweed. Excellent control of *H. aurantiacum*

and *H. caespitosum* was obtained by applying picloram or a picloram/2.4-D mixture and good control by using clopyralid (Noel et al., 1979; Whitson et al., 1999-2000). However, because these weeds are especially abundant in low productivity areas, herbicide application is often not economical (Grundy, 1989).

Mechanical control of hawkweeds has had limited success for two reasons. Firstly, the low-growing rosettes escape mowing blades and grazing, and secondly digging or disturbance by machinery may only spread the weeds by dividing the plants (Wilson and Callihan, 1999). Moreover, recommendations concerning grazing regimes are contradictory. Makepeace (1985a) showed that removal of immature inflorescences of *H. pilosella* to simulate the effects of grazing increased the number and doubled the length of stolons produced. In contrast, Espie's (1992) conclusions for management are that seed is important in establishment of new hawkweed populations and that spring and early summer grazing may reduce hawkweed expansion rate by removing flowering culms and limiting seed production.

Apart from using herbicides, the main method to control hawkweeds at present is to develop land by oversowing with pasture species and application of fertilizer to increase the competitive ability of the more desirable species (Scott, 1993). Makepeace et al. (1985) found that *Trifolium hybridum* L. is the best competitor of the plant species investigated. Competition experiments carried out by Moen and Meurk (2001) indicate that fertilization alone as a strategy to control *Hieracium* in short-tussock grasslands may be counter-productive for low-growing native species, since *Hieracium* is likely to benefit more. Thus, hawkweeds remain a problem in areas unsuitable for economic development, retired land, reserves and national parks (Grundy, 1989). In contrast to the situation for farmers, using exotic grass and legume species to replace hawkweeds does not solve the conservation problem. Thus, the use of specific biological control agents might solve the problem and give native species a chance to re-establish or increase (Scott, 1993).

1.5 Classical biological weed control

Biological control is the use of herbivores, parasitoids, predators, and pathogens to maintain another organism's population density at a lower average than would occur in their absence (De Bach, 1964). Approaches are (i) conservation, i.e. the protection or maintenance of existing populations of biological control agents, (ii) augmentation through

releases of a biological control agent, and (iii) classical biological control (Nordlund, 1996). Classical biological weed control is the import and establishment of specialised natural enemies, e.g. arthropods, pathogens or nematodes from the area of origin of the plant, to control weed populations (Nordlund, 1996; Unruh and Woolley, 1999). Since classical biological control agents are imported without their natural enemies, they often benefit, at least temporarily, from the absence of predators, parasitoids and diseases and can therefore reach outbreak densities. A successful importation program generally results in the re-establishment of a natural regulatory relationship between the weed and the herbivore (Nordlund, 1996).

Advantages of biological weed control are (i) the introduced agents can perpetuate and distribute themselves throughout the weed's range; (ii) the impact of host-specific agents is focused on a single weed species without harm to other plants; (iii) the cost of developing biological control is relatively inexpensive compared to much higher costs for other approaches; (iv) the agents are non-polluting, energy efficient, and biodegradable; and (v) the knowledge generated during pre-release and evaluation studies contributes to improved understanding of weed ecosystems and environmental factors regulating natural communities (Cruttwell McFadyen, 1998; Goeden and Andrés, 1999 and references therein).

However, there are also risks involved such as (i) once established in an area, an introduced agent cannot be recalled or limited to parts of the target weed's range; (ii) a host-specific agent may control only one species in a weed species complex; (iii) agent impact is often slow and may require 3-4 years before local control is attained; (iv) an agent may feed and reproduce on closely related non-target plants (Goeden and Andrés, 1999 and references therein). However, an integrated weed management strategy incorporating different management practices such as biological control, mechanical and cultural control, e.g. sound grazing management, prevention and chemical control, is needed in most cases to not only successfully control a weed but also to prevent future infestation by other weedy plants (Adkins, 1997). In any case, preventing the introduction and establishment of exotic pests should continue to be the first line of defense (Ehler, 1998; Wittenberg and Cock, 2001).

An important pre-requisite for the release of exotic control organisms is a restricted host

range. Host-specificity investigations carried out prior to release aim to predict the potential host range of biological control agents (Harris, 1991). The range of plants selected for host-specificity tests has changed over the last 30-40 years. Weed biological control has always required the use of agents having a host range that precludes significant damage to desirable plants, but currently there is more concern for native congeneric plants than there has been in the past (Wan and Harris, 1997). Prior to 1970, crops and ornamentals were the main species tested regardless of their phylogenetic relationship with the target weed (McFadyen and Willson, 1997). Nowadays, closely related plants (namely natives), plants growing in the same habitat, rare and endangered species, host plants of close relatives of the potential biocontrol agent, and plants with morphological and biochemical similarities with the target weed are the main focus of testing (Cullen, 1990; Harris and Zwölfer, 1968; Heard, 1997; Wapshere, 1974; Waphere, 1989). Preliminary information about the host range of a potential agent are obtained from literature and field surveys. In order to design meaningful host range tests, the biology of the insect has to be studied beforehand, addressing the following questions: which stages of the insect damage the plant? Which developmental stages of the insect are involved in the host selection process? Which plant parts are infested? In which phenological stage is the plant being attacked?

Generally, two types of host range tests can be distinguished: (1) no-choice tests, i.e. adults or immature stages (larvae, nymphs) of the insect are exposed to a single plant species, and (2) choice tests where the insect is exposed to two or more plant species. No-choice tests evaluate the fundamental host range of an organism, i.e. the range of plant species that support its development (Cullen, 1990; Schaffner, 2001). No-choice tests are considered conservative since they provoke strong oviposition or feeding pressure. Insects thus often develop on plants, especially congeners, they would not attack in nature. However, no-choice tests are a useful indicator for plant species on which development is not possible, and which therefore can be excluded from further testing. Choice tests generally give a narrower range of acceptable plant species but results can still be misleading if the insects cannot express their natural host-selection behavior due to caging etc. (Wapshere, 1989). There are three types of choice tests: multiple-choice tests (more than one test plant species and control combined), single-choice tests (one test plant species and control combined), and choice minus control (choice of several test plant species excluding the control). An overview of the different test designs is given in Heard

and van Klinken (1998). Host range testing normally follows the centrifugal phylogenetic testing method proposed by Wapshere (1974), i.e. the potential agent is exposed to a sequence of plants from the most closely related to the weed species, progressing to successively more and more distantly related plants until the host range has been adequately circumscribed. Potential biological control agents not showing the required level of specificity are rejected to avoid feeding on plant species other than the target weed (McFadyen and Weggler-Beaton, 2000).

1.6 The project for the biological control of hawkweeds in New Zealand and North America

Since chemical and mechanical control of hawkweeds are ineffective and/or not economical, a program to develop biological control of hawkweeds with insects and a pathogen was started in 1992 for New Zealand (Syrett and Smith, 1998). Surveys carried out in New Zealand showed that *H. pilosella*, *H. caespitosum, H. praealtum* and *H. lepidulum* are not attacked to any noticeable degree by phytophagous insects in New Zealand, and that none of the insects found is specialized on hawkweeds (Syrett and Smith, 1998). *Hieracium* species may therefore have a competitive advantage over native rangeland species. Biocontrol practitioners generally agree that not all plant species are equally suitable for biological control, e.g. in terms of finding a sufficiently host-specific biological control agent due to the existence of closely related native plants (Peschken and McClay, 1995). In a cost-benefit evaluation of the potential for biological control of *Hieracium* in New Zealand hawkweeds scored relatively high because they are causing severe economic losses over considerable areas, they are still spreading, there are no native species in the genus *Hieracium*, hawkweeds are more abundant and aggressive in New Zealand than in their native range, and they are growing in relatively stable habitats (i.e. extensively used grasslands and conservation areas) where agents have a higher chance to establish and persist (Grundy, 1989). The selection of weed targets that have few or no native congeners augments the chance of finding host-specific candidates (Pemberton, 2000).

The European rust *Puccinia hieracii* var. *piloselloidarum* (Probst) Jørst. was the first organism chosen as a potential biological control agent. However, during investigations prior to introduction, it appeared that the rust fungus was already present in New Zealand (Morin and Syrett, 1996). Differences in the susceptibility of various *H. pilosella* populations

occurring in New Zealand towards the rust indicate the existence of miscellaneous genotypes of the weed. For this reason, more isolates will have to be introduced to secure effective control (Morin and Syrett, 1996).

During the search for potential biological control agents, insects developing in the vegetative parts of *H. pilosella* were prioritized for two reasons. Firstly, vegetative reproduction within existing populations appears to be much more important than propagation by seeds, and, secondly, experimental removal of flower heads increases the number and length of stolons (Makepeace, 1985a). After literature and field surveys in Europe, five insect species associated with *H. pilosella* in Central Europe were chosen for further investigations: a plume moth, a gall wasp, a gall midge and two hoverfly species, for all of which permission for field release in New Zealand has now been obtained (Großkopf, 1996; Grosskopf, 1997) (see Table 1.1).

Table 1.1. Phytophagous insect species studied at the CABI Bioscience Switzerland Centre for their use as biological control agents of *Hieracium* spp. in New Zealand (NZ) and their prospects for North America (NA). The species that were investigated in detail during this study are highlighted.

Species	Order, family	Status in NZ*	Status in NA
Oxyptilus pilosellae	Lep., Pterophoridae	released in 1999	not specific enough
<u>*Aulacidea subterminalis*</u>	Hym., Cynipidae	released in 1999	still investigated
Cheilosia urbana	Dipt., Syrphidae	released in 2002	still investigated
Cheilosia psilophthalma	Dipt., Syrphidae	release permit obtained in 2001	still investigated
<u>***Macrolabis pilosellae***</u>	Dipt., Cecidomyiidae	released in 2002	not specific enough

* Year of first field release in New Zealand; underlined: species considered as established (Lindsay Smith, Landcare Research, personal communication).

Oxyptilus pilosellae Zeller (Lep., Pterophoridae) is a univoltine plume moth, the larvae of which feed on the above-ground plant parts (Syrett et al., 1999). *Aulacidea subterminalis* Niblett (Hym., Cynipidae) is a univoltine, parthenogenetic gall wasp, which galls the stolon tips of *H. pilosella* and *H. aurantiacum* (Klöppel et al., 2003; Syrett et al., 1999). *Macrolabis pilosellae* (Dipt., Cecidomyiidae) is the only multivoltine biological control agent of the suite of five insects and it induces galls on rosettes, stolon tips and leaf axils of *H. pilosella*. Larvae of *Cheilosia urbana* (Dipt., Syrphidae) feed externally on the roots, those of *Cheilosia psilophthalma* (Dipt., Syrphidae) on the above-ground plants parts, i.e. rosette centre, stolon tips and leaf axils.

If a potential biological control agent proves to be a promising candidate, a petition to import and release the insect is submitted to the responsible authorities in the land of introduction. The petition gives a detailed overview on the taxonomic position of the candidate, its origin, life cycle, biology, host-specificity and mortality factors. Based on the information given in the petition, a committee consisting of scientists, members of governmental agencies and representatives of different organisations decide if the agent is to be released or not. In certain cases host range investigations are considered incomplete and the testing of additional plant species is proposed. In New Zealand, the import of any exotic organism has to be approved by the Environmental Risk Management Authority (ERMA). In North America, APHIS (Animal and Plant Health Inspection Service) is responsible for issuing release permits of control agents. However, the Technical Advisory Group for Biological Control Agents of Weeds (TAG) reviews petitions and provides an exchange of views, information and advice to researchers and those in APHIS (see also http://www.aphis.usda.gov/ppq/permits/tag/).

1.7 Outline of this thesis

With this thesis I want to contribute data on the ecology of three Diptera species associated with *H. pilosella*, i.e. *C. urbana*, *C. psilophthalma* and *M. pilosellae*. Since *H. pilosella* is not of economic importance in its native range and probably due to its inconspicuous growth, no quantitative data is available of its associated herbivores. Moreover, during my studies, the larval feeding niches of both *Cheilosia* species were discovered for the first time.

The current chapter introduces the theory of classical biological weed control, the problems hawkweeds cause outside their native range and the project aiming at the biological control of this group of plants. The potential biological control agents investigated so far are briefly described. Chapter 2 presents comparative data of the life history of the two sympatric phytophagous hoverfly species associated with hawkweeds. To date, detailed investigations on phytophagous hoverflies are scarce. Field observations suggest that most *Cheilosia* species seem to have a narrow host range. This taxonomic group could thus harbor further potential biological control agents. Host-specificity investigations carried out to explore the safety of the two hoverflies for release in New Zealand are described in chapter 3.

Chapter 4 presents the biology and host-specificity of the gall-inducing midge *M. pilosellae*, which has been field-released in New Zealand. Gall-inducing herbivores provoke the development of plant deformations, which are organ-, plant- and insect-specific. Gall formation is generally not comparable with e.g. external feeding or cutting. Its impact on the growth of *H. pilosella* is presented in the following chapter. Chapter 6 is dedicated to host-specificity investigations of *M. pilosellae* with regard to its suitability as potential biocontrol agent of invasive hawkweeds in North America. The fundamental difference between the screening programs for New Zealand and North American is the existence of native North American *Hieracium* species, and the implications are discussed. In chapter 7, the suitability of all three insect species for use as biological control agents is discussed in a broader context.

2 Biology and life history of *Cheilosia urbana* (Meigen) and *Cheilosia psilophthalma* (Becker), two sympatric hoverflies approved for the biological control of hawkweeds (*Hieracium* spp.) in New Zealand[2]

2.1 Introduction

During surveys for specialized phytophagous insects associated with *H. pilosella* in its native range, *Cheilosia* larvae were frequently found feeding on the aerial plant parts of mouse-ear hawkweed in the Swiss Jura and the Black Forest. Determination of *Cheilosia* females from *H. pilosella* rosettes during oviposition revealed a small number of *C. psilophthalma* females among a majority of *C. urbana* females, indicating that larvae of both species develop on *H. pilosella*. However, only oviposition records of *C. urbana*, syn. *Cheilosia praecox* (Zetterstedt), have been recorded on *H. pilosella* (Claußen, 1980). Moreover, the feeding niches of *C. urbana* and *C. psilophthalma* larvae were unknown and no information was available on the host plant of the larvae of *C. psilophthalma*. During these studies the larvae of a third *Cheilosia* species, *Cheilosia mutabilis* (Fallén), were also found to develop on mouse-ear hawkweed.

Cheilosia is one of the largest genus in the family syrphidae. There is a large discrepancy between the number of *Cheilosia* spp. described and the number of larval hosts known. Two-hundred-ninety *Cheilosia* spp. are listed in the catalogue of Palaearctic Diptera (Peck, 1988), but the larvae of only 51 European species can be reliably assigned to a host (Doczkal, 2002; Grosskopf et al., 2002; Schmid, 2000; Stuke, 2000 and references therein; Stuke and Carstensen, 2000). Most *Cheilosia* larvae feed on plants (Doczkal, 2002; Grosskopf et al., 2002; Schmid, 2000; Stuke, 2000 and references therein; Stuke and Carstensen, 2000), but there are also species that feed on mushrooms or on plant resin (Rotheray, 1993; Smith, 1979; Stuke, 2000). Although *Cheilosia* is the most species-rich genus in the family Syrphidae, the biology of a few *Cheilosia* spp. has been studied in

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[2] The data presented in this chapter were published as:

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detail, e.g. *Cheilosia fasciata* Schin. & Egg., a leaf miner on *Allium ursinum* L. (Hövemeyer, 1987; 1992), or *Cheilosia grossa* (Fallén), syn. *Cheilosia corydon* (Harris), the larvae of which feed in the stems and roots of several *Carduus* spp. (Rizza et al., 1988; Sheppard et al., 1995). In general, *Cheilosia* spp. seem to have a restricted host range since they are usually associated with a single plant species or a range of closely related plants (Doczkal, 1996; Rotheray, 1993; Smith, 1979; Stuke, 2000). Because of their narrow host range, more *Cheilosia* species could be of interest as potential biological control agents. *Cheilosia grossa* was the first *Cheilosia* species considered as a biological control agent (Rizza et al., 1988). The fly was first released in the United States in 1990 to control musk thistle, *Carduus nutans* L. (Julien and Griffiths, 1998). *Cheilosia pascuorum* (Becker) is being studied as a potential biological control agent of hounds-tongue, *Cynoglossum officinale* L. (Boraginaceae), a poisonous, invasive weed in North America (Hinz et al., 2003).

Cheilosia urbana and *C. psilophthalma* are both restricted to species within the genus *Hieracium* (Grosskopf et al., 2002), and are therefore considered safe for field-release in New Zealand where no native hawkweed species occur (Webb et al., 1988). Field-release of both hoverfly species was approved by ERMA New Zealand (Environmental Risk Management Authority of New Zealand) in June 2001 (ERMA New Zealand, 2001).

The aim of the present paper is to improve the knowledge of this large genus by describing the biology and feeding niches of these two sympatric *Cheilosia* species.

2.2 Materials and Methods

2.2.1 Adult emergence

Adult emergence of *C. urbana* and *C. psilophthalma* was recorded from immature stages used in rearing and host-specificity tests at Delémont, Switzerland. All larvae and puparia retrieved from different *Hieracium* spp. in late summer and autumn were kept individually in vials (6.6 cm length, 2.2 cm diameter) or up to ca. 30 individuals in cylinders (16 cm height, 11 cm diameter) half-filled with sieved, damp commercial potting soil. The containers were overwintered under semi-natural temperature conditions. Emergence was checked daily from early April onwards.

2.2.2 Longevity

Newly-emerged adults of *C. urbana* and *C. psilophthalma* were individually maintained in vials (2.2 cm diameter, 6.6 cm height), provided with a daisy flower head (*Bellis perennis* L., Asteraceae), a *H. pilosella* leaf, and a moistened cotton wool pad of 10% honey solution as a food/water source. Vials were maintained at 20 ± 1 °C in an incubator with a photoperiod of 16:8 h (L:D). Food and *H. pilosella* leaves were renewed every day. Daily checks were made and mortality recorded. Longevity data obtained was log-transformed to obtain normally distributed data, analyzed by ANOVA and the means were compared with Tukey's HSD test.

2.2.3 Experiments to obtain mating in captivity

Freshly-emerged *C. urbana* adults were kept in different cages and containers to obtain mating behavior. Cages (31 cm x 31 cm x 54 cm) and cylinders (11 cm diameter, 16 cm height) were kept in an unheated greenhouse and in the lab. Field cages [(200 cm x 200 cm x 200 cm) and (100 x cm x 100 cm x 100 cm)] were set up in the Centre's garden. All adults were provided with dandelion flower heads (*Taraxacum officinale* Weber, Asteraceae), cotton wool soaked with honey water as food sources and potted *H. pilosella* plants which had not yet flowered. In April 2003, 370 *C. psilophthalma* adults (180 males and 190 females) reared from overwintered puparia were transferred into a gauze cage (2 x 4 x 1.7m) in the Centre's garden. Thirty-six potted *Hieracium* plants comprising six different species were placed in one part of the cage for a multiple-choice oviposition test. Five pots containing *H. caespitosum*, and five pots containing *H. pilosella* were embedded in the second half of the cage. These plants were regularly checked for eggs. To provide food and shelter, *T. officinale* flower heads and a flowering willow were provided. The cage was partly covered with shading nets to prevent overheating and to create a mix of shady and sunny spots.

2.2.4 Release of marked females and field collection of gravid females

Since techniques for mating *Cheilosia* spp. in captivity could not be developed, naturally occurring gravid *C. urbana* and *C. psilophthalma* females as well as marked and subsequently field-released females from rearings were caught during oviposition on *Hieracium* plants in the field.

Cheilosia urbana and *C. psilophthalma* adults, which emerged from rearing and from host-

specificity tests, were marked on the thorax with a dot (ca. 0.5-1 mm diameter) of quick drying lacquer (acrylic lacquer M Color®) and released on a south facing plot (2 x 4 m) in the Centre's garden planted with *Hieracium* spp., including *H. aurantiacum*, *H. caespitosum*, *H. praealtum*, *H. pilosella*, *H. sabaudum*, and *H. ´ stoloniflorum*. The plot was located in a meadow 32 - 38 m away from a mixed forest. In order to determine the date of emergence of recaptured flies, a different color was used every one to two days (almost every day in 1997), and a sample of each color painted onto a sheet of paper for later comparison. The marked flies were released in the late afternoon of the day of emergence or during morning hours of the following day. Release during the cool part of the day discouraged immediate dispersal. Until their release, the marked flies were provided with dandelion flower heads, a common plant flowering during the flight period of both species.

Gravid *C. urbana* and *C. psilophthalma* females were attracted to the *Hieracium* plots. They were caught by hand using a transparent plastic cylinder (16 cm height, 11 cm diameter). Due to time constraints and the need to catch as many gravid females as possible, a regular sample regime for the capture of gravid females was not feasible. Flies were caught between 11.30 a.m. and 5.30 p.m. *Cheilosia urbana* and *C. psilophthalma* females did not visit the plots when it was raining, windy, or chilly, noticeable by the absence of flower-visiting insects like syrphids and honey-bees. Female flies were trapped while ovipositing by placing the cylinder over the plant. This way, only flies which were ovipositing could be easily caught. The date of emergence of marked flies could then be ascertained to the nearest 48h (or 24h in 1997), based on the lacquer color. Gravid females were also caught at three different field sites during field trips to the Black Forest, southern Germany, and at one field site in the Swiss Jura.

Cheilosia urbana and *C. psilophthalma* were identified to species using the key from Claussen and Kassebeer (1993).

2.2.5 Life span and fecundity of field-collected females

To estimate longevity and realized fecundity, both marked and unmarked field-collected females, i.e. females with known age (marked females) and unknown age (unmarked, naturally occurring females), were individually maintained in plastic vials (2.2 cm diameter, 6.6 cm height) provided with a *H. pilosella* leaf, a *B. perennis* flower head and a moistened cotton wool pad of honey solution as a food/water source. Food and leaves were renewed

daily. The vials were kept in a shaded, polythene-covered garden tunnel under semi-natural conditions. Female survival and number of eggs laid were recorded daily. However, the number of eggs laid in captivity is likely an underestimate of the females' fecundity since they already could have laid a significant number of eggs in the field. The number of eggs laid by *C. urbana* and *C. psilophthalma* females and the number of days alive in captivity were analyzed with one-way-ANOVAs followed by Scheffé multiple comparison tests, marked and unmarked *C. urbana* females were compared using Mann-Whitney *U* test.

In an oogenesis trial, 14 *C. urbana* females were dissected within 24h after emergence, while another 16 females were maintained in plastic vials (2.2 cm diameter, 6.6 cm height) for five days at 20 °C (18:6/L:D) and provided with honey water, a *B. perennis* flower head and a *H. pilosella* leaf prior to dissection. During this period there was no oviposition. Eggs of dissected females were classified as partly or fully developed.

To investigate whether there is a positive relationship between pupal weight and the number of eggs in the abdomen of five-day-old-females, 100 *C. urbana* puparia were weighed on 23 March 2000. Once the flies emerged, 35 freshly emerged females were kept for five days at 20 °C (18:6/L:D), provided with food as described above. The flies were dissected between 19 and 28 April 2000 and the number of eggs recorded. The pupal weights of females containing no developed eggs and the pupal weights of females containing partly and fully developed eggs were compared using *t*-tests for unequal variances.

2.2.6 Egg morphology and development

Egg size was recorded by measuring the length and the longest width with a micrometer mounted on a dissecting microscope. To record duration of egg development, freshly laid eggs were placed in tightly-closing plastic Petri dishes (diameter 5.5 cm) lined with moist filter paper to prevent desiccation. The eggs were incubated at 12, 15, 18, 20 and 25 °C (± 1 °C). Petri dishes were checked daily for larvae. Duration of egg development was compared using Mann-Whitney *U* tests.

2.2.7 Phenology of C. urbana and C. psilophthalma

Between 29 April and 1 May 1997, *H. pilosella* plants growing in individual clay pots (diameter at top: 18 cm) were each infested with 25 neonate *C. urbana* larvae. Due to a shortage of *H. pilosella* plants, H*. caespitosum* plants growing in clay pots (diameter: 13

cm) were used for neonate *C. psilophthalma* larvae between 6 and 15 May 1997. *Hieracium caespitosum* plants were chosen since heavy attack by *C. psilophthalma* had been observed on this plant species under field conditions (G. Grosskopf, unpublished data). All infested pots were covered with mesh bags and kept in a garden bed. The gauze was intended to protect the plants from attack by *C. urbana* and *C. psilophthalma* occurring naturally in the Centre's garden.

Pots were checked for immature stages of *C. urbana* and *C. psilophthalma* at 11-day intervals to follow the development and the location of the larvae. The aerial plant parts were searched for larvae using a binocular dissecting microscope and the soil checked several times for larvae and puparia. Soil was sieved to expose the larvae (mesh: ca. 1.5 mm x 2 mm) once they were approximately 3 mm long.

To determine the number of larval instars, larvae were preserved in Pampelsche solvent (Klausnitzer, 1991) and the apical widths of the sclerotized posterior respiratory processes were measured using an eye-piece micrometer. The sizes of *C. urbana* and *C. psilophthalma* puparia were compared using Student's *t*-test.

2.2.8 Impact on H. pilosella

A controlled experiment was set up in spring 2001 to evaluate the potential impact of *C. urbana* and *C. psilophthalma* on the growth of *H. pilosella*. Forty-eight clay-pots (13 cm diameter), each containing a rosette of *H. pilosella* with 6 to 17 leaves planted in standard potting soil, were assigned to a full-factorial design. Factors included herbivory by *C. urbana* and herbivory by *C. psilophthalma*. Between 16 and 18 May, 12 plants were infested each with either ten neonate *C. urbana* larvae, ten neonate *C. psilophthalma* larvae, or ten neonate larvae of both *C. urbana* and *C. psilophthalma*. Twelve plants were left uninfested as controls. Neonate larvae were carefully transferred into leaf axils with a moist paint brush. All pots were then covered with mesh gauze bags, kept shaded for one week to protect the larvae from extreme weather conditions and then embedded in a garden bed in the Centre's garden. Plants were checked for flower head and seed production at 2-3 week intervals throughout the summer. Between 14 and 16 November, all plants were harvested and all plant material and soil checked for *Cheilosia* puparia. *Cheilosia urbana* puparia were distinguished from *C. psilophthalma* puparia by their setae, in contrast to *C. psilophthalma* which are glabrous. The anterior respiratory stigmata of *C. urbana* are also

bright brown whereas those of *C. psilophthalma* are dark brown and shiny. The harvested plants were assessed as follows: number of leaves, number of vegetative reproductive organs, i.e. rosettes, stolon tips and rosettes or stolon tips in leaf axils, above-ground and root biomass. The biomass was dried at 80 °C for 24h and the weight taken on a micro-scale, to the nearest 0.001 g. The data was analyzed with a two-way-ANOVA. The number of vegetative reproductive organs was log-transformed, the data for above- and below-ground biomass was square-root transformed, and the number of flower heads and the number of seeds was $(x + 0.5)^{1/2}$ -transformed.

2.2.9 Parasitoids

Mature *C. urbana* larvae and puparia were collected from *H. pilosella* plants naturally infested in the Centre's garden and kept in 1.3 l cylinders half-filled with damp soil. Parasitoids were reared from overwintered puparia.

Three field collections of *C. psilophthalma* larvae were made in the Southern Black Forest and the Swiss Jura in 2002. A site near Neuenweg (47°47,721'N, 7°50.152'E) was visited on 25 July (*n* = 126 larvae), the site near Mutterslehen (47°45.811'N, 8°04.522'E), also in the Black Forest on 27 August (*n* = 63 larvae) and the site "Gorges du Pichoux" in the Swiss Jura on 15 July (*n* = 7 larvae). The rosette centres, leaf axils and leaves of *H. pilosella* and *H. lactucella* Wallr. plants were visually checked for *C. psilophthalma* larvae. All hoverfly larvae, including the rosette on which they had been feeding, were collected. To allow completion of larval development, between 7 and 24 field-collected larvae were transferred onto potted *H. caespitosum* plants. The pots (13 cm diameter), covered with gauze bags, were kept embedded in the Centre's garden. Between 20 and 27 September, all pots were checked for immature stages of *C. psilophthalma*, which were then transferred onto sieved soil in Petri dishes (9 cm diameter). The length and width of non-parasitized puparia and mummified larvae were taken on 7 November and compared with Student's *t*-test. All immature stages retrieved were overwintered under semi-natural temperature conditions and parasitoid emergence was checked in spring 2003.

Two parasitoids were reared from normal-sized and normal-looking *C. psilophthalma* puparia on 20 September 2002.

2.3 Results

2.3.1 Adult emergence

Adults of *C. urbana* and *C. psilophthalma* emerged in April and had a protandric emergence pattern. Fifty percent of *C. urbana* males emerged 7-13 days earlier than 50% of *C. urbana* females in the corresponding year (Table 2.1). The sex ratio of *C. urbana* adults (females:males) ranged from 1:1.15 to 1:0.69 , resulting in an equal long-term sex ratio over a three year period with 392 females and 391 males. Similar emergence patterns were recorded for *C. psilophthalma*. The sex ratio of *C. psilophthalma* (females:males) ranged from 1:1.83 to 1:0.56 between 1997 and 1999 with an equal long term sex ratio of 78 females and 74 males.

Four *C. mutabilis* adults were reared from *H. pilosella* and *H. praealtum*. Larvae were found on 3 May and 10 June. The four *C. mutabilis* females emerged between 7 and 27 June, i.e. approximately two months later than the *C. urbana* and *C. psilophthalma* adults.

Table 2.1. Sex ratio and date by when 50 % of *C. urbana* and *C. psilophthalma* adults from rearings had emerged (E_{50}).

		Total No. females/males emerged	Sex ratio	E_{50} females	E_{50} males
C. urbana	1997	158/181	1:1.15	12.4.97	4.4.97
	1998	162/160	1:0.99	23.4.98	16.4.98
	1999	72/50	1:0.69	23.4.99	10.4.99
C. psilophthalma	1997	6/11	1:1.83	10.4.97	4.4.97
	1998	29/39	1:1.34	25.4.98	18.4.98
	1999	43/24	1:0.56	24.4.99	10.4.99

2.3.2 Longevity

At 20 °C there were significant differences in the longevity of *C. urbana* males and females, and *C. psilophthalma* males and *C. urbana* females ($F_{3,136}$ = 6.253, P = 0.001) (Figure 2.1). *Cheilosia urbana* males lived 9.5 ± 0.47 days (mean ± SE, n = 43), *C. urbana* females 13.0 ± 0.94 days (n = 35), *C. psilophthalma* males 8.8 ± 0.59 days (n = 33), and *C. psilophthalma* females 11.0 ± 0.84 days (n = 29).

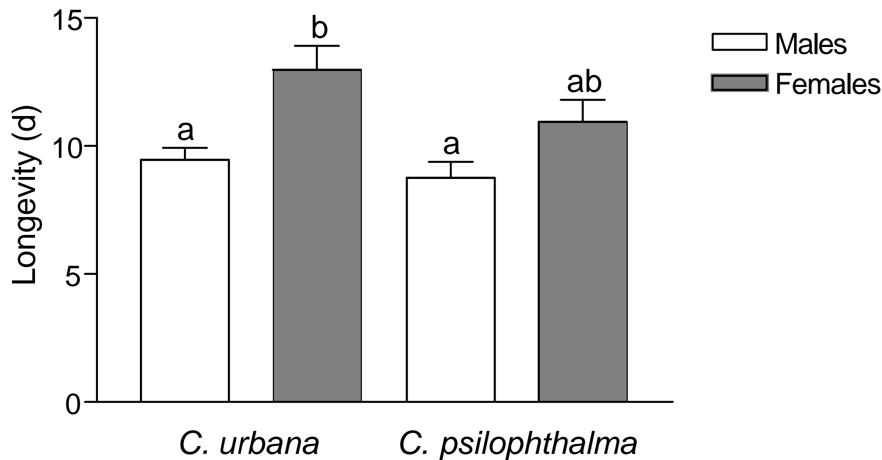

Figure 2.1. Longevity of *C. urbana* males (*n* = 43) and females (*n* = 35), and *C. psilophthalma* males (*n* = 33) and females (*n* = 29). Means with the same letter are not significantly different (Tukey's HSD test).

2.3.3 Experiments to obtain mating in captivity

Mating behavior was never observed in the garden, in the field or under controlled conditions in the greenhouse, laboratory or in field cage. Under semi-natural conditions, both *C. urbana* males and females were predominantly sitting on the gauze of their confinement. Females were observed to visit *Hieracium* rosettes and showing oviposition behavior, i.e. walking with extended ovipositor on the rosette leaves. However, only two eggs were recovered in 2003, one on *H. caespitosum* and the other on *H. pilosella*; neither was fertile (Grosskopf et al., 2004). Under all rearing conditions adults were observed to visit flower heads and cotton wool soaked with honey water for food-intake.

2.3.4 Release of marked females and field collection of gravid females

Cheilosia urbana and *C. psilophthalma* females were the only *Cheilosia* females caught in the attraction plot of *Hieracium* plants. Gravid *C. urbana* and *C. psilophthalma* females typically landed on a rosette leaf, rested or groomed for a moment or immediately started to walk downwards to the base of the leaf with an extended ovipositor. Females then turned around and moved backwards to lay an egg at the lower part of the rosette.

In three subsequent years between 17.2 and 25% of the marked and subsequently released *C. urbana* females were caught on the *Hieracium* patches during oviposition (Table 2.2). The proportion of marked *C. urbana* females caught in the Centre's garden was approximately 40% in all three years. Fewer marked *C. psilophthalma* females (i.e. 16.6%,

3.4% and 4.7%) were caught than *C. urbana*. Of all *C. psilophthalma* females caught in the Centre's garden, between 3.2 and 7.4% were marked. From 1996 throughout 2000 more *C. urbana*, i.e. between 54.2 and 90.9%, than *C. psilophthalma* females were caught in the Centre's garden (Table 2.3). *Cheilosia urbana* females were also more abundant than *C. psilophthalma* females during field collections in the Black Forest and the Swiss Jura.

Gravid *C. urbana* and *C. psiloptthalma* females were caught on the *Hieracium* patch at the Centre between 10 April and 16 May. In 1997, gravid *C. urbana* and *C. psilophthalma* females started visiting the *Hieracium* patch earlier than in the following two years (Figure 2.2). In the Black Forest *C. urbana* females were caught also in late May (Table 2.3).

Table 2.2. Number of marked *C. urbana* and *C. psilophthalma* females released and numbers of marked/unmarked females caught on the *Hieracium* attraction plot at Delémont.

	C. urbana			*C. psilophthalma*		
	1997	1998	1999	1997	1998	1999
	4 - 29 April[a]	1 April – 3 May	18 – 29 April	9 – 16 April	21 April – 1 May	17 - 29 April
No. marked females released	151	132	52	6	29	43
Total No. females caught	68	68	32	29	31	27
Marked	26 (38.2%)	27 (39.7%)	13 (40.6%)	1 (3.4%)	1 (3.2%)	2 (7.4%)
Unmarked	42 (61.8%)	41 (60.3%)	19 (59.4%)	28 (96.6%)	30 (96.8%)	25 (92.6%)
No. marked females caught[b]	26 (17.2%)	27 (20.5%)	13 (25%)	1 (16.6%)	1 (3.4%)	2 (4.7%)

[a] Release period of marked females.

[b] The total number of marked females released corresponds to 100%.

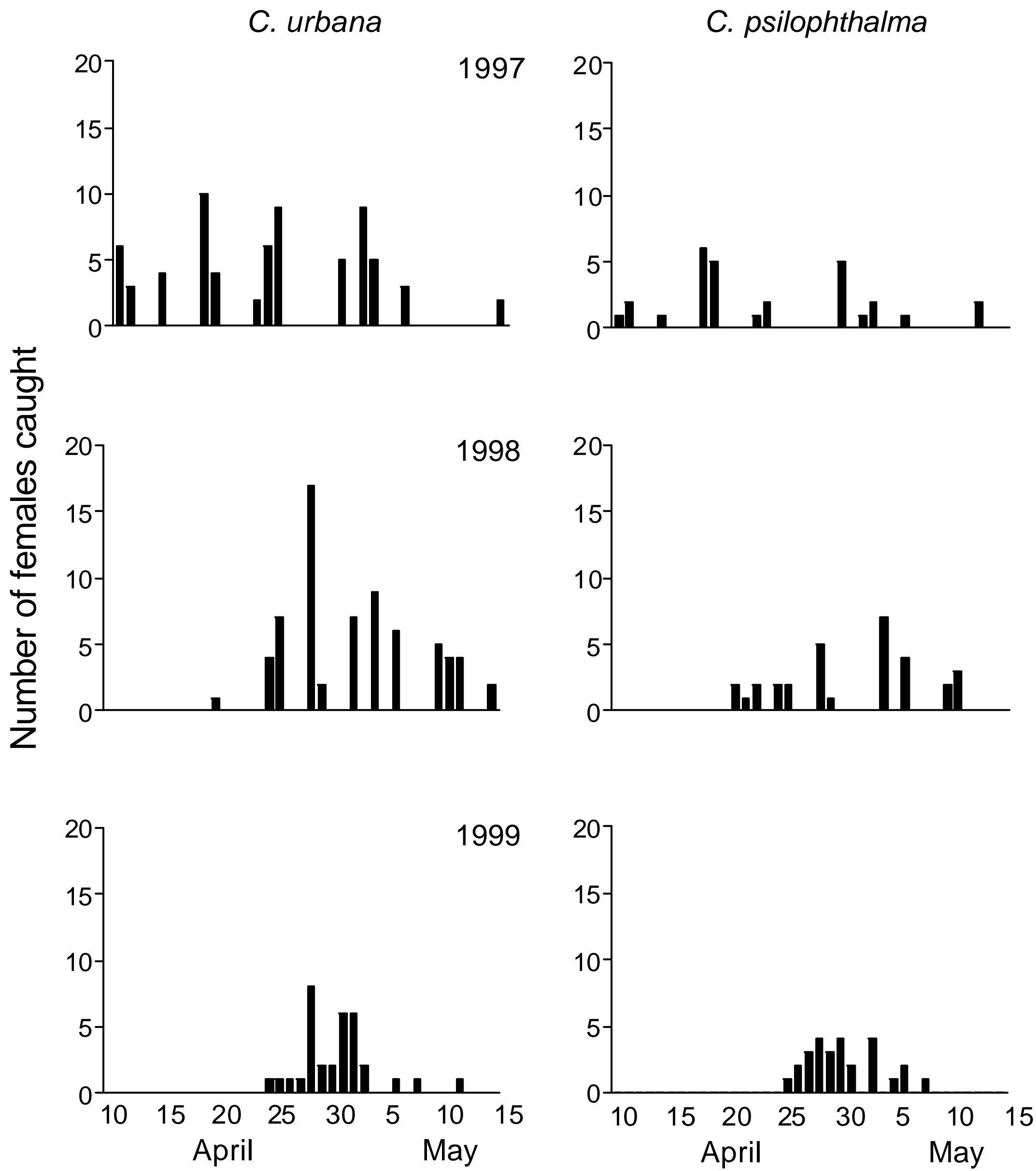

Figure 2.2. Capture of ovipositing *C. urbana* and *C. psilophthalma* females at Delémont between 1997 and 1999. The sampling regime was not consistent.

Table 2.3. Capture of ovipositing *C. urbana* and *C. psilophthalma* females at the Centre and different field sites.

Location	Region	Date or year	No. flies caught	% Flies caught (*n*)	
				C. u.	*C. psi.*
Delémont, garden	Jura (CH)	1996	22	90.9 (20)	9.1 (2)
Delémont, garden	Jura (CH)	1997	97	70.1 (68)	29.9 (29)
Delémont, garden	Jura (CH)	1998	99	68.7 (68)	31.3 (31)
Delémont, garden	Jura (CH)	1999	59	54.2 (32)	45.8 (27)
Delémont, garden	Jura (CH)	2000	108	70.4 (76)	29.6 (32)
sum			**385**	**68.6 (264)**	**31.4 (121)**
Gorges du Pichoux	Jura (CH)	7.5.1996	1	100 (1)	-
Gorges du Pichoux	Jura (CH)	13.5.1999	1	100 (1)	-
sum			**2**	**100 (2)**	**-**
Bernau	Black Forest (D)	29.5.1996	3	100 (3)	-
Bernau	Black Forest (D)	24.5.1999	7	100 (7)	-
Bernau	Black Forest (D)	10.5.2000	23	100 (23)	-
sum			**33**	**100 (33)**	**-**
near Neuenweg	Black Forest (D)	20.5.1996	1	100 (1)	-
near Neuenweg	Black Forest (D)	18.5.1999	11	81.8 (9)	18.2 (2)
near Neuenweg	Black Forest (D)	24.5.1999	11	81.8 (9)	18.2 (2)
near Neuenweg	Black Forest (D)	10.5.2000	12	75 (9)	25 (3)
sum			**35**	**80.0 (28)**	**20.0 (7)**
Wies	Black Forest (D)	18.5.1999	1	100 (1)	-

2.3.5 Life span and fecundity of field-collected females

The youngest marked *C. urbana* females caught at the *Hieracium* attraction patches in the three different years were between three and six days old (Table 2.4). One of the four marked *C. psilophthalma* females was caught after 5.5 days, the remaining ones up to 13.5 days after emergence. There was no significant difference in the longevity of marked and unmarked *C. urbana* females caught at Delémont. Marked *C. urbana* females kept in vials lived on average 16.3 days, unmarked females lived 15.1 days (U = 3574, P = 0.343). In captivity, marked *C. psilophthalma* females lived 13.3 days, unmarked females lived 13.6 days. There was no significant difference in longevity between *C. urbana* and *C. psilophthalma* females caught in the same year (Figure 2.3 A). However, there was a significant difference in-between years ($F_{5,\ 242}$ = 7.94, P < 0.001). The shortest life span of marked *C. urbana* females, i.e. the number of days in the field plus the number of days in captivity was 14.5 days, the longest 46.5 days with a mean of 27 days (Table 2.4).

Table 2.4. Fecundity and life span of marked and unmarked *C. urbana* and *C. psilophthalma* females caught at Delémont between 1997 and 1999.[a]

	Marked/unmarked	*n*	No. eggs laid in captivity[b]	No. days alive in captivity[b]	No. days emergence-capture	No. days emergence-death
C. urbana	marked	62	83.9 ± 4.31 (1-147)[**]	16.3 ± 0.99 (4-36)[n.s.]	10.7 ± 0.48 (3-19.5)	27.0 ± 1.14 (14.5-46.5)
	unmarked	99	69.2 ± 3.32 (0-184)	15.1 ± 0.71 (2-36)	-	-
	all	161	74.0 ± 2.68 (0-184)	15.5 ± 0.58 (2-36)	-	-
C. psilophthalma	marked	4	67.0 ± 23.59 (26-126)	13.3 ± 2.59 (7-18)	10.1 ± 1.7 (5.5-13.5)	23.4 ± 2.93 (16.5-28.5)
	unmarked	83	53.7 ± 4.68 (0-158)	13.6 ± 0.90 (2-37)	-	-
	all	87	54.3 ± 4.57 (0-158)	13.6 ± 0.87 (2-37)	-	-

[a] Mean values with standard error are given, numbers in brackets indicate the range.

[b] Significance level of comparison marked/unmarked *C. urbana* females (Mann-Whitney *U* test), n.s., $P = 0.05$; **, $0.001 < P < 0.05$.

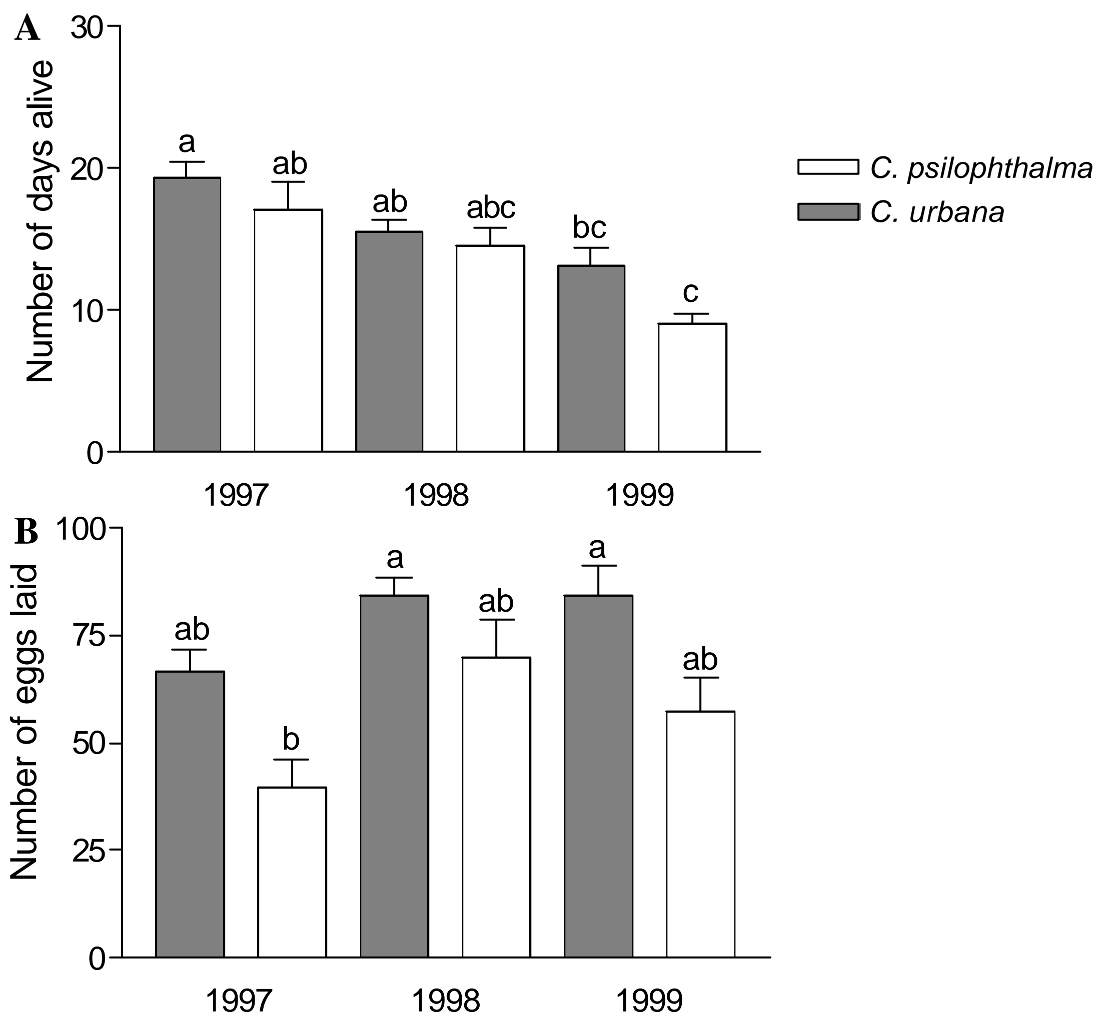

Figure 2.3. Longevity (A) and fecundity (B) of *C. urbana* and *C. psilophthalma* females caught on the attraction plot at Delémont between 1997 and 1999 and kept under semi-natural conditions. Means with the same letter are not significantly different (Scheffé multiple comparison test).

After being transferred into plastic vials, some *C. urbana* and *C. psilophthalma* females started laying eggs immediately. Between 96.8 and 100% of the *C. urbana* females caught at Delémont laid eggs in 1997-1999. Similar results were obtained for *C. psilophthalma* females with between 90.3 and 100% of the field-collected females laying eggs. The oviposition period of *C. urbana* and *C. psilophthalma* started with the capture of the first gravid female and lasted until mid/end of May. The maximum number of eggs laid by a *C. urbana* female was 184 in comparison to 158 of *C. psilophthalma*. Marked *C. urbana* females laid significantly more eggs than unmarked females when data of all three years are pooled ($U = 2930.5$, $P = 0.005$), however, when analyzed for each year individually there is no significant difference. There was a significant difference between the number of eggs

laid by *C. psilophthalma* females in 1997 and the number of eggs laid by *C. urbana* females in 1998 and 1999 ($F_{5,\ 242}$ = 6.82, P < 0.001), but the number of eggs laid by *C. urbana* and *C. psilophthalma* females caught in the same year did not differ significantly (Figure 2.3 B). There was a linear relationship between the number of days alive in captivity and the number of eggs laid by field-collected *C. urbana* and *C. psilophthalma* females (Figure 2.4).

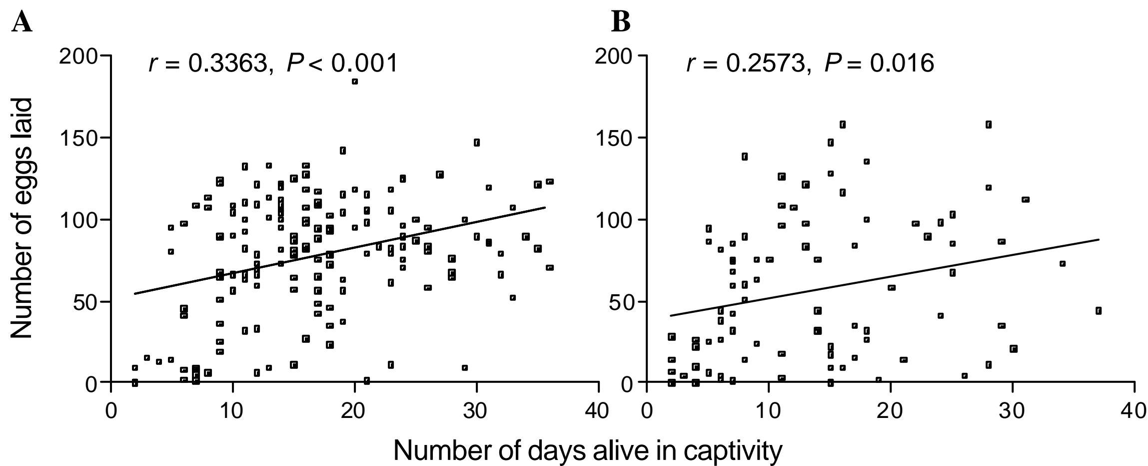

Figure 2.4. Relationship between longevity and number of eggs laid in captivity between 1997-1999, A. *C. urbana*, y = 51.03 + 1.59 x, r = 0.3363, P < 0.0001, n = 161; B. *C. psilophthalma*, y = 37.72 + 1.33 x, r = 0.2573, P = 0.0161, n = 87.

Freshly emerged *C. urbana* females contained no partly or fully developed eggs. All eggs found in the abdomen were very small and transparent. All five-day-old females contained fully and partly developed eggs, except one extremely small female. Five-day-old females contained 32.5 ± 3.50 fully developed eggs (mean ± SE, n = 16, range 0-52) and 15.1 ± 1.73 partly developed eggs (range 0-26). The total number of fully and partly developed eggs was 47.6 ± 4.48 (range 0-69). The puparium of the female which had developed no eggs after five days weighed 7.5 mg, i.e. only one third of the average weight of puparia in this study (21.2 mg). There is a linear relationship between the weight of puparia and the total number of partially and fully developed eggs in the abdomen of five-day-old females (r = 0.7993, P < 0.001, df = 33) (Figure 2.5 A, B). Nine females (25.7%) had no eggs and the weight of their puparia was significantly lower at 10.70 ± 0.513 mg (mean ± SE, n = 9) compared to 14.12 ± 0.699 mg (n = 26) of the puparia from which the five-day old females containing eggs emerged (t = 3.95, P < 0.001).

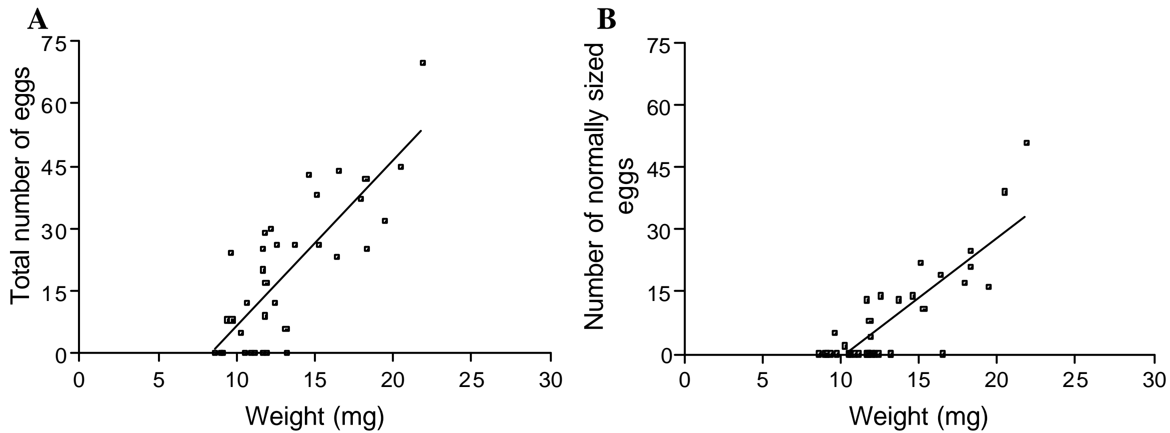

Figure 2.5. Relationship between pupal weight and the number of eggs found in the abdomen of 5-day-old *C. urbana* females. A. Total number of eggs, y = -33.51 + 4.00 x, r = 0.7992, P < 0.001, x-intercept 8.37, n = 35; B. Number of normally sized eggs, y = -30.37 + 2.93 x, r = 0.8373, P < 0.001, x-intercept 10.37, n = 35.

2.3.6 Egg morphology and development

Eggs of *C. urbana* and *C. psilophthalma* are elongated and oval. The surface has a netted structure. Freshly laid eggs are first white but turn gray if they are fertile. *Cheilosia urbana* eggs measure 0.86 ± 0.008 mm (mean ± SE, n = 68) in length and 0.32 ± 0.002 mm in width. *Cheilosia psilophthalma* eggs are similar: 0.84 ± 0.008 mm long and 0.31 ± 0.002 mm (n = 41) wide.

When incubated at 20 °C, all *C. urbana* eggs hatch in 5 days ± 0.0 (mean ± SD, n = 76), whereas *C. psilophthalma* eggs hatch in 4 days ± 0.0 (n = 114) (Figure 2.6). At the lowest incubation temperature (12 °C) *C. urbana* eggs (n = 133) hatched after 10.1 ± 0.26 days whereas *C. psilophthalma* eggs (n = 154) took 9.8 ± 0.55 days to hatch (U = 7368.5, P < 0.001). At the highest temperature used in this experiment (25 °C) *C. urbana* larvae needed on average 4.0 ± 0.19 days (n = 110) to hatch but *C. psilophthalma* larvae needed only 3.1 ± 0.33 days (n = 56) (U = 371.0, P < 0.001). No significant difference in the duration of egg development was measured at 18 °C (U = 450, P = 0.62). The developmental threshold for *C. urbana* eggs is 5.4 °C, and for *C. psilophthalma* eggs the development threshold is 2.3 °C (Figure 2.6).

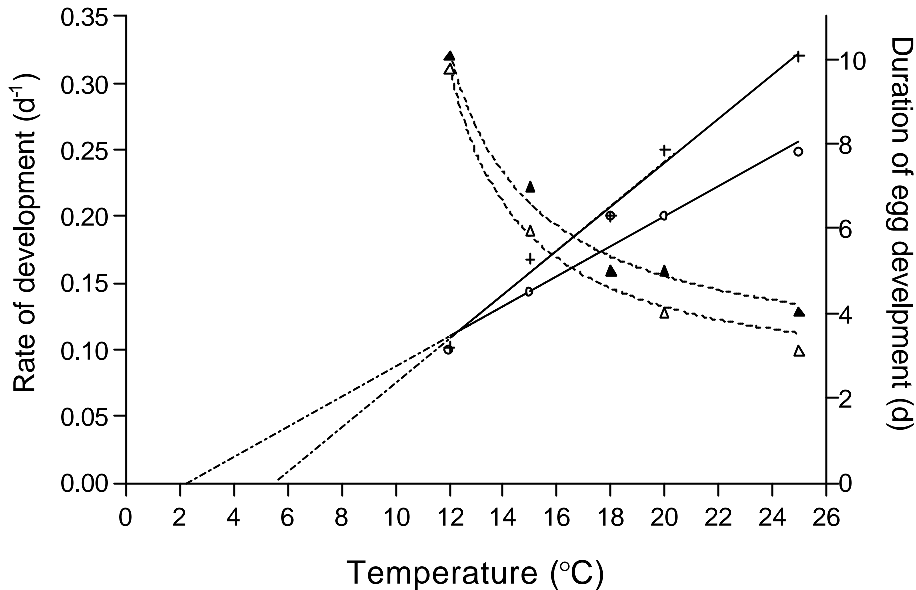

Figure 2.6. Effect of temperature on duration of egg development (time period from oviposition to larval hatch, triangles) and rate of egg development (time period from oviposition to larval hatch^{-1}, circles and crosses) of *C. urbana* (filled triangles and crosses) and *C. psilophthalma* (empty symbols); *C. urbana*, y = -0.09 + 0.0167 x, r = 0.9947, P < 0.001, n = 443; *C. psilophthalma*, y = -0.03 + 0.0113 x, r = 0.9746, P < 0.01, n = 542.

2.3.7 Phenology of C. urbana and C. psilophthalma

Dissections at 11-day intervals throughout summer 1997 revealed that *C. urbana* and *C. psilophthalma* are both univoltine species, with three larval instars of which the third is the longest duration, and they overwinter in the pupal stage (Figure 2.7). *Cheilosia urbana* larvae live in the soil and feed externally on the roots. Due to their small size early instar larvae are difficult to detect and therefore only five first instar larvae were found during the first dissection date on 12 May (Figure 2.7 A). From the third dissection date onwards, when all larvae were big enough to be reliably detected, the survival rate to mature larvae and/or puparium stage varied between 72 and 88% in the different pots. *Cheilosia urbana* puparia were found in the soil very close to the surface. *Cheilosia psilophthalma* larvae were exclusively found on the aerial plant parts feeding in rosette centres, leaf axils, stolon tips and base, and on the leaves. The number of immature stages of *C. psilophthalma* found declined drastically over time, and by 25 September and 4 November, survival was just 8% (Figure 2.7 B). On 15 October, the first *C. psilophthalma* puparia were found on the soil surface where they are always formed.

Cheilosia urbana puparia measure 5.59 ± 0.071 mm (mean ± SE, n = 38) in length and 2.59 ± 0.037 mm in width, whereas *C. psilophthalma* puparia are significantly larger with

5.94 ± 0.059 mm in length (t = -3.87, P < 0.001) and 2.98 ± 0.031 mm in width (n = 35) (t = -7.89, P < 0.001).

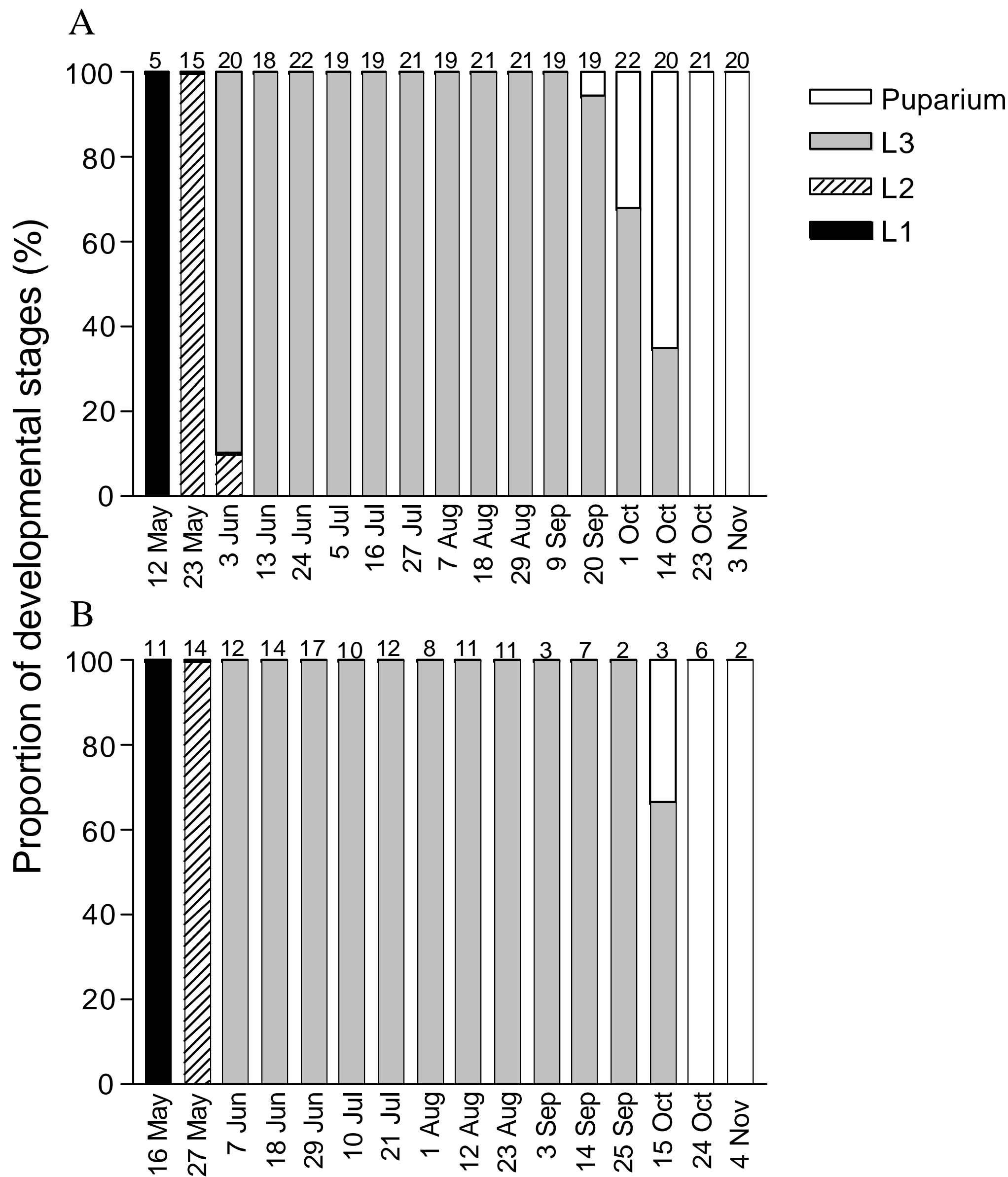

Figure 2.7. Phenology of (A) *C. urbana* and (B) *C. psilophthalma*. Data are from dissection of artificially infested plants dissected at 11-day-intervals in 1997 including inspection of the soil. Total number of individuals found are given on top of each bar.

2.3.8 Impact on H. pilosella

Below-ground herbivory by *C. urbana* reduced above-ground biomass of *H. pilosella* by 20% and below-ground biomass by 19% (Figure 2.8). In contrast, above-ground herbivory by *C. psilophthalma* increased above-ground biomass by 6% and below-ground biomass by 3% compared to uninoculated plants. *Cheilosia psilophthalma* reduced flower head and

seed production by 39%, while *C. urbana* reduced seed and flower head production by 24%. However, neither of the two hoverflies had a significant effect on the below-ground biomass, the number of meristems, the number of leaves or the number of flower heads (Table 2.5). Only below-ground herbivory by *C. urbana* had a significant effect on the above-ground biomass, and feeding by *C. psilophthalma* significantly reduced seed production.

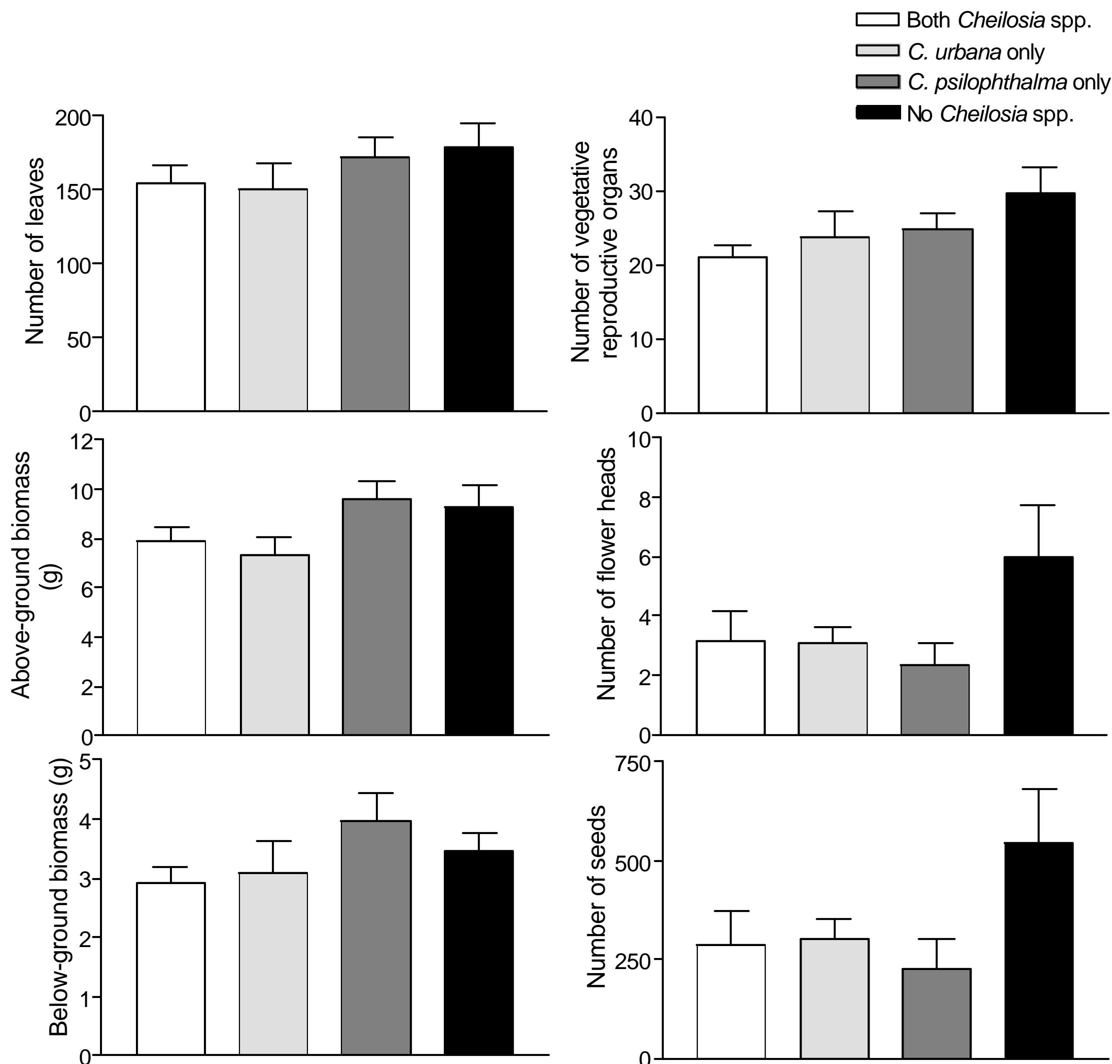

Figure 2.8. The effect of herbivory by *C. urbana* and *C. psilophthalma* on different plant parameters of potted *H. pilosella* plants (in all graphs mean ± SE are given).

The pupation rates of *C. urbana* (55%) and of *C. psilophthalma* larvae (25%), when transferred alone, were comparable to survival rates recorded on *H. pilosella* in no-choice larval transfer tests with 52.2% for *C. urbana* and 21.2% for *C. psilophthalma* (Grosskopf and Murphy, 1999). In contrast, the survival rate of *C. psilophthalma* in the presence of *C. urbana* larvae was extremely low at only 3.3%, while the survival rate of *C. urbana* was 35.8% in the presence of *C. psilophthalma* larvae.

Table 2.5. Results of two-factor ANOVA for the effects of herbivory by *C. psilophthalma* and *C. urbana* on *H. pilosella* plant size parameters.

		Number of leaves		Number of vegetative reproductive organs		Above-ground biomass		Below-ground biomass		Number of flower heads		Number of seeds	
Source of variation	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Above-ground herbivory (*C. p.*)	1	0.004	0.949	1.294	0.261	0.495	0.485	0.174	0.678	3.687	0.061	5.063	0.029
Below-ground herbivory (*C. u.*)	1	2.419	0.127	3.410	0.072	6.048	0.018	3.195	0.081	0.522	0.474	1.628	0.209
C. p. x *C. u.*	1	0.107	0.745	0.214	0.646	0.028	0.869	0.200	0.657	2.106	0.154	4.244	0.045
Error	44												

2.3.9 Parasitoids

Two *Phygadeuon* species (Hymenoptera, Ichneumonidae) and a braconid were the only parasitoids reared from immature stages of *C. urbana* and *C. psilophthalma*. *Phygadeuon* sp. 1 was reared from overwintered *C. urbana* puparia. The parasitoids emerged from mid May onwards until early June. The flies were parasitized before October but since larvae and puparia were not segregated, it is uncertain which stage of the immature insect was attacked. Two adults of *Phygadeuon* sp. 2 emerged from *C. psilophthalma* puparia in late summer.

All braconids reared from mummified *C. psilophthalma* larvae emerged between the beginning and middle of May with a protandric emergence pattern. Mummified *C. psilophthalma* larvae (length: 4.2 ± 0.03 mm (mean ± SE, $n = 110$), width: 2 ± 0.015) were significantly smaller than unparasitized puparia (length: 5.8 ± 0.071 mm, width: 2.9 ± 0.037, $n = 26$, length: $P < 0.001$, $t = -22.85$, width: $P < 0.001$, $t = -25.54$). Parasitization rates of recovered *C. psilophthalma* larvae were 94.7% ($n = 94$ retrieved larvae from Neuenweg), 51.2% ($n = 43$ retrieved larvae from Mutterslehen) and 0% ($n = 1$ retrieved larva from the Swiss Jura).

2.4 Discussion

Cheilosia urbana and *C. psilophthalma* are sympatric species that coexist in the Swiss Jura, the Black Forest and probably elsewhere in their distribution. *Cheilosia urbana* occurs throughout Europe from Scandinavia to Spain and Italy (Peck, 1988) reaching Northern Asia (Lundbeck, 1916) including alpine regions (Claussen and Kassebeer, 1993). The distribution of *C. psilophthalma* is also reported to cover most of Europe, i.e. Ireland (Speight, 1996), Germany (Ssymank et al., 1999), France (Speight et al., 1998), Poland, the former Czechoslovakia and the Tatra Mountains in Hungary which are the type locality (Peck, 1988). Neither immature stages nor adults of either species were recorded during a three-year survey for insects associated with mouse-ear hawkweed in northern central Hungary (Sárospataki, 1999). However, it is possible that *C. urbana* larvae might not have been detected due to their concealed feeding niche, e.g. if soil was not included in the root samples. *Cheilosia mutabilis*, the third *Cheilosia* species associated with hawkweeds, can be found throughout Europe, including northern Sweden and Finland, where adults fly between 26 June and 9 August (Lundbeck, 1916). Rossi (1848) reports *Carduus*

acanthoides L. as a host for *C. mutabilis*, but this is doubtful. The phytophagous insect complex associated with thistles, many of which are introduced weed species in North America (Beck, 1999; Morishita, 1999), is well studied in Europe (Freese, 1995; Rizza et al., 1988). Several *Cheilosia* spp. have been reared from both *Carduus* and *Cirsium* spp. (Dušek and Laska, 1962; Freese, 1995; Rotheray, 1988), but *C. mutabilis* has not yet been reared from thistles indicating that Rossi's findings are perhaps based on a misidentification.

During field investigations in the Swiss Jura and the Black Forest, *C. psilophthalma* females were less abundant than *C. urbana* females. One reason might be higher mortality of immature stages of *C. psilophthalma*. Due to their external, above-ground feeding mode, *C. psilophthalma* larvae are exposed to parasitoids and predators. In contrast, *C. urbana* larvae move into the soil immediately after eclosion, which could make them less susceptible to natural above-ground enemies. The parasitoids recorded during these studies were two parasitic species of *Phygadeuon*, and a braconid. *Phygadeuon* species emerge from puparia of *Cheilosia* spp. and other Diptera, including Muscidae and Anthomyiidae (Horstmann, 1986; Rizza et al., 1988). Predators and parasitoids, unless very small, were excluded from the phenology experiment described here, as well as from the controls of the host-specificity studies with these two species (Grosskopf et al., 2002), yet there was still a clear difference in survival rates between the two *Cheilosia* spp. *Cheilosia psilophthalma* larvae feeding above-ground are likely to be more affected by temperature and physical disturbance, including wind, rain and trampling by animals but this has not been investigated. Furthermore, *C. psilophthalma* larvae pupate on the soil surface without any protection from predators, e.g. arthropods, mice or birds, whereas *C. urbana* larvae pupate within the soil very close to the surface.

The main ecological difference between *C. urbana* and *C. psilophthalma* is that their larvae exploit different feeding niches and occupy different pupation locations. Exploitation of different niches on the same host plant is known for other *Cheilosia* spp. e.g. *C. canicularis* (Panzer), *C. himantopus* (Panzer), and *C. orthotricha* Vujic & Claussen on *Petasites hybridus* (L.) Gaertner. et al. (Stuke and Claußen, 2000). In some *Cheilosia* spp. e.g. *Cheilosia rhodiolae* Schmid, adults feed on pollen of the same plant species on which their larvae develop (Schmid, 2000), which is not the case for *C. urbana* and *C. psilophthalma*.

At Delémont, as well as sites in Germany and Switzerland, *H. pilosella* plants do not start flowering until the end of the oviposition period of both hoverfly species. Male *C. urbana* or *C. psilophthalma* were never observed on *H. pilosella* plants except right after emergence from the puparium in the morning hours, indicating that mating occurs elsewhere.

The release/recapture experiments give valuable indications for future field-release strategies for *C. urbana* and *C. psilophthalma*. Since mating was not obtained in captivity, flies can not be cultured under caged conditions. Therefore F1 puparia and larvae obtained from field-collected gravid females should be introduced into New Zealand and the F1 males and females emerging from these should be released. One major factor in the establishment success of biological control agents can be the number of individuals released (Williamson and Fitter, 1996). Since the number of insects available for field-release is usually limited, there is a trade-off between the release size and the number of releases (Grevstad, 1996). The high recapture rates obtained for gravid *C. urbana* females, i.e. between 17 and 25%, suggest that even releases of small quantities of flies can lead to mating which is a prerequisite for successful establishment. I would recommend releasing into relatively isolated patches in order to concentrate flies. A large number of small releases onto isolated hawkweed patches preferably similar to the conditions at our reliable field sites, e.g. close climatic match and proximity to a forest margin, appears to be most promising for establishment of the two hoverfly species. Capture of gravid *C. urbana* and *C. psilophthalma* females followed by laboratory rearing or direct release of field-collected gravid females at different field sites in New Zealand could be a helpful method to re-distribute *C. urbana* and *C. psilophthalma*. *Cheilosia grossa* has been released in North America for thistle control. In 1990, puparia were sent to Maryland and Oregon for field release. Establishment has been confirmed in Oregon and Maryland (personal communication Dr. Gaetano Campobasso 2005, USDA-ARS-EBCL), but there is no published information about its impact and host use in the field in North America.

Cheilosia spp. can have significant impacts on plants. In manipulative experiments, *C. grossa* reduced seed production of *C. nutans* by up to 45% (Sheppard et al., 1995). Its attack upsets the apical dominance of branching on the primary stem and young host plants were occasionally killed by several *C. grossa* larvae (Sheppard et al., 1995). *Hieracium pilosella* plants responded to root and above-ground herbivory by *C. urbana* and *C.*

psilophthalma, but aside from seed production and above-ground biomass, plant production was not significantly affected by the feeding of larvae, highlighting the flexibility and compensatory power of the plant in response to herbivore damage. However, the results cannot be extrapolated directly to field conditions. The *H. pilosella* plants used in the experiment were grown in potting soil resulting in a more vigorous growth than plants in the field (G. Grosskopf, personal observations). In addition, other factors in the field, such as the presence of competitive plants, may also have a negative effect on growth of *Hieracium*, especially when effects are additive or even synergistic. Reduced seed production may limit long distance seed dispersal but it is unlikely that it has an effect on the establishment of new rosettes within mouse-ear hawkweed patches. Makepeace (1985a) found that within existing *H. pilosella* populations, spread occurs mainly by vegetative means, i.e. stolon production, whereas rosettes originating from seeds accounted for only 1 % of total new plants in field plots in the Mackenzie Basin, New Zealand.

Apart from *C. urbana* and *C. psilophthalma*, three other agents have been approved for field release in New Zealand (see chapter 1). By combining several agents, the likelihood of reducing the density of hawkweeds in New Zealand is expected to increase to levels comparable with those found in the insects' native region.

3 Host range of *Cheilosia urbana* and *Cheilosia psilophthalma* (Diptera: Syrphidae), candidates for the biological control of invasive alien hawkweeds in New Zealand[3]

3.1 Introduction

Cheilosia psilophthalma and *C. urbana* were selected as potential biological control agents to complement three other agents screened for the control program in New Zealand because they occupy feeding niches not yet or only partially covered by the other herbivores. While predatory larvae of hoverflies are considered important antagonists of pest insects in crops and ornamental cultures (Franz and Krieg, 1982), to date only one hoverfly, *C. grossa*, has been used as a weed biological control agent (Julien and Griffiths, 1998). *Cheilosia grossa* was first released in 1990 (Sheppard et al., 1995) to control musk thistle (*Carduus nutans* group) in the United States (Rizza et al., 1988; Julien and Griffiths, 1998).

The purpose of this study, carried out from 1997 to spring 2000, was to determine the host range of *C. urbana* and *C. psilophthalma* in order to assess their safety for release in New Zealand. Host-specificity testing is driven by two potentially conflicting needs: first, not to introduce an agent that may cause unacceptable damage to a nontarget plant, and second, not to reject a potentially effective agent unnecessarily (Briese, 1999). It is widely believed that, under caged conditions, the results of no-choice and choice host-specificity trials can be ambiguous, giving false positive or false-negative results (Heard and van Klinken, 1998; Marohasy, 1998; Withers, 1999). Since many insect species feed and develop readily under these conditions, the results of such tests could lead to the rejection of potentially safe biological control agents and such results must therefore be supplemented by less restrictive tests (Cullen, 1990; Harris and Zwölfer, 1968; Marohasy, 1998; McEvoy, 1996; Wapshere, 1989). Starvation tests, with all their inadequacies, are quick and easy, give valuable information about the potential host range of the biological control agent, and can

[3] The data presented in this chapter were published as:

Grosskopf, G., Smith, L.A., Syrett, P., 2002. Host range of *Cheilosia urbana* (Meigen) and *Cheilosia psilophthalma* (Becker) (Diptera: Syrphidae), candidates for the biological control of invasive alien hawkweeds (*Hieracium* spp., Asteraceae) in New Zealand. Biol. Control 24, 7-19.

provide assurance that non-target species, especially unrelated economic species, will not be at risk of attack (Cullen, 1990; Heard, 1997). However, in comparison with starvation tests, results from sequential no-choice feeding trials should be carefully evaluated since insects might increasingly accept lower ranked host plants after food deprivation, which, using just-fed insects, might lead to false-negative results (Withers, 1999). Hence this study is based on a combination of approaches: no-choice larval development tests to eliminate obviously unsuitable hosts and determination of the physiological host range, and choice oviposition tests to allow behavioral cues to influence selection for oviposition.

3.2 Materials and Methods

3.2.1 General

A basic test plant list was compiled following the procedures outlined by Wapshere (1974) and Harris and Zwölfer (1968). Plant species known as hosts of other *Cheilosia* spp. (Doczkal, 1996; Rotheray, 1993; Schwarzländer et al., 1994; Smith, 1979) were added to the list. Test plant species from 29 families were included to provide a broad range (Table 3.1). Most plants belonged to the family Asteraceae and in particular the tribe Lactuceae. All *Hieracium* spp. adventive to New Zealand were included, except *H. pollichiae* for which no seeds were available. The abbreviations EUR, NZ, and USA refer to the origin of the hawkweed seeds from which the test plants were grown, i.e., Europe, New Zealand, and the United States. Taxonomy of the plants followed Tutin et al. (1964, 1968, 1972, 1976, 1980) and Hegi (1987) for European species, and Allen (1982), Healy and Edgar (1980), Moore and Edgar (1970), and Webb et al. (1988) for the New Zealand test plant species. All single-choice oviposition tests, open-field tests and the majority of no-choice larval transfer tests were carried out at the CABI Bioscience Switzerland Centre in Delémont, Switzerland. All garden beds in the Centre's garden containing *Hieracium* plants were covered with gauze nets from April to June to prevent the plants from being attacked by naturally occurring *C. urbana* and *C. psilophthalma*. No-choice larval transfer tests were also carried out in quarantine facilities at Landcare Research Ltd. at Lincoln, New Zealand, with test plant species not available at Delémont.

Table 3.1. Test plant list and number of replicates set up in no-choice larval transfer tests conducted with *C. urbana* and *C. psiloptthalma* in Switzerland and New Zealand between 1997 and 1999.

Plant species[a,b]	Category[c,d]	No. replicates *C. urbana*	No. replicates *C. psilophthalma*
Asteraceae			
Tribe: Lactuceae			
Subgenus *Pilosella*			
Hieracium pilosella L. EUR[e]	Target weed	41	34
H. pilosella L. NZ[a]	Target weed	5	5
H. pilosella L. NZ	Target weed	9	15
H. aurantiacum L.	Target weed	9	13
H. caespitosum Dumort. EUR	Target weed	7	5
H. caespitosum Dumort. NZ	Target weed	7	4
H. caespitosum Dumort. US	Target weed	6	5
H. praealtum Vill. ex Gnochat	Target weed	11	10
H. × *stoloniflorum* Waldst. & Kit.	Naturalized[c]	10	9
Subgenus *Hieracium*			
H. argillaceum Jordan	Naturalized[c]	8	9
H. lepidulum (Stenström) Omang	Target weed	9	12
H. murorum L.	Naturalized[c]	8	9
H. sabaudum L.	Naturalized[c]	8	9
Cichorium intybus L.	Cultivated	9	9
Embergeria grandifolia (Kirk) Boulos	Native	8	3
Hypochoeris radicata L.	Naturalized	8	9
Kirkianella novae-zelandiae (Hook. f.) Allan[a]	Native	5	5
Lactuca sativa L.	Cultivated	6	4
Leontodon taraxacoides (Vill.) Mérat	Naturalized	8	8
Microseris scapigera (Sol. ex A. Cunn.) Sch. Bip.	Native	8	7
Picris hieracioides L.	Native	8	6
Sonchus kirkii Hamlin	Native	7	8
Sonchus oleraceus L.	Naturalized	7	9
Taraxacum officinale Weber	Naturalized	6	6
Tragopogon porrifolius L.	Cultivated	7	8
Tribe: Anthemideae			
Artemisia dracunculus L.	Cultivated	10	6
Chrysanthemum cinerariifolium (Trev.) Vis.	Cultivated	7	10
Tripleurospermum perforatum (Mérat) Laínz	*Cheilosia* sp.	6	5
Tribe: Astereae			
Olearia avicenniaefolia (Raoul) Hook. f.	Native	6	0
Tribe: Heliantheae			
Helianthus annuus L.	Cultivated	6	6
Tribe: Inuleae			
Helichrysum bellidioides (Forster f.) Willd.[a]	Native	5	5
Helichrysum bracteatum (Vent.) Andrews	Cultivated	8	6
Gnaphalium audax D. Drury	Native	5	1
Raoulia hookeri Allan[a]	Native	5	5
Tribe: Senecioneae			
Senecio monroi Hook. f. (= *Brachyglottis monroi*)	Native	6	11
Petasites paradoxus (Retz.) Baumg.	*Cheilosia* sp.	6	0
Senecio jacobaea L.	*Cheilosia* sp.	9	6
Tribe: Cardueae			
Carduus acanthoides L.	*Cheilosia* sp.	6	3
Carduus nutans L.	*Cheilosia* sp.	6	6
Carthamus tinctorius L.	Cultivated	6	3
Cirsium palustre (L.) Scop.	*Cheilosia* sp.	6	6
Cirsium vulgare (Savi) Ten.	*Cheilosia* sp.	6	6
Cynara scolymus L.	Cultivated	8	6
Apiaceae			
Aegopodium podagraria L.	*Cheilosia* sp.	6	0
Petroselinum crispum (Miller) A. W. Hill	Cultivated	6	3
Boraginaceae			
Cynoglossum officinale L.	*Cheilosia* sp.	5	3
Brassicaceae			
Brassica oleracea L.	Cultivated	6	6
Cannaceae			
Canna indica L.	Cultivated	7	6

Table 3.1. (continued)

Plant species[a,b]	Category[c,d]	No. replicates	
		C. urbana	*C. psilophthalma*
Crassulaceae			
Sempervivum sp. L.	*Cheilosia* sp.	6	3
Caryophyllaceae			
Dianthus barbatus L.	Cultivated	6	9
Cyperaceae			
Carex testacea Boott	Native	6	3
Ericaceae			
Gaultheria crassa Allan[a]	Native	5	5
Fabaceae			
Trifolium repens L.	Cultivated	6	6
Iridaceae			
Gladiolus communis L.	Cultivated	6	3
Lamiaceae			
Mentha sp. L.	Cultivated	6	6
Liliaceae			
Allium cepa L.	Cultivated	7	6
Malvaceae			
Althea rosea L.	Cultivated	6	6
Myrtaceae			
Leptospermum scoparium J. R. & G. Forst.	Native	6	6
Oleaceae			
Olea europaea L.	Cultivated	6	6
Poaceae			
Festuca novae-zelandiae J. B. Armstr.	Native	6	3
Poa colensoi Hook. f.	Native	6	3
Agrostis tenuis Sibth.	Cultivated	6	3
Polygonaceae			
Rumex acetosella L.		6	2
Primulaceae			
Primula sp. L.	*Cheilosia* sp.	5	5
Proteaceae			
Knightia excelsa R. Br.[a]	Native	5	5
Ranunculaceae			
Clematis forsteri Gmel.	Native	5[f]	0
Clematis paniculata Gmel.	Native	1[f]	0
Ranunculus repens L.	*Cheilosia* sp.	6	6
Rhamnaceae			
Discaria toumatou Raoul	Native	2	0
Discaria toumatou Raoul[a]	Native	5	5
Rosaceae			
Filipendula ulmaria Maxim.	*Cheilosia* sp.	6	3
Rutaceae			
Citrus sp.	Cultitvated	6	6
Scrophulariaceae			
Antirrhinum majus L.	Cultivated	9	3
Theaceae			
Camellia japonica L.	Cultivated	6	0
Solanaceae			
Lycopersicon esculentum Miller	Cultivated	6	0
Urticaceae			
Urtica dioica L.		6	1
Vitaceae			
Vitis vinifera L.	Cultivated	8	3

[a] Plant species tested in quarantine facilities of Landcare Research, New Zealand.

[b] Taxonomy of the plants follows Tutin et al. (1964, 1968, 1972, 1976, 1980) and Hegi (1987) for European species, and Allen (1982), Healy and Edgar (1980), Moore and Edgar (1970) and Webb et al. (1988) for New Zealand species.

[c] *Hieracium* spp. not regarded as a noxious weed in New Zealand but attack by *Cheilosia* spp. is desired.

[d] "*Cheilosia* sp." refers to recorded host plants of other *Cheilosia* spp.

[e] EUR, NZ, and USA refer to the origin of the seeds from which the plants were grown, i.e. Europe, New Zealand and United States.

[f] Fifteen instead of five neonate larvae were transferred per replicate.

3.2.2 No-choice larval transfer tests in Switzerland

Since *C. urbana* and *C. psilophthalma* do not mate in captivity, gravid females were caught in the garden of CABI Bioscience Switzerland Centre on *Hieracium* patches planted to attract gravid females and on *H. pilosella* plants in the Black Forest. They were identified under a stereo-microscope using the key in Claussen and Kassebeer (1993). All *Cheilosia* adults which emerged from no-choice tests and rearings were released in the Centre's garden to support the local breeding population since females were being caught for host-specificity investigations. Gravid females were kept in plastic vials (6.6 cm length, 2.2 cm width) provided with a leaf of *H. pilosella*, a flower head of *B. perennis* and a cotton wool pad moistened with a honey solution as a food and water source. The plastic vials were kept in a shaded, polythene-covered tunnel at Delémont. Eggs of both species were removed daily and placed in tightly closing plastic petri dishes (5.5 cm diameter) lined with moist filter paper and kept at 20 °C with a photoperiod of 18:6 (L:D).

All plants used in the tests were potted and grown in standard potting compost. Most test plants, including all Lactuceae, were grown in clay-pots measuring 13 cm in diameter and 12 cm in height. Bigger clay or plastic pots (16-22 cm in diam., 19-25 cm in ht) were used for growing *Camellia japonica*, *Carduus nutans*, *Cirsium vulgare*, *Clematis* spp., *Cynoglossum officinale*, *Filipendula ulmaria*, *Gladiolus communis*, and *Urtica dioica*. The age of the plants varied from several weeks or months to years. All plants used in tests were sufficiently big to support the development of either five *C. urbana* or *C. psilophthalma* larvae.

No-choice larval transfer tests with *C. urbana* in Switzerland were conducted using potted plants of 70 species/biotypes in 27 families (Table 3.1). In the case of rosette plants, five freshly hatched larvae were transferred onto the leaf axils of each potted test plant using a moist paint brush. If plants had another growth pattern, larvae were placed onto the stem near the soil, preferably onto leaf axils. The plants were covered with gauze bags and kept under a shelter for three to seven days to protect the larvae from extreme weather conditions. The pots were then embedded in raised beds in the centre's garden until evaluation of the tests. Tests were set up between April and the beginning of June. Infested plants were evaluated between the middle of August and the end of September. The soil of each pot was sieved and then checked visually two or three times for immature stages of *C. urbana*. The weight of each larva was recorded to assess whether larvae reared on plant

species of genera other than *Hieracium* had a lower weight than those reared from the target plants. The larvae were then individually transferred into vials (6.6 cm length, 2.2 cm width) half-filled with damp soil. At the beginning of November, the vials were placed in an underground shelter for hibernation until the beginning of April, when they were moved to a wooden shed and their emergence checked daily. No-choice larval transfer tests with *C. psilophthalma* were set up the same way, except that the freshly hatched larvae were transferred onto leaf axils at terminal and axillary buds. For evaluation, the above-ground plant parts were carefully examined visually and the number of larvae recorded. The soil was sieved once and examined for larvae and puparia. Sixty-two plant species/biotypes in 24 families were tested (Table 3.1).

The number of *C. urbana* and *C. psilophthalma* larvae retrieved from each test plant and adult survival of *C. urbana* were analyzed by analysis of variance (ANOVA) and the means were compared with Tukey-HSD test. The data for larval survival of *C. psilophthalma* was $(x + 0.5)^{1/2}$-transformed to obtain homogeneity of variances. Since homogenous variances of adult survival rates of *C. psilophthalma* could not be obtained, data was analyzed using the Kruskal-Wallis test.

3.2.3 No-choice larval transfer tests in New Zealand

Cheilosia urbana and *C. psilophthalma* eggs obtained from females caught at Delémont were imported from CABI Bioscience Switzerland into Landcare Research's containment facility at Lincoln where they were maintained on moist filter paper in Petri dishes under warm, spring conditions (18 °C at day, 12 °C at night and a photoperiod of 12:12 (L:D)). As larvae emerged, they were transferred to leaf axils of potted test plants and these were then arranged at random in plastic (Perspex®) boxes (50 cm x 50 cm x 75 cm) and maintained in the containment cell under the same conditions as the eggs. Five larvae were placed on each of five replicates for each test plant. After 12 to 16 weeks, the test plants were removed from their pots, the soil sieved, and all parts of the plant examined for larvae and pupae. Those retrieved were weighed. Six plant species native to New Zealand were tested with *H. pilosella* as natural host plants (Table 3.1).

3.2.4 Single-choice oviposition tests in Switzerland

During daylight hours, one female hoverfly was transferred onto a pot (18-cm diam.)

containing the target weed, *H. pilosella*, and a test plant grown in standard potting compost covered with a gauze bag. Two cotton wool pads, one soaked in honey-water and the other in fresh water, and a *B. perennis* flower head were offered to the flies as food and water sources. The pots were either kept outside under a semi-transparent roof between two greenhouses, or, during cold days, when females would not oviposit outside, in an insectary with artificial light for approximately 8 h. Afterwards, the number of eggs laid onto each plant was counted.

To find out whether females perceive both plants when offered simultaneously, pots containing two rosettes of *H. pilosella* without a test plant were also set up. Only pots with at least one egg were analyzed. Apart from *Hieracium* spp. belonging to the subgenera *Pilosella* and *Hieracium*, *Embergeria grandifolia* (Asteraeae), and *Filipendula ulmaria* (Rosaceae) were used in single-choice oviposition tests carried out with *C. urbana*. *Embergeria grandifolia* was tested since one extremely small *C. urbana* larva was found in the soil of a potted *E. grandifolia* plant during preliminary no-choice larval transfer tests in 1996. This larva did not pupate and had an extremely low weight of 8.5 mg in comparison to an average 20.9 mg of *C. urbana* larvae extracted from the soil of the target weed *H. pilosella* (Grosskopf, 1996). *Filipendula ulmaria* was included in the tests because egg-laying behavior of a *C. urbana* female in the field has been reported by Kassebeer (1993). The Wilcoxon Signed Ranks Test was applied for test plant species with at least six replicates, i.e., *H. lepidulum*, *H. murorum*, and *H. sabaudum*.

3.2.5 Open-field tests in Switzerland

Five plots, each measuring 1 m^2 and containing 16 pots with four different plant species arranged in a randomized complete block design, were exposed in the Centre's garden from April until evaluation in the second half of September. *Cheilosia urbana* and *C. psilophthalma* females occur naturally in the Centre's garden in April and May. The above-ground plant parts and the soil were checked for larvae and puparia. Plots were established in the Centre's garden, two in 1998 and three in 1999. Since *E. grandifolia* plants used in 1998 started to die in July but still had excellent root systems, they were evaluated at the end of July, whereas the other plants were checked two weeks later in mid August.

3.3 Results

3.3.1 No-choice larval transfer tests in Switzerland

Neonate *C. urbana* larvae developed to maturity larvae on eight of the nine *Hieracium* spp. tested but not on *H. murorum* (Table 3.2) or on plants outside the genus *Hieracium* (data not shown). The survival rate of *C. urbana* larvae on the various *Hieracium* spp. differed significantly ($F_{11, 121} = 6.97$, $P < 0.001$) and ranged from 0.6 larvae (12.5% survival) per replicate on *H. argillaceum* to 4.3 larvae (86.7% survival) on *H. caespitosum* USA. On average 3.1 larvae (62% survival) were found on the target weed, the European accession of *H. pilosella* (Table 3.2). Fewer larvae survived on *H.* × *stoloniflorum*, *H. argillaceum*, and *H. murorum* than on the target plant, the European *H. pilosella*. *Cheilosia urbana* adults emerged from all *Hieracium* species on which larvae had been found during evaluation of the tests. Survival from neonate larva to adult varied between hawkweed species ($F_{11, 121} = 5.76$, $P < 0.001$) and ranged from 2.5% on *H. argillaceum* to 80.0% on *H. caespitosum* (USA) to 46.8% on *H. pilosella* (Europe). Fewer adults emerged from *H. argillaceum*, *H.* × *stoloniflorum*, a hybrid between *H. aurantiacum* L. and *H. pilosella*, and *H. murorum* than from *H. pilosella* (Europe).

Mature *C. psilophthalma* larvae were found on all nine hawkweed species tested (Table 3.3), whereas plants outside the genus *Hieracium* proved to be unsuitable larval hosts (data not shown). Larval survival varied between *Hieracium* species ($F_{11, 122} = 5.40$, $P < 0.001$). In comparison to *C. urbana*, survival rates of *C. psilophthalma* larvae were lower on *H. pilosella* (Europe) ($t = 5.83$, $P < 0.001$), *H. caespitosum* (New Zealand) ($t = 2.79$, $P < 0.05$), *H. caespitosum* (USA) ($t = 3.28$, $P < 0.05$) and *H. lepidulum* ($U = 11.5$, $P < 0.01$) but no difference was recorded on the other *Hieracium* species. Survival of *C. psilophthalma* larvae was lowest on *H. argillaceum* with an average 0.1 larvae per replicate (2.2% survival) and highest on *H. pilosella* (New Zealand) with 2.7 larvae per pot (54.7% survival) (Table 3.3). More larvae survived on *H. pilosella* (New Zealand) in the subgenus *Pilosella*, than on *H. argillaceum*, *H. lepidulum*, *H. murorum,* and *H. sabaudum* in the subgenus *Hieracium*. *Cheilosia psilophthalma* adults emerged from all *Hieracium* spp. on which larvae had been found during evaluation of the tests (Table 3.3) but survival from neonate larva to adulthood differed between *Hieracium* species ($H_{11, 122} = 47.71$, $P < 0.001$).

3.3.2 No-choice larval transfer tests in New Zealand

Neither *C. urbana* nor *C. psilophthalma* larvae developed on test plants outside the genus *Hieracium*. On average 2.8 *C. urbana* larvae (56% survival) were retrieved per replicate in comparison to 2.0 *C. psilophthalma* larvae (40% survival) per replicate. The 14 *C. urbana* larvae were on average 14.7 ± 1.19 mg in mass in comparison to 21.7 ± 1.58 mg (n = 10 larvae) for *C. psilophthalma*.

3.3.3 Single-choice oviposition tests in Switzerland

In five out of seven cases, both *H. pilosella* rosettes offered simultaneously were accepted for oviposition by gravid *C. urbana* females (Table 3.4). *Cheilosia urbana* females oviposited exclusively onto *Hieracium* spp. whereas no eggs were laid on *F. ulmaria* or on *E. grandifolia*. The lowest proportions of eggs were found on *H. murorum* and *H. praealtum* and the highest on *H. caespitosum* and *H. aurantiacum*. The target weed *H. pilosella* was preferred over *H. murorum* ($P < 0.01$) and *H. lepidulum* ($P < 0.05$) but no significant difference was recorded between *H. pilosella* and *H. sabaudum* ($P = 0.312$).

Fewer tests were conducted with *C. psilophthalma*. All plant species offered were accepted for oviposition in the presence of the target weed *H. pilosella* (Table 3.5). The highest proportion of eggs was recorded on *H. aurantiacum* and the lowest on *H. lepidulum*.

Table 3.2. Results of no-choice larval transfer tests with *C. urbana* conducted during 1997-1999.[a]

Test plant	Total No. L1 transferred	No. plants[b]	Mature larvae recovered		Weight of retrieved larvae		No. developing to adult	
			Mean ± SE[c]	%[d]	*n*	Mean ± SE	Mean ± SE[c]	%
Subgenus *Pilosella*								
Hieracium pilosella EUR	205	41	3.1 (0.20) ab	62.0	127	21.7 (0.52)	2.3 (0.24) ab	46.8
H. pilosella NZ	45	9	2.8 (0.47) abc	55.6	25	19.5 (0.96)	2.2 (0.57) ac	44.4
H. aurantiacum	45	9	2.6 (0.56) abcd	51.1	23	24.6 (0.64)	2.2 (0.55) ac	44.4
H. caespitosum EUR	35	7	2.9 (0.55) abcd	57.1	20	19.5 (1.22)	2.3 (0.42) ad	45.7
H. caespitosum NZ	35	7	2.9 (0.34) abcd	57.1	20	16.0 (0.55)	2.1 (0.51) ad	42.9
H. caespitosum USA	30	6	4.3 (0.33) a	86.7	26	23.2 (0.65)	4.0 (0.37) a	80.0
H. praealtum	55	11	2.5 (0.47) abcd	50.9	28	21.6 (1.18)	1.9 (0.46) ad	38.2
H. ×*stoloniflorum*	50	10	1.4 (0.45) ce	28.0	14	16.5 (0.77)	0.6 (0.31) cd	12.0
Subgenus *Hieracium*								
H. argillaceum	40	8	0.6 (0.50) de	12.5	5	15.1 (0.70)	0.1 (0.13) cd	2.5
H. lepidulum	45	9	2 (0.41) be	40.0	18	20.0 (1.25)	1.2 (0.32) bcd	24.4
H. murorum	40	8	0 e	0	-	-	0 d	0
H. sabaudum	40	8	1.9 (0.52) be	37.5	15	20.3 (0.92)	1.5 (0.46) bcd	30.0

[a] Plants outside the genus *Hieracium* remained free of attack (see Table 3.1 for plant species tested and the number of replicates).

[b] Five newly hatched *C. urbana* larvae were transferred onto each plant.

[c] ANOVA followed by Tukey-HSD multiple comparison test, homogenous groups are represented by equal letters, $P < 0.05$.

[d] Percentage number of larvae retrieved divided through total number of neonate larvae transferred.

Table 3.3. Results of no-choice larval transfer tests with *C. psilophthalma* conducted during 1997-1999.[a]

Test plant	Total No. L1 transferred	No. plants[b]	Mature larvae/puparia recovered		Weight of retrieved larvae		Development to adult	
			Mean ± SE[c]	%[d]	*n*	Mean ± SE	Mean ± SE	%
Subgenus *Pilosella*								
Hieracium pilosella EUR	170	34	1.4 (0.22) abc	27.1	45	26.3 (1.00)	0.9 (0.18)	17.6
H. pilosella NZ	75	15	2.7 (0.38) a	54.7	37	29.1 (0.73)	2.3 (0.32)	45.3
H. aurantiacum	65	13	2.3 (0.43) ad	46.2	30	31.2 (31.2)	2.2 (0.39)	43.1
H. caespitosum EUR	25	5	1.8 (0.58) abc	36.0	9	28.3 (1.34)	1.8 (0.58)	36.0
H. caespitosum NZ	20	4	1.3 (0.48) abc	25.0	4	24.7 (3.96)	0.8 (0.48)	15.0
H. caespitosum USA	25	5	2.4 (0.51) ab	48.0	10	25.7 (2.74)	1.6 (0.68)	32.0
H. praealtum	50	10	2.2 (0.51) ab	44.0	20	32.4 (1.54)	2.1 (0.53)	42.0
H. ×*stoloniflorum*	45	9	1.4 (0.58) abc	28.9	12	23.0 (1.40)	1.4 (0.58)	28.9
Subgenus *Hieracium*								
H. argillaceum	45	9	0.1 (0.11) c	2.2	1	30.6	0.1 (0.11)	2.2
H. lepidulum	60	12	0.3 (0.18) c	5.0	3	34.1 (5.41)	0.1 (0.08)	1.7
H. murorum	45	9	0.4 (0.29) c	8.9	2	26.2 (1.10)	0.2 (0.15)	4.4
H. sabaudum	45	9	0.8 (0.28) bcd	15.6	7	25.8 (1.97)	0.6 (0.24)	11.1

[a] Plants outside the genus *Hieracium* remained free of attack (see Table 3.1 for plant species tested and the number of replicates).

[b] Five newly hatched *C. psilophthalma* larvae were transferred onto each plant.

[c] ANOVA followed by Tukey-HSD multiple comparison test, homogenous groups are represented by equal letters, $P < 0.05$.

[d] Percentage number of larvae and puparia retrieved divided through total number of neonate larvae transferred.

Table 3.4. Results of single-choice oviposition tests offering a control plant, *H. pilosella* EUR, and a test plant simultaneously to a field-collected *C. urbana* female in Switzerland in 1999.

Test plant	No. eggs laid on test/control plant										No. eggs laid on		Percentage of eggs laid on		Factor of acceptance[a]
	Replicates										Test	Control	Test	Control	
<u>Subg. *Pilosella*</u>															
H. pilosella	22/5	0/25	6/7	1/0	30/5	3/48	9/26				-	-	-	-	-
H. aurantiacum	10/0	5/1	3/0	3/12	0/3						21	16	56.8	43.2	1.31
H. caespitosum	45/0	36/1	32/8								113	9	92.6	7.4	12.56
H. praealtum	3/22	0/2	3/5	0/47	0/40						6	116	4.9	95.1	0.05
<u>Subg. *Hieracium*</u>															
H. lepidulum	0/2	1/4	1/2	0/1	0/20	0/16	0/3	8/8	0/4		10	60	14.3	85.7	0.17
H. murorum	0/5	0/9	0/5	0/1	1/56	0/5	0/1	0/7	0/30	1/10	2	129	1.5	98.5	0.02
H. sabaudum	0/7	4/1	0/4	0/36	0/7	12/5	0/1	0/32	10/1		26	94	21.7	78.3	0.28
E. grandifolia	0/22	0/4	0/4	0/4	0/4	0/31	0/22	0/35			0	126	0	100	0
F. ulmaria	0/31	0/8	0/25	0/3	0/9	0/1					0	77	0	100	0

[a] No. eggs laid on the test plant divided by the number of eggs laid on the control plant: factor of acceptance = 1, no preference; factor of acceptance > 1, test plant was preferred; factor of acceptance < 1, control was preferred.

Table 3.5. Results of single-choice oviposition tests offering a control plant, *H. pilosella* (Europe), and a test plant simultaneously to a field-collected *C. psilophthalma* female in Switzerland in 1999.

Test plant	No. eggs laid on test/control plant				No. eggs laid on		Percentage of eggs laid on		Factor of acceptance[a]
	Replicates				Test	Control	Test	Control	(control = 1)
Subgenus *Pilosella*									
H. aurantiacum	10/5	7/30	0/1	45/5	62	41	60.2	39.8	1.51
Subgenus *Hieracium*									
H. lepidulum	5/20	0/6	0/8		5	34	12.8	87.2	0.15
H. murorum	0/45	0/8	13/4		13	57	18.6	81.4	0.23
H. sabaudum	1/2	2/1	18/3	7/44	28	50	35.9	64.1	0.56

[a] No. eggs laid on the test plant divided by the number of eggs laid on the control plant: factor of acceptance = 1, no preference; factor of acceptance > 1, test plant was preferred; factor of acceptance < 1, control was preferred.

3.3.4 Open-field tests in Switzerland

Apart from one *C. psilophthalma* larva feeding on *H. aurantiacum*, only *C. urbana* larvae were found when plants were checked in 1998 and 1999. In general, the number of *C. urbana* larvae retrieved was low and varied considerably within a single plant species between both years and plots, e.g., *H. pilosella* and *H. praealtum* (Table 3.6). In 1998, larvae were found on *H. pilosella*, *H. praealtum*, and a single larva each on *H. caespitosum* and *H. lepidulum*. In contrast, *H. murorum* and *H. sabaudum* were not attacked. The highest number of larvae was found on *H. praealtum* with an average of 2.5 larvae per pot. However, only half of the *H. pilosella* pots contained larvae. In 1999, most larvae were found on *H. aurantiacum* and on *H. pilosella*, both of which are weeds in New Zealand but no larvae were present *on E. grandifolia*, *F. ulmaria*, *H. argillaceum*, *H. lepidulum*, *H. praealtum*, and *H. murorum*. Only one larva was found on *H. caespitosum*.

Table 3.6. Results of open-field tests conducted in the garden of CABI Bioscience Switzerland Centre in 1998 and 1999.[a]

Plot No. (year)	Test plant	No. pots with larvae[b]	Total No. larvae[b] found	Mean No. larvae[b]/pot
1 (1998)	*Hieracium pilosella*	2	2	0.5
	H. murorum	0	0	0
	H. caespitosum	1	1	0.25
	Embergeria grandifolia	0	0	0
2 (1998)	*Hieracium pilosella*	2	3	0.75
	H. sabaudum	0	0	0
	H. lepidulum	1	1	0.25
	H. praealtum	4	10	2.5
3 (1999)	*Hieracium pilosella*	0	0	0
	H. aurantiacum	3	7	1.75
	H. lepidulum	0	0	0
	Embergeria grandifolia	0	0	0
4 (1999)	*Hieracium pilosella*	2	2	0.5
	H. caespitosum	1	1	0.25
	H. murorum	0	0	0
	H. sabaudum	0	0	0
5 (1999)	*Hieracium pilosella*	4	5	1.25
	H. praealtum	0	0	0
	H. argillaceum	0	0	0
	Filipendula ulmaria	0	0	0

[a] In all plots (1 x 1 m) four pots of each plant species were exposed to the naturally occurring populations of *C. urbana* and *C. psilophthalma* females, and the number of larvae attacking each plant was recorded in August and September.

[b] Only larvae of *C. urbana* were found.

3.4 Discussion

3.4.1 Estimating physiological host range

Since host records were limited to field observations of *C. urbana* females ovipositing on *H. pilosella* rosettes (Claußen, 1980; Doczkal, 1996), and no records were available for *C. psilophthalma*, we conducted no-choice larval-transfer tests to determine the physiological host range (i.e., plants species on which larvae can develop) of *C. urbana* and *C. psilophthalma*. Our no-choice larval transfer tests (starvation tests) confirmed that the physiological host range of *C. urbana* and *C. psilophthalma* larvae is restricted to species in the genus *Hieracium*. Plants of economic and ecological importance in New Zealand were unable to sustain these insects. *Hieracium murorum* was the only one of the nine *Hieracium* spp. tested on which *C. urbana* larvae could not develop. In contrast, *C. psilophthalma* larvae developed on all nine species tested. Although egg-laying behavior of a *C. urbana* female on *F. ulmaria* (Rosaceae) in the field has been reported by Kassebeer (1993), no-choice larval transfer tests demonstrate that larval development on this plant species does not occur. The negative test results and the fact that during the field observation mainly *Cheilosia vernalis* (Fallén) specimens were caught indicate that *C. urbana* might have been confused with *C. vernalis,* the females of which are of similar size and have a broad host range (Kassebeer, 2000). Additional single-choice oviposition tests showed that *C. urbana* females do not accept *F. ulmaria* for oviposition in the presence of the target weed *H. pilosella*. Negative results in no-choice larval transfer tests, single-choice oviposition tests and an open-field test were also obtained for *E. grandifolia. Cheilosia grossa*, tested in the early 1980s for the biological control of musk thistles in the United States, developed on various *Carduus* spp. and on *Cirsium crassicaule* (Greene) Jeps., a native North American thistle, in no-choice larval development tests. The survival rates to mature larvae ranged from 8% on *C. crassicaule* to 80% on the target weed *C. nutans* (Rizza et al., 1988). In our no-choice larval transfer tests carried out at in Switzerland, the survival rates to mature larvae ranged from 12.5% on *H. argillaceum* to 86.7% on *H. caespitosum* (North American biotype) for *C. urbana*, and from 2.2% on *H. argillaceum* to 54.7% on *H. pilosella* (New Zealand biotype) for *C. psilophthalma*.

3.4.2 Predicting field host range and conclusions

Since all 10 *Hieracium* species adventive to New Zealand are of Eurasian origin and no

native *Hieracium* spp. exist in this country (Webb et al., 1988), potential agents that are at least genus-specific are considered safe for field-release. We conclude that *C. urbana* and *C. psilophthalma* are safe for release in New Zealand and are likely to attack not only *H. pilosella* but a range of weedy *Hieracium* species there. Results of open-field tests suggest that *H. pilosella*, *H. praealtum,* and *H. aurantiacum* are likely to be attacked by *C. urbana* under field conditions, but possibly also *H. caespitosum* which was preferred over *H. pilosella* in single-choice oviposition tests with 92.6% of the eggs laid onto this plant species and two *C. urbana* larvae found in open-field tests. *Hieracium lepidulum* from which only one larva was extracted in open-field tests might be a potential field host. However, due to the low level of attack, the open-field tests are of limited value, although they are considered to give valuable results since females have the opportunity to express their complete host-choice behavior (Briese, 1999; Cullen, 1990). The results of the garden study are not representative for low attack rates in the field. During preliminary no-choice larval transfer tests with *C. urbana* in 1995, during which three eggs were transferred per potted plant, and neither pots nor garden beds were protected with gauze bags or nets, up to 14 *Cheilosia* larvae were found per pot indicating additional natural attack, mainly by *C. urbana* (Grosskopf, unpublished data). One reason for the low attack rates in the open-field tests may be the capture of gravid *C. psilophthalma* and *C. urbana* females in the Centre's garden, although we tried to compensate these losses in naturally occurring *Cheilosia* females with the release of additional *C. urbana* and *C. psilophthalma* flies in the Centre's garden which emerged from no-choice tests and rearing plants.

Hawkweeds of Eurasian origin in the subgenus *Pilosella* are severe pasture weeds in North and South America as well (Wilson and Callihan, 1999). *Hieracium caespitosum*, meadow hawkweed, as well as *H. aurantiacum*, orange hawkweed, are among the eleven species of highly invasive hawkweeds intoduced into North America from Europe and targeted for biological control (Wilson and Callihan, 1999). In contrast to the situation in New Zealand, native North and South American hawkweed species do occur (Fernald, 1950; Scoggan, 1979). Native American *Hieracium* spp. belong exclusively to the subgenera *Chionoracium* and *Hieracium* (Gottschlich, 1996). None of these are listed in the "Threatened and Endangered Species System (TESS)" of the U.S. Fish and Wildlife Service (http://www.fws.gov/endangered/). Species in the subgenus *Chionoracium* are restricted to

Asia (Japan, Kamchatka) and North, Central, and South Americas with most species occurring in the Andes. The 600 *Hieracium* spp. occurring in Europe belong either to the subgenus *Hieracium* or the subgenus *Pilosella* (Gottschlich, 1996). It is not clear from tests described here whether *C. urbana* and *C. psilophthalma* would be suitably host-specific for release in North and South Americas where *Hieracium* spp. are exotic weeds and native relatives occur. Native *Hieracium* spp. in the subgenera *Hieracium* and *Chionoracium* will need to be tested as well as other members of North American Asteraceae. In the United States and Canada seven hawkweed species are placed in the subgenus *Hieracium*: four of them are nonindigenous, i.e. *H. murorum*, *H. vulgatum* Fries, *H. groenlandicum* Arv.-Touv., and *H. sabaudum*, and three are native, i.e., *Hieracium umbellatum* L., *Hieracium robinsonii* Zahn, and *Hieracium canadense* Michx. (Fernald, 1950). None of the four *Hieracium* spp. in the subgenus *Hieracium* adventive to New Zealand and used in our tests, i.e., *H. argillaceum*, *H. lepidulum*, *H. murorum,* and *H. sabaudum* are native in North America. Therefore, *H. umbellatum*, *H. robinsonii,* and *H. canadense* would have to be tested to assess if they are at risk. A program for the biological control of invasive *Hieracium* spp. of Eurasian origin in North America was started in 2000. One part of the program which is carried out at the CABI Bioscience Switzerland Centre is to assess the host range of *C. urbana* and *C. psilophthalma* within the genus *Hieracium* using native North American hawkweed species (Grosskopf et al., 2000).

Mating of *Cheilosia* in captivity has not yet been seen (C. F. Kassebeer, University of Kiel, personal communication) and no literature regarding this topic is available. Several attempts were made to mate *C. urbana* males and females emerged from puparia at the CABI Bioscience Switzerland Centre using screened rearing cages in the laboratory and in the greenhouse and field cages (measuring 1m x 1m x 1m and 2 m x 2 m x 2.2 m) in the Centre's garden, but the adults did not mate (G. Grosskopf, personal observation). Mating of these species was not observed in the field. Gruhl (1959) observed *Cheilosia proxima* (Zetterstedt) males on a clearing in a forest alternating between resting on leaves and flying up for a short time, forming a loose group of up to 10 males, presumably waiting for female *C. proxima* to pass by. Schmid (2000) recorded similar behavioral patterns for *C. rhodiolae* males which used *Rhodiola rosea* L. (Crassulaceae), the larval host plant, for resting and its flowers for feeding. If *C. urbana* and *C. psilophthalma* need similar conditions for mating, this will be difficult to reproduce. However, to obtain *C. urbana* and *C. psilophthalma*

females for field release in New Zealand, fertile eggs laid by field-collected females in Europe and mature larvae or puparia reared from eggs – all stages protected from parasitoids - can be shipped to New Zealand and adult flies emerging from this material could be field-released after being checked for pathogens.

4 Life history and host-specificity of *Macrolabis pilosellae*, a biological control agent of hawkweeds in the subgenus *Pilosella* in New Zealand

4.1 Introduction

During surveys for potential biological control agents of *Hieracium* spp. in Central Europe, the area of origin of several invasive *Hieracium* spp., rosettes, stolon tips and meristems in leaf axils with galls induced by *M. pilosellae* (Diptera, Cecidomyiidae) were recorded on *H. pilosella*, *H. lactucella*, and *H. glomeratum*. According to literature records, 13 gall midge species are associated with *Hieracium* spp. (Table 4.1), three of which belong to the genus *Macrolabis*. Larvae of *M. pilosellae* feed gregariously in galls of *Hieracium* spp. in the subgenus *Pilosella* and galls can be found from June onwards until late autumn throughout most of Europe and Great Britain (Buhr, 1964). Larval attack on the apical bud at the growing point leads to coalescence of young leaves. Galled leaves are wrinkled, remain furled, and their margins can be enrolled. The upper surface of galled leaves is covered with an abnormal dense layer of short hairs instead of sparse long hairs. The enrolled, furled leaves enclose the larvae, which feed within the cavities among the leaves. The gall becomes progressively larger and more hairy with time, and attacked stolons remain stunted. Gall-inducing insects from different taxa have been used as biological control agents; e.g. Curculionidae, Cecidomyiidae or Eurytomidae. There are a number of economically important gall midge pests suggesting that this group might harbour promising biological control agents. Some of the most damaging gall midge species are pests of cereal crops, e.g. *Mayetiola destructor* (Say) attacking wheat and barley in Europe and North America, *Haplodiplosis marginata* (von Roser) infesting these two crops in Europe, and *Contarinia sorghicola* (Coquillett) attacking sorghum, mainly in the tropics and sub-tropics (Skuhravá et al., 1984). *Contarinia nasturtii* (Kieffer) was recently identified as a pest on broccoli crops in Canada (Hallett and Heal, 2001).

The genus *Macrolabis* comprises 56 species (Fedotova, 2004). Herbivorous species in the genus *Macrolabis* appear to have a narrow host range since they are recorded developing on only one or a few plant species; e.g. *Macrolabis corrugans* (Löw) is associated with *Pastinaca sativa* L. (Apiaceae), *Macrolabis stellariae* (Liebel) with *Stellaria media* (L.) Vill., and *Myosoton aquaticum* (L.) Moench. (Caryophyllaceae) and *Macrolabis*

ruebsaameni Hdck. with *Prunella vulgaris* L. and *Prunella grandiflora* (L.) Scholl in the family Lamiaceae (Buhr, 1965).

The current study presents data on the life history and host range of *M. pilosellae* and assesses its potential for the biological control of *H. pilosella* and other alien invasive hawkweeds in New Zealand.

Table 4.1. Literature records of gall midges associated with *Hieracium* spp.

Gall midge species	Host plant	Mode of life[a]	References
Arthrocnodax hieraciis Fedotova	*H. echioides* Lumnitzer, *H. kirghisorum* Juxip, *H. virosum* L.	h	Fedotova (2000)
Cecidiomyiidae sp.	*H. procerum* Fries	h	Fedotova (2000)
Contarinia pilosellae Kieffer	*H. caespitosum* Dumort., *H. flagellare* Willd., *H. pilosella* L., *H. lachenalii* Gmel., *H. murorum* L., *H. umbellatum* L.	h	Buhr (1964)
Cystiphora sanguinea (Bremi) (=*C. hieracii* Löw, *C. pilosellae* Kieffer)	*H. aurantiacum* L., *H. lactucella* Wallr., *H. murorum, H. pilosella, H. virosum, Hieracium* spp. in subgenus *Hieracium*	h	Buhr (1964), Fedotova (2000), Skuhravá and Skuhravý (1997)
Cystiphora virosa Fedotova	*H. virosum*	h	Fedotova (2000)
Dasyneura compositarum (Kieffer)	*H. pilosella*	i?	Buhr (1964)
Jaapiella cirsiicola (Rübsaamen)	*H. pilosella*	i?	Buhr (1964)
Jaapiella compositarum (Kieffer)	*Hieracium* sp.	h	Skuhravá and Skuhravý (1997)
Macrolabis pilosellae (Binnie)	*Hieracium* spp. in subgenus *Pilosella*	h	Buhr (1964)
Macrolabis hieraciflorae Fedotova	*H. virosum*	h	Fedotova (2000, 2004)
Macrolabis hieracii Rübsaamen	*Hieracium* spp. in subgenus *Hieracium, H. umbellatum*	h	Buhr (1964), Skuhravá and Skuhravý (1997)
Mycodiplosis sp.	*H. korshinskyi* Zahn	h	Fedotova (2000)
Mycodiplosis sp.	*H. strictissimum* Froel.	h	Fedotova (2000)

[a] h: herbivorous species; i?: possibly inquiline species.

4.2 Materials and Methods

4.2.1 Life cycle and phenology

Adult gall midges that emerged from field-collected galls originating from the Black Forest and the Swiss Jura were repotted and kept under semi-natural conditions at the CABI Bioscience Switzerland Centre in Delémont. Depending on the availability of adults, up to 30 females and males were released onto potted *H. pilosella* plants (diameter: 13 and 18 cm) covered with gauze bags. Galled plants were kept out-of-doors, underneath a shelter, for one week to protect the adults from extreme weather conditions while they were laying eggs, and the potted plants were then embedded in sawdust in a garden bed. Once empty galls were detected, indicating that the mature larvae had moved into the soil for pupation, the pots were transferred into screened rearing cages under a shelter between two greenhouses. Several times between 10 a.m. and 3 p.m., freshly emerged adults were collected from the plants, and from the gauze and pots, using an aspirator.

Three series of clay pots, i.e. 10, 14 and 9 pots (18 cm diameter), containing *H. pilosella* rosettes covered with gauze bags were exposed to 15 females and a varying number of males during the 1998 field season. Pots with adults from the first generation were set up on 8 and 9 May, with adults of the second generation on 19 and 20 July, and between 9 and 12 September with adults of the third generation. The pots were kept outside under a plastic roof for up to one week and were then embedded in a garden bed in the Centre's garden. Plants of the different series were dissected under a stereo-microscope at weekly intervals with the first plant being evaluated six to ten days after incubation of adult gall midges. The following developmental stages were distinguished: eggs, early instar larvae within galls, mature larvae within galls, mature larvae or pupae in cocoons on the plant, and larvae in cocoons in the soil. In order to extract cocoons from the soil, the content of each pot was floated on a riddle system with three different meshes (5 mm, 1 mm, 0.5 mm) to separate the light cocoons from bigger and smaller soil particles (Nissen, 1997). The soil was transferred into a two litre conical flask located on a riddle system and mixed with water. A hose with moderately flowing water was inserted into the flask until touching the bottom. Due to the water current, light soil particles were flushed out and were running down on the outside of the flask, whereas sand and heavier soil particles remained within the flask. The different riddles caught soil particles according to their size. The soil was checked for

cocoons the first time when mature larvae were found in the galls. Although this method was suitable for extracting *Dasineura brassicae* Winn. cocoons from the soil (Nissen, 1997), it did not work reliably for extracting *M. pilosellae* cocoons from the soil. Soil particles attached to the cocoons of *M. pilosellae* can prevent floating of the cocoons.

One pot of each series was not dissected and transferred into a gauze cage to record adult emergence.

In order to investigate how the midge overwinters in the soil, 50 mature *M. pilosellae* larvae from rearing pots were transferred onto sieved, damp soil in plastic cups (6.5 cm diameter, 8 cm height) on 20 and 21 October 1998. The cups were checked for immature stages and adults between 23 November 1998 and 25 May 1999, at approximately 2-3 weeks intervals, by floating the soil in a riddle system as described above and by checking the soil visually.

4.2.2 Dissection of field-collected galls

Between 1998 and 2001, *M. pilosellae* galls were collected at four field sites in the southern part of the Black Forest: near Marzell (46°46.444'N, 7°44.539'E), near St. Blasien (47°45.272'N, 8°05.498'E), Belchen (47°47,721'N, 7°50.152'E), and near Mutterslehen (47°45.811'N, 8°04.522'E). Twenty to 36 rosettes were randomly sampled per site. Due to the low number of galls available, only five to 16 galls were collected from the site near Marzell. Field-collected galls were wrapped in moist paper towel and brought back to the laboratory. Galls were opened under a stereo-microscope the same day they were collected, or kept at 10 °C and opened the next day. To assess the proportion of each developmental stage found within the galls, the number of eggs, early instar larvae, mature larvae, and cocoons containing larvae or pupae were recorded.

4.2.3 Longevity and fecundity of egg-laying females

The ovaries of freshly emerged females from rearing cages were dissected in a drop of water under a stereo-microscope to record the number of eggs in the abdomen. Freshly laid eggs retrieved from cut *H. pilosella* rosettes and stolon tips previously exposed to females were transferred onto black filter paper to measure egg size and into tight-closing Petri dishes to record egg development at 20 °C.

Males and females were kept in pairs in small plastic cups (5.5 cm diameter, 8 cm height) at 15, 20 and 25 °C and long-day regime (16:8/L:D). The adults were provided every day

with a freshly cut *H. pilosella* rosette on moist filter paper. Daily checks were made for mortality, and the number of eggs laid onto the rosette. Only those females that laid at least one egg onto the exposed plant part, and their mates, were included in the analysis. Longevity and fecundity data obtained at the different temperatures were analyzed by ANOVA and the means were compared with Tukey's HSD test.

On 22 July 2000, 26 potted *H. pilosella* plants were exposed to newly emerged pairs of *M. pilosellae*. All pots were kept under a plastic roof between two greenhouses under semi-natural conditions. All plants were dissected under a stereo-microscope 3.5 weeks later and the number of larvae on individual plants were recorded.

4.2.4 Mortality factors

To measure rates of parasitization, immature stages of *M. pilosellae* found in field-collected galls were dissected in water under a dissecting microscope. Mummified larvae were kept in the cavities of microtiter plates closed with foam stoppers for emergence of adult parasitoids. Since unparasitized mature larvae leave the galls to pupate in the soil but parasitized larvae stay on the plant, rates of parasitization may be lower in some of the dissected galls.

4.2.5 Host range investigations

Host range testing of *M. pilosellae* followed generally the same basic test plant list as used for the hover flies *C. urbana* and *C. psilophthalma* (Grosskopf et al., 2002). Four plant species known as hosts of other *Macrolabis* spp., i.e. *Cirsium vulgare* for *Macrolabis cirsii* (Rübsaamen) (Buhr, 1964), *Aegopodium podagraria* for *Macrolabis podagrariae* Stelter (Buhr, 1964; Skuhravá and Skuhravý, 1997), *Stellaria media* for *M. stellariae* (Buhr, 1965; Skuhravá and Skuhravý, 1997), and *Lamium pupureum* L. for *Macrolabis lamii* (Rübsaamen) (Buhr, 1964; Skuhravá and Skuhravý, 1997) were added to the list. Altogether 73 plant species or biotypes from 30 families were tested, 35 of which belong to the family Asteraceae. Tests were mainly carried out at the CABI Bioscience Switzerland Centre in Delémont, Switzerland. In addition, nine native New Zealand plant species that could not be grown in Switzerland and the target weed, *H. pilosella* NZ, were tested by Lindsay Smith in quarantine facilities of Landcare Research Ltd., Lincoln, New Zealand.

No-choice gall development tests I

Between 1996 and 1999, potted tests plants grown in clay-pots (13 cm diameter) were covered with mesh gauze bags, and three newly emerged *M. pilosellae* females and two or three *M. pilosellae* males were transferred onto each plant. These plants were then kept under a transparent shelter for up to one week and later outside in the garden. Three to four weeks after incubation of adults, all test plants were checked for gall development. Galled plants were transferred into screened rearing cages (43 x 43 x 79 cm) when adults were expected to emerge. Each test plant species was maintained in a separate cage. Adult emergence was recorded daily. Plants tested in the invertebrate containment facility at Lincoln were covered individually with Perspex® barrels (20 cm diameter, 19 cm height) instead of mesh gauze bags.

No-choice gall development tests II

Between 18 and 20 August 1999, gall development tests were set up with nine of the ten *Hieracium* spp. naturalized in New Zealand. Three female, with three male gall midges were transferred onto each potted test plant covered with a gauze bag. The adults were left on the plant and the pots kept outside under a shelter for about one week and afterwards in the garden. On 24 September, i.e. four weeks after introduction of the adults, all plants were dissected under a stereo-microscope. The following plant and gall midge parameters were recorded: number of galls, plants with feeding marks, number of early instar larvae and number of late instar larvae.

No-choice gall development tests III

Ten female and five male gall midges were transferred onto potted plants covered with gauze bags of the following species in the family Asteraceae: *H. pilosella*, *Microseris scapigera*, *Cichorium intybus*, *Tripleurospermum perforatum*, *Hypochoeris radicata*, *Sonchus kirkii*, *S. oleraceus*, *Helichrysum bracteatum*, *Cynara scolymus*, *Gnaphalium audax*, *Leontodon taraxacoides,* and *Taraxacum officinale*. Four pots of each test plant species (except *G. audax* with only two) were set up between 28 and 30 August 1999. On 25 September 1999, the plants were dissected under a stereo-microscope. The number of galls, early and late instar larvae and the presence of feeding marks were recorded.

4.3 Results

4.3.1 Life cycle and phenology

In Delémont, *M. pilosellae* had three generations (Figure 4.1). Adults of *M. pilosellae* usually emerged in the morning or late morning, males and females together. Adults of the first generation emerged in late April and May, adults of the second generation emerged in late June and July and adults of the third generation emerged in August and September. In all years, the sex ratio was in favor of females and ranged from 1.01:1 to 1.39:1 (females:males).

Oocytes of *M. pilosellae* are mature at emergence and there is no further oogenesis. Mating occurs soon after emergence. Females of *M. pilosellae* have a long, versatile ovipositor, and lay their eggs into the leaf axils in the rosette centre, stolon leaves and stolon tips. The young gall midge larvae evoke gall development.

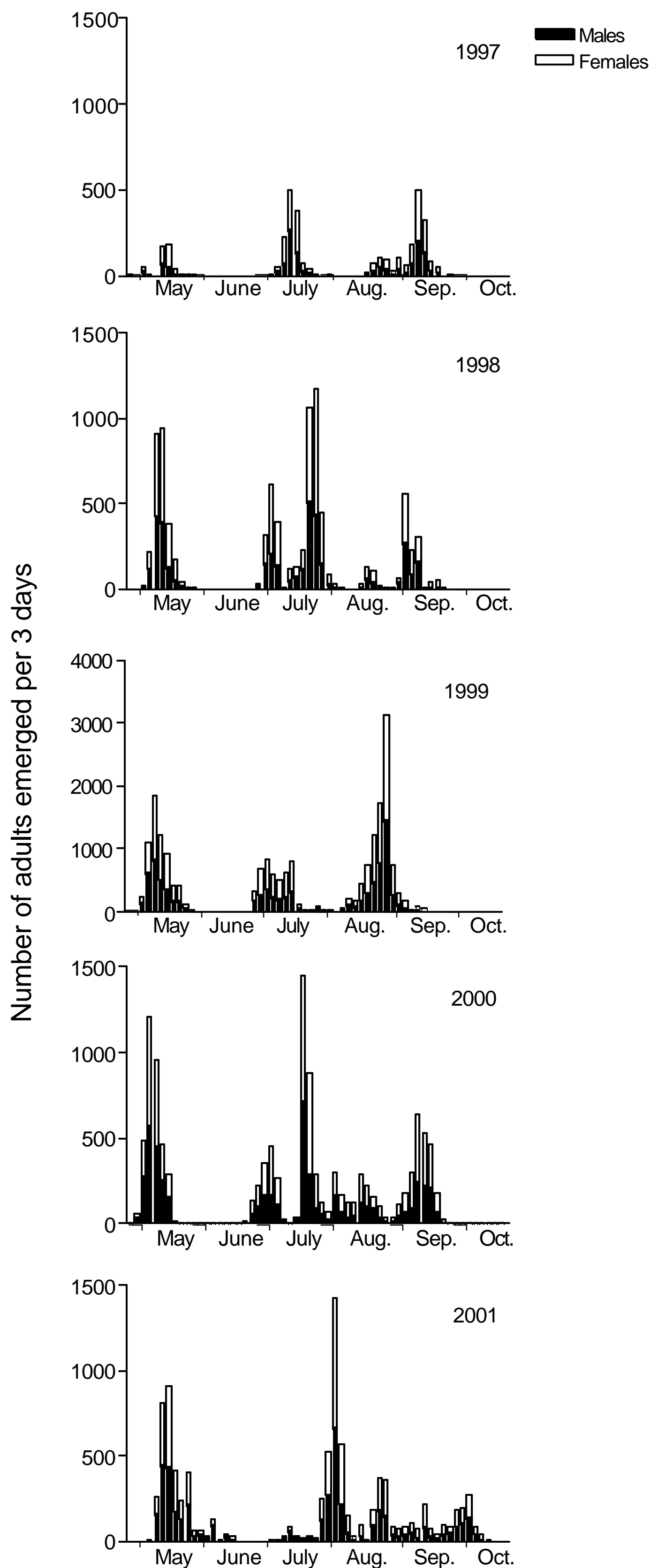

Figure 4.1. Emergence of *M. pilosellae* under semi-natural conditions between 1997 and 2001 reared at Delémont (Switzerland).

During the first two dissection dates, eggs and early instar larvae, or early instar larvae exclusively, were found (Figure 4.2 A-C). Late instar larvae were first recorded three to four weeks after incubation of adults. The presence of cocoons and the substantial reduction of immature stages from 8 June onwards suggest that mature larvae had already moved into the soil for pupation. Cocoons containing late instar larvae or pupae were found within galls of the first two series but no cocoons were retrieved from the soil (Figure 4.2 A, B). However, the low number of cocoons recorded on the above-ground plant parts and personal observations indicate that the majority of mature larvae move into the soil for pupation, and that the floating method is not appropriate to retrieve these. In contrast, from 20 October onwards, cocoons containing mature larvae were successfully retrieved from the soil of pots of the third series, i.e. 4, 145, and 69 cocoons, respectively, all containing mature larvae. The floating method did not allow older larvae and pupae that did not form a cocoon to be extracted from the soil as well as cocoons to which soil particles, e.g. sand, were attached. Therefore, the occurrence of these could not be quantified. Emergence of adults of the second generation was observed between late June and late July, and of the third generation between late August and early October (Figure 4.3).

Regular examination of the soil in plastic cups revealed that overwintering occurs in the larval stage within a silken cocoon. Pupation occurred from late April/early May onwards (Figure 4.4).

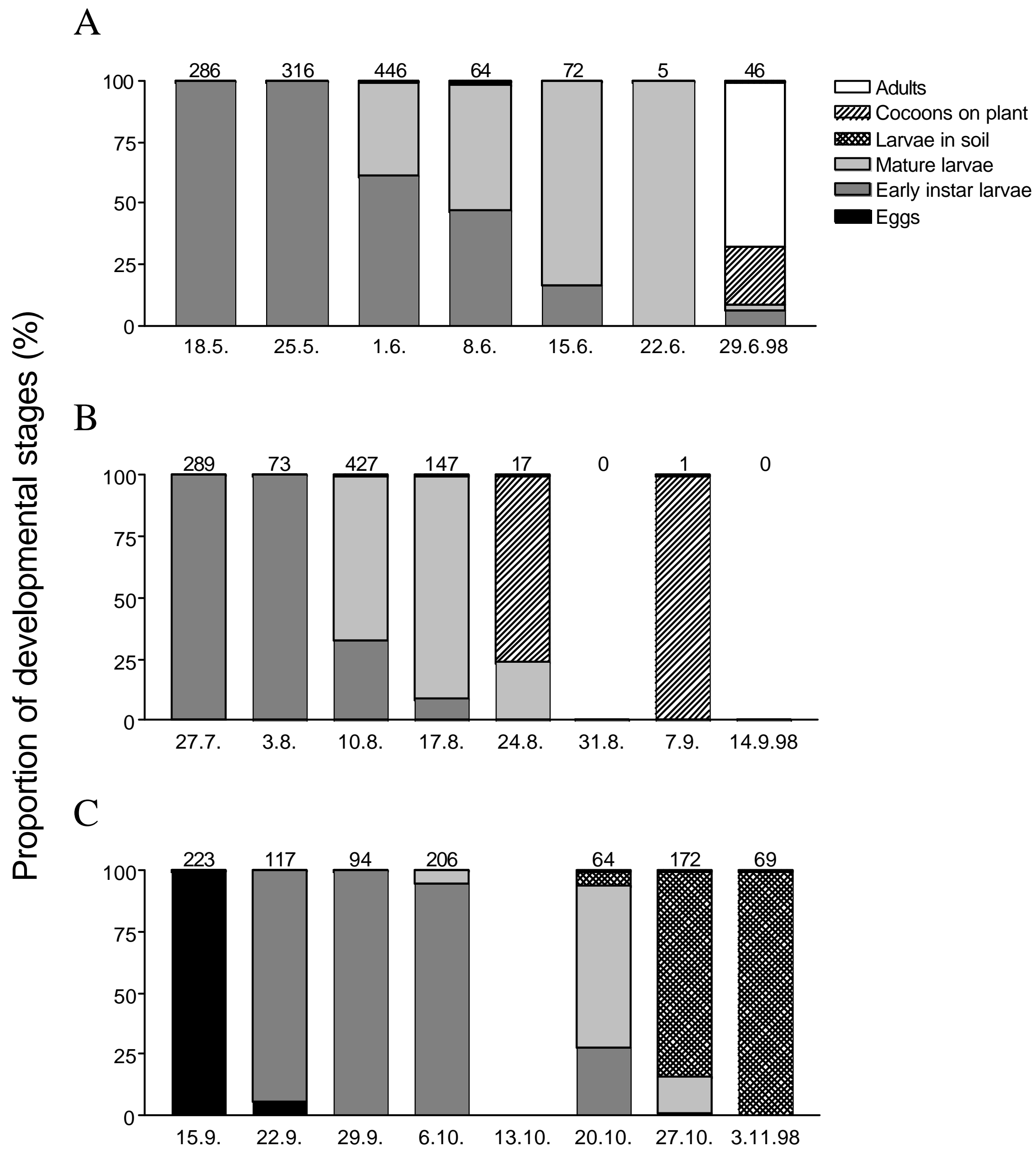

Figure 4.2. Results from weekly dissection of *H. pilosella* plants artificially infested with the gall midge in 1998. Total number of individuals recorded per pot are given on top of each bar. Pots of series A were incubated on 8 and 9 May, pots of series B were set up on 19 and 20 July and pots of the third series between 9 and 12 September.

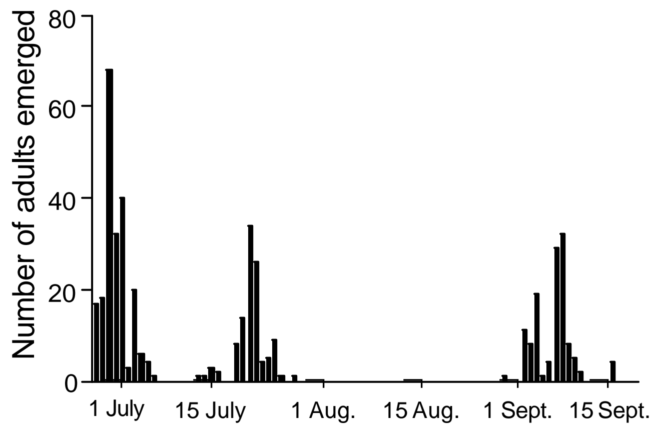

Figure 4.3. Emergence of the second (June, July) and third generation (August, September) of *M. pilosellae*.

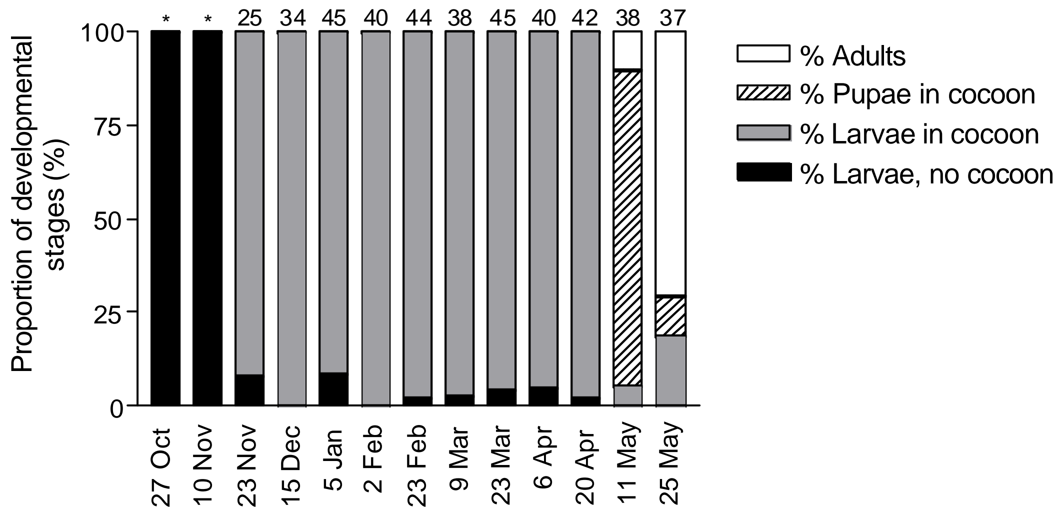

Figure 4.4. Overwintering of immature stages of *M. pilosellae* (stars above the columns indicate that only mature larvae without cocoon were found but they were not quantified). Total number of individuals found per cup are given on top of each bar.

4.3.2 Dissection of field-collected galls

Hieracium rosettes galled by *M. pilosellae* were found mainly in proximity to shrubs, but also among mosses and herbs. Galls are usually not found at extremely dry sites. Due to the multivoltine life cycle of *M. pilosellae*, larvae were found in galls throughout the sampling period (Figure 4.5 A-D). Up to 24 individuals per gall were recorded, with the highest number of early instar larvae per gall being 16, and a maximum of 17 mature larvae per gall. On average 4.7 ± 0.19 immature stages of the gall midge were found per gall (empty galls were excluded from the analysis). In 21.3% of the galls only one gall midge was found (Figure 4.6), and all samples contained galls with no midges.

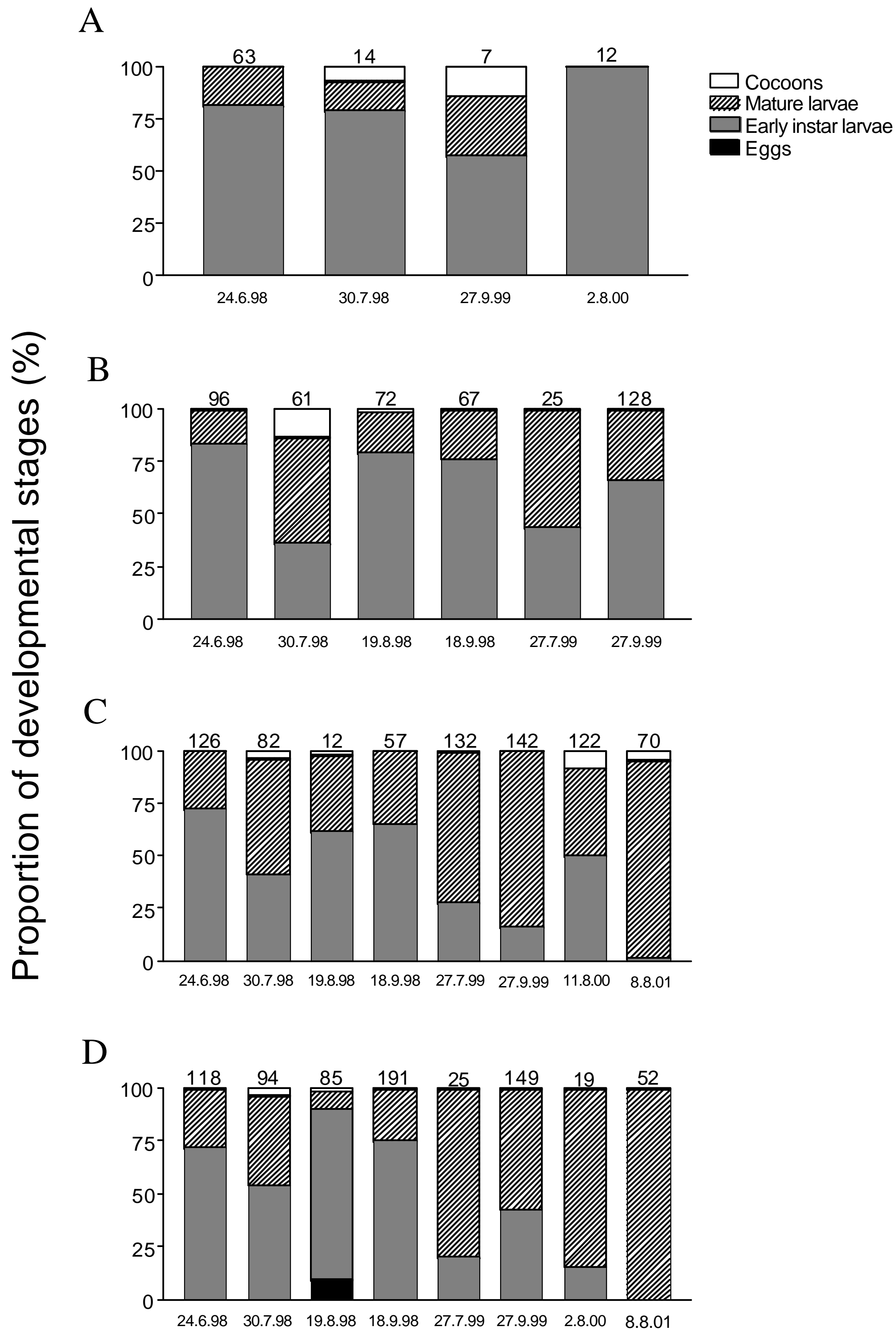

Figure 4.5. Phenology of *M. pilosellae* at four different sites in the Black Forest between 1998 and 2001. (A) Marzell, (B) St. Blasien, (C) Mutterslehen, (D) Belchen. Total number of immature stages found per sampling date are given on the top of each bar.

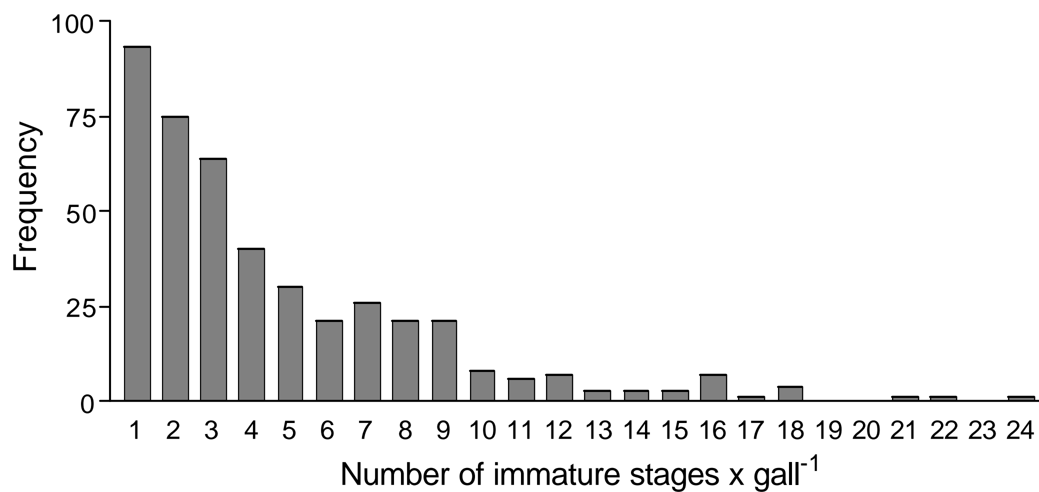

Figure 4.6. Frequency distribution of immature stages found in field-collected *M. pilosellae* galls (mean: 4.7 ± 0.19, n = 436 galls).

4.3.3 Longevity and fecundity of egg-laying females

The abdomen of freshly emerged females contained on average 67.7 ± 2.51 eggs (mean ± SE, n = 53, range: 22-120). Freshly laid eggs are whitish-translucent. They measure 0.35 ± 0.020 mm in length (mean ± SE, n = 92) and 0.09 ± 0.001 mm in width. Incubated at 20 °C, eggs hatch after 4 days.

Eight (25%) of the 32 females incubated at 15 °C did not lay any eggs onto the rosettes offered and, together with the corresponding males, were not included in the analysis. The same applied to one (3.2%) out of 31 females at 20 °C and one (2.8%) out of 36 at 25 °C. Most eggs were laid at 25 °C with 56.8 ± 2.96 eggs (mean ± SE, n = 35) whereas at 15 °C 34.5% fewer eggs were laid with on average 37.2 ± 4.55 eggs (n = 24) (Figure 4.7). Under all three temperature regimes, males lived significantly longer, i.e. between 1.5 and 3 days, than the corresponding females ($F_{2, 86}$ = 7.05, P = 0.001). Both males and females had a shorter life span with increasing temperature ($F_{5, 172}$ = 49.08, P < 0.001). The shortest life span was recorded for *M. pilosellae* females at 25 °C with 3.4 ± 0.20 days (mean ± SE, n = 35) and the longest for males at 15 °C with 10.1 ± 0.60 days (n = 24). Males and females live twice as long at 15 °C as at 25 °C.

Twenty of the 26 potted plants set up to explore fecundity contained larvae. The mean number of larvae was 26.3 ± 4.4 (range 2-70).

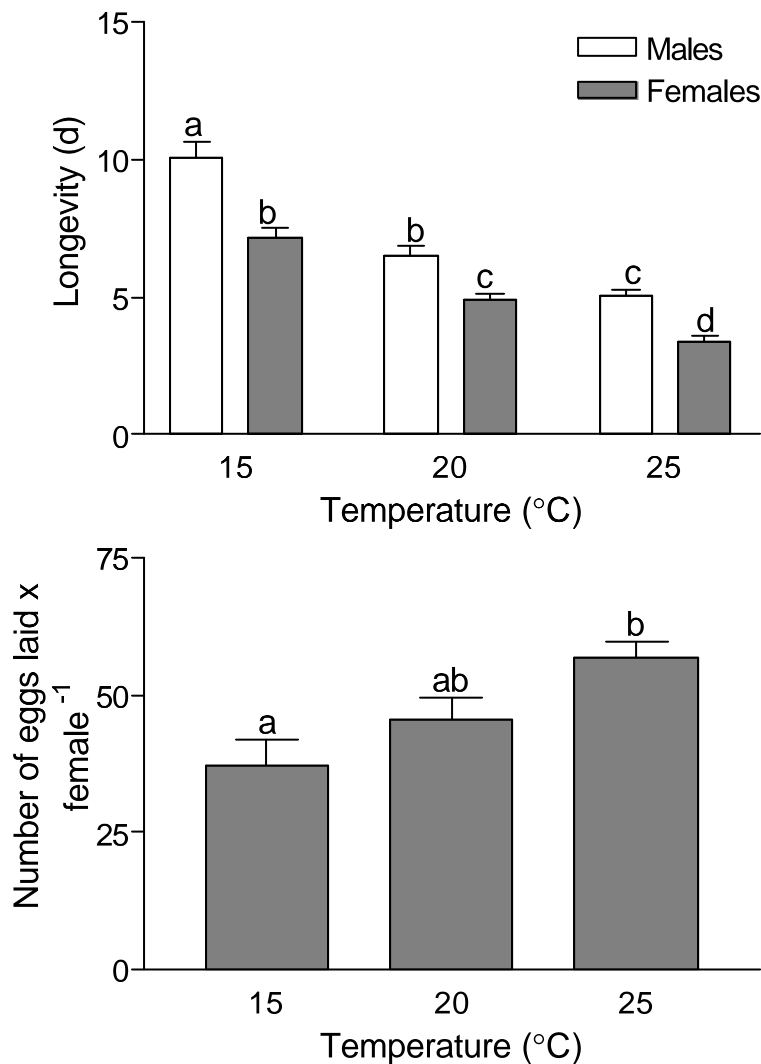

Figure 4.7. Longevity and fecundity of *M. pilosellae* at three different temperatures (in both graphs mean ± SE are given). Bars with the same letter are not significantly different at the 5% level (Tukey HSD test).

4.3.4 Mortality factors

A larval endoparasitoid, an *Aprostocetus* species (Eulophidae, Tetrastichinae) which is possibly not yet described, was reared from *M. pilosellae* (identification Hannes Baur, Naturhistorisches Museum Bern). Parasitized *M. pilosellae* larvae did not leave the gall to spin a cocoon in the soil, but they stayed in the gall where they mummified. Parasitoids either emerged in the same year or, if they developed in the last generation of *M. pilosellae*, in the following year after overwintering. Six of 13 samples dissected contained parasitoids and parasitization rates ranged from 0 to 92.9% (Table 4.2).

Table 4.2. Rates of parasitization of *M. pilosellae* by *Aprostocetus* sp., a larval endoparasitoid.

Site	Coll. date	No. immature stages per gall[a] (mean ± SE)	Total No. immature stages found	Rate of parasitization (%)
Marzell	2.8.2000	6.00 ± 2.00	12	0
	27.9.1999	1.75 ± 0.48	7	0
Belchen	27.7.1999	2.78 ± 0.70	25	4.0
	2.8.2000	2.38 ± 0.53	19	0
	8.8.2001	4.00 ± 1.00	52	0
	27.9.1999	5.73 ± 0.92	149	0
St. Blasien	27.7.1999	1.79 ± 0.19	25	12.0
	27.9.1999	5.57 ± 1.10	128	0
Mutterslehen	27.7.1999	5.92 ± 0.86	142	8.5
	27.9.1999	5.30 ± 0.93	122	80.3
	11.8.2000	2.00 ± 0.37	12	50.0
	8.8.2001	3.50 ± 0.61	70	92.9
Les Genevez	24.7.2000	1.50 ± 0.29	6	0

[a] Empty galls were excluded.

4.3.5 Host range investigations

No-choice gall development tests I

Only three *Hieracium* spp., i.e. *H. pilosella*, *H. caespitosum* and *H. praealtum*, all belonging to the subgenus *Pilosella*, showed “normal” gall development and adults emerged from these plants (Table 4.3). Gall development occurred to a lesser extent on *H. stoloniflorum* but development to adult did not occur.

Table 4.3. Results of no-choice gall development tests between 1996 and 1999.

Plant species [a]	No. plants inc.	No. plants galled	No. females inc.	No. adults em.
Asteraceae				
Tribe: Lactuceae				
Subgenus *Pilosella*				
[b]*Hieracium pilosella* L. EUR	56	53	159	894
[b]*H. pilosella* L. NZ	11	9	33	114
[b]*H. pilosella* L. NZ*	20	18	60	n.r.
[c]*H. aurantiacum* L.	12	0	34	0
[b]*H. caespitosum* Dumort. EUR	6	6	17	124
[b]*H. caespitosum* Dumort. US	6	6	15	53
[b]*H. praealtum* Vill. ex Gnochat	6	5	16	27
[c]*H.* × *stoloniflorum* Waldst. & Kit.	12	2	36	0
Subgenus *Hieracium*				
[c]*H. argillaceum* Jordan	6	0	18	0
[b]*H. lepidulum* (Stenström) Omang	6	0	17	0
[c]*H. murorum* L.	8	0	24	0
[c]*H. sabaudum* L.	6	0	16	0
Cichorium intybus L.	8	0	24	0
Embergeria grandifolia (Kirk) Boulos	7	0	21	0
Hypochoeris radicata L.	7	0	21	0
Lactuca sativa L.	5	0	35	0
Leontodon taraxacoides (Vill.) Mérat	6	0	18	0
Kirkianella novae-zelandiae (Hook. f.) Allan*	5	0	15	n.r.
Microseris scapigera (Sol. ex A. Cunn.)Sch. Bip.	7	0	21	0
Picris hieracioides L.	6	0	24	0
Sonchus kirkii Hamlin	9	0	27	0
Sonchus oleraceus L.	6	0	18	0
Taraxacum officinale Weber	7	0	21	0
Tragopogon porrifolius L.	7	0	21	0
Tribe: Anthemideae				
Artemisia dracunculus L.	6	0	24	0
Chrysanthemum cinerariifolium (Trev.) Vis.	9	0	27	0
Tribe: Astereae				
Celmisia semicordata (Petrie) Cheesem.*	5	0	15	n.r.
Olearia avicenniaefolia (Raoul) Hook. f.	6	0	18	0
Tribe: Heliantheae				
Helianthus annuus L.	6	0	18	0
Tribe: Inuleae				
Helichrysum bracteatum (Vent.) Andrews	6	0	18	0
Gnaphalium audax D. Drury	6	0	18	0
Raoulia hookeri Allan*	5	0	15	n.r.
Tribe: Senecioneae				
Senecio monroi Hook. f. (= *Brachyglottis monroi*)	10	0	30	0
Tribe: Cardueae				
Carthamus tinctorius L.	7	0	21	0
Cirsium vulgare (Savi) Ten.	6	0	18	0
Cynara scolymus L.	9	0	27	0
Apiaceae				
Aegopodium podagraria L.	6	0	18	0
Petroselinum crispum (Miller) A. W. Hill	6	0	18	0
Betulaceae				
Betula sp.	3	0	9	0
Betula pendula Roth	3[d]	0	18	0
Brassicaceae				
Brassica oleracea L.	6	0	18	0
Cannaceae				
Canna indica L.	6	0	18	0
Caprifoliaceae				
Alseuosmia macrophylla A. Cunn.*	5	0	15	n.r.

Table 4.3. (continued)

Plant species	No. plants inc.	No. plants galled	No. females inc.	No. adults em.
Caryophyllaceae				
Dianthus barbatus L.	6	0	18	0
Stellaria media (L.) Vill.	6	0	18	0
Cyperaceae				
Carex paniculata L.	8	0	24	0
Ericaceae				
Gaultheria crassa Allan*	5	0	15	n.r.
Euphorbiaceae				
Euphorbia glauca Forst. f.*	5	0	15	n.r.
Fabaceae				
Trifolium repens L.	6	0	18	0
Grossulariaceae				
Ribes nigrum L.	3[e]	0	36	0
Ribes rubrum L.	3	0	9	0
Iridaceae				
Libertia grandiflora (R. Br.) Sweet*	5	0	15	n.r.
Lamiaceae				
Lamium purpureum L.	10	0	30	0
Mentha sp.	9	0	27	0
Liliaceae				
Allium cepa L.	6	0	18	0
Malvaceae				
Althea rosea L.	6	0	18	0
Myrtaceae				
Leptospermum scoparium J. R. & G. Forst.	9	0	27	0
Oleaceae				
Olea europaea L.	6	0	18	0
Poaceae				
Festuca novae-zelandiae J. B. Armstr.	6	0	18	0
Poa colensoi Hook. f.	6	0	18	0
Agrostis tenuis Sibth.	6	0	18	0
Polygonaceae				
Rumex acetosella L.	6	0	18	0
Proteaceae				
Knightia excelsa R. Br.*	5	0	15	n.r.
Ranunculaceae				
Clematis forsteri Gmel.	3	0	9	0
Clematis paniculata Gmel.	1	0	3	0
Rhamnaceae				
Discaria toumatou Raoul	2	0	6	0
Discaria toumatou Raoul*	5	0	15	n.r.
Rutaceae				
Citrus sp.	6	0	18	0
Salicaceae				
Salix sp.	3	0	9	0
Salix viminalis L.	4	0	12	0
Scrophulariaceae				
Antirrhinum majus L.	10	0	30	0
Solanaceae				
Lycopersicon esculentum Miller	5	0	15	0
Theaceae				
Camellia japonica L.	6	0	18	0
Urticaceae				
Urtica dioica L.	6	0	18	0
Vitaceae				
Vitis vinifera L.	9	0	27	0

[a]Plants with an asterix were tested in quarantine facilities at Lancare Research Ltd., New Zealand, plants without an asterix were tested at CABI Bioscience Switzerland at Delémont; [b]target weed; [c]naturalized *Hieracium* sp.; [d]6 females incubated per branch and tree; [e]12 females incubated per shrub; n.r. not recorded.

No-choice gall development tests II

All *H. pilosella*, *H. praealtum,* and *H. caespitosum* plants offered were heavily galled and showed distinct feeding marks (Table 4.4, no-choice test II). The highest number of galls was recorded on *H. pilosella* with on average 9.3 galls and 6.3 late instar larvae per plant. Empty galls with distinct feeding marks were recorded on *H. pilosella*, *H. praealtum,* and *H. caespitosum* indicating that the larvae had already moved into the soil for pupation. One slightly developed gall, as well as deformations, feeding marks, and larvae were recorded on *H. aurantiacum* plants. Five percent of the 60 larvae reared on orange hawkweed were late instar larvae. It might therefore be possible for development to adulthood to occur on *H. aurantiacum*. However, due to the absence of galls, most of the retrieved larvae fed externally on the leaves and the size of many of the early instar larvae was comparable to that of neonate larvae, indicating that they failed to feed and develop successfully. *Hieracium argillaceum* was the only hawkweed species in the subgenus *Hieracium* from which larvae were retrieved. None of the plants were galled and all larvae were early instar larvae. It is therefore not likely that the larvae found on *H. argillaceum* would have been able to complete their development before the end of the field season. No galls, feeding marks or larvae were found on *H. sabaudum*, *H. lepidulum*, *H. stoloniflorum* or *H. murorum*.

No-choice gall development tests III

Feeding marks, larvae and galls were recorded only on *H. pilosella* plants, with all plants offered being attacked (Table 4.4, no-choice test III). One *H. pilosella* plant died during the studies and was thus not included in the analysis. The mean number of galls per *H. pilosella* plant was 8.3 ± 0.88. The mean number of early instar larvae per plant was 77.0 ± 31.90 and the mean number of late instar larvae 28.0 ± 11.72. All other plant species had neither galls, deformations, feeding marks nor larvae.

Table 4.4. No-choice gall and larval development tests with *M. pilosellae* in 1999.[a]

Test plant	No. plants		No. plants with	Mean No.	Mean No. empty	Mean No. larvae x plant $^{-1}$	
	incubated	with galls	feeding marks	galls x plant^{-1}	galls x plant $^{-1}$	early instar	late instar
No-choice test II [b]							
Hieracium pilosella EUR	6	6	6	9.3 ± 2.01	7.3 ± 2.26	2.7 ± 1.41	6.3 ± 2.44
H. aurantiacum	6	1	6	0.2 ± 0.17	0	9.5 ± 2.72	0.5 ± 0.34
H. caespitosum NZ	6	6	6	7.8 ± 1.01	7.5 ± 1.06	0	0.8 ± 0.65
H. praealtum	6	6	6	5.5 ± 0.67	3.7 ± 0.99	1.2 ± 0.6	5.8 ± 4.11
H. × *stoloniflorum*	6	0	0	0	0	0	0
H. argillaceum	6	0	2	0	0	1.7 ± 0.99	0
H. lepidulum	6	0	0	0	0	0	0
H. murorum	5	0	0	0	0	0	0
H. sabaudum	6	0	0	0	0	0	0
No-choice test III [c]							
Hieracium pilosella EUR	3	3	3	8.3 ± 0.88	0	77.0 ± 31.9	28.0 ± 11.72
Cichorium intybus	4	0	0	0	0	0	0
Hypochoeris radicata	4	0	0	0	0	0	0
Leontodon taraxacoides	4	0	0	0	0	0	0
Microseris scapigera	4	0	0	0	0	0	0
Sonchus kirkii	4	0	0	0	0	0	0
Sonchus oleraceus	3	0	0	0	0	0	0
Taraxacum officinale	4	0	0	0	0	0	0
Tripleurospermum perforatum	3	0	0	0	0	0	0
Helichrysum bracteatum	4	0	0	0	0	0	0
Gnaphalium audax	2	0	0	0	0	0	0
Cynara scolymus	4	0	0	0	0	0	0

[a] Mean ± SE are given; [b] three pairs were transferred per pot; [c] ten females and five males were incubated per pot.

4.4 Discussion

One the most successful gall-inducing biological control agent is the pteromalid *Trichilogaster acaciaelongifoliae* (Froggatt) (Hymenoptera: Pteromalidae), which is effectively controlling *Acacia longifolia* (Andr.) Willd. in South Africa (Dennill, 1988; Julien and Griffiths, 1998). In conjunction with the Australian weevil *Melanterius ventralis* Lea, the wasp reduces seed production to only 1% of levels formerly found in South Africa (Julien and Griffiths, 1998). The gall midge *Cystiphora sonchi* (Bremi) reduced the density of perennial sow-thistle, *Sonchus arvensis* L., on unmowed sites in Nova Scotia by 50% and flowering by 80% (Julien and Griffiths, 1998). However, the ecology of gall makers is largely determined by trophic levels above and below them, with the main mortality factor being parasitoids and predators (Weis et al., 1988). High rates of parasitization by the larval endoparasitoid *Aprostocetus* sp. near *atticus* Graham is likely to limit the abundance of *C. sonchi* (McClay and Peschken, 2002). First field observations show that *Rhopalomyia tripleurospermi* Skuhravá, introduced into Canada for the biological control of *T. perforatum*, might also experience losses due to parasitism in the area of release (McClay et al., 2002). There are several *Aprostocetus* spp. in New Zealand (e.g. Withers et al., 2000). However, if larvae of *M. pilosellae* will be parasitized by *Aprostocetus* spp. or other parasitoids in New Zealand has to be verified in post-release studies. The univoltine biological control agent *T. acaciaelongifoliae* is protected from generalist parasitoids by its asynchronized phenology and by producing multi-chambered galls of which the deep-seated chambers have a low parasitism rate (Manongi and Hoffmann, 1995). Due to *M. pilosellae*'s multivoltine life cycle, larvae are repeatedly exposed to parasitoid attack. However, not only parasitoids cause mortality in gall midges, but also adverse weather conditions, e.g. drought, and predators. According to Skuhravý et al. (1996) the following gall midge stages are especially vulnerable to unfavourable conditions: newly hatched larvae which look for an appropriate feeding site, mature larvae which leave the gall to pupate in the soil, and during and shortly after adult emergence.

Several generations of cecidomyiids are common on herbaceous plants and grasses that continue growing throughout the season (Rohfritsch, 1992). Since *M. pilosellae* induces galls on rosettes and stolons, the gall midge is not dependent on the availability of a certain plant stage. The emergence period of each of the three different generations of *M.*

pilosellae extends over several weeks, thus increasing the chances of finding suitable oviposition sites, and, with regard to the last generation, completion of larval development of at least part of the generation before wintertime. Other examples of gall midges which are able to produce several generations per year are *Dasineura affinis* Kieffer associated with *Viola odorata* L. (Birch et al., 1992) and *R. tripleurospermi* on *T. perforatum* (Hinz, 1998). The European gall midge *C. sonchii* has the same number of generations in Canada and in the Czech Republic (Peschken et al., 1989). It is assumed that, depending on site characteristics such as climate and altitude, *M. pilosellae* will have 2-3 generations in New Zealand.

An important prerequisite for the release of an exotic biological control agent is a restricted host range to avoid harm of non-target plants (Cruttwell McFadyen, 1998). Since all hawkweed species occurring in New Zealand are naturalized (Webb et al., 1988) and none of them is of economic importance, a broader host range within the genus *Hieracium* is preferred. Host-specificity tests carried out with nine of the ten *Hieracium* spp. naturalized in New Zealand demonstrate that *M. pilosellae* induces fully-developed galls from which adults emerge exclusively on *Hieracium* spp. that are in the subgenus *Pilosella*, i.e. *H. pilosella*, *H. caespitosum* and *H. praealtum*, all of which are weedy species. This corresponds well with the information given in Buhr (1964) that *M. pilosellae* is restricted to species in the subgenus *Pilosella*. However, although *H. aurantiacum* and *H. stoloniflorum* are also in the subgenus *Pilosella*, they proved not to be suitable hosts of the gall midge in this study. *Hieracium argillaceum, H. lepidulum, H. murorum* and *H. sabaudum*, i.e. *Hieracium* spp. tested in the subgenus *Hieracium*, are not predicted to be field hosts of *M. pilosellae* either from host records in Buhr (1964) or from this study. A close relative of *M. pilosellae*, *M. hieracii*, is known to develop on hawkweed species in the subgenus *Hieracium* (Buhr, 1964).

Conclusions for biological control and outlook

A petition to release *M. pilosellae* in New Zealand was made to ERMA (Environmental Risk Management Authority) in June 2001 and the first field releases were made in 2002 (personal communication L. Smith, Landcare Research, Ltd.). Due to its multivoltine life cycle, its impact on plant growth and the potential to develop on several weedy *Hieracium*

spp. in the subgenus *Pilosella*, *M. pilosellae* is considered to be a promising biological control agent for alien invasive hawkweeds in New Zealand. Its host range is not so narrow as the host range of *A. subterminalis* (Hym., Cynipidae), another biological control agent released in New Zealand that only attacks *H. pilosella* and *H. aurantiacum* (Syrett et al., 1999). So *M. pilosellae* will affect more problem species but its host range is not so broad as to attack non-target plants. To date *M. pilosellae* has been released at 132 field sites throughout New Zealand with establishment confirmed at 60% of sites monitored after one winter. Sites have also been established to monitor gall midge impact on vegetation but insect numbers are yet to reach measurably damaging levels (personal communication L. Smith, Landcare Research Ltd.).

5 The impact of *Macrolabis pilosellae* herbivory on plant parameters of mouse-ear hawkweed

5.1 Introduction

Besides a restricted host range, a negative impact on the growth of the target weed is an important criterion for the selection of potential biological control agents (Harris, 1991). Impact studies carried out prior to release give valuable information as to how the insect damages the plant. Herbivory by gall-inducing insects has the potential to result in a number of negative impacts on their host plants, e.g. reduced seed production (Dennill, 1988; Erasmus et al., 1992), stunting of the plant (Birch et al., 1992; Caresche and Wapshere, 1975; Cullen et al., 1982; Hinz, 1999), breakage and mortality of branches due to the weight of the galls (Dennill, 1988), or even mortality of plants (Erasmus et al., 1992; Hinz, 1999). However, results for a certain gall insect can not be generalized for other gall-inducing insects, as the effect of each cecidogenous organism will vary depending upon its seasonal cycles, host clonality, organs attacked, tissues stimulated, and the degree of resources mobilized (Abrahamson and McCrea, 1986).

Gall midge larvae reduce the production of normal plant structures by utilizing plant resources for production of galls. In certain gall midge species, dry weight of foliage with galls may be two to five times higher than that without galls (Skuhravá et al., 1984). In addition, the gall-inducing organism consumes nutrients that usually would be available for the production of normal plant tissue. Depending on the taxon, initiation of gall development is either associated with oviposition by the adult (i.e. sawflies, cynipids, and some beetles) or with the activity of first-instar larvae (i.e. cecidomyiids and coccids) (Rohfritsch, 1992). The eggs of most cecidomyiids are deposited on the surface of plant tissues and the neonate larvae search for the right place to initiate the gall (Dreger-Jauffret and Shorthouse, 1992) but females of *Cystiphora sonchi* place their eggs inside the leaf via the stomata (DeClerck and Steeves, 1988). Gall midge larvae have reduced mouthparts and feed by sucking exuding fluids from cells of the nutritive tissue without provoking cell necrosis (Dreger-Jauffret and Shorthouse, 1992; Rohfritsch, 1992). Gall development is provoked by mechanical and chemical stimuli, i.e. by wiggling of the neonate larva and exuding saliva (Rohfritsch, 1992).

Macrolabis pilosellae was chosen as potential biological control agent of mouse-ear hawkweed due to its observed damage to the plant in the field and in the laboratory. The gall midge induces galls on rosettes and stolon tips. *Macrolabis pilosellae* can develop on several *Hieracium* species and has three generations in Switzerland allowing repeated

attack over the growing season. Manipulative experiments with potted plants were carried out to investigate the effect of gall formation on plant parameters of *H. pilosella*.

5.2 Materials and Methods

Two different experiments were carried out to measure the impact of *M. pilosellae*-induced gall formation on different plant parameters of *H. pilosella*.

5.2.1 Experiment 1

On 14 August 2000, *H. pilosella* daughter rosettes were separated from their mother plants and individually planted into the cavities (diameter 4.3 cm) of a planting tray. On 3 September, the young plants were planted into clay pots (13 cm diameter) with standard potting soil. Thirty-six rosettes having no stolons were chosen for the experiment. The number of leaves of each rosette was recorded. All pots were covered with gauze bags and 18 of them randomly chosen and infested with two male and three female *M. pilosellae* gall midges on 5 and 6 September whereas the other 18 pots were kept as control pots. The pots were kept in a greenhouse until 14 September and were then embedded in the garden. Length of all stolons was recorded at 7-day-intervals. On 9 and 10 November, the number of flower heads, stolons, leaves and vegetative meristems (stolon tips, rosettes, buds or rosettes in leaf axils), and the stolon lengths were recorded. Above-ground plant parts and roots were dried separately and the dry weight was taken with a microbalance after 24 h at 80 °C. The difference in the survival of plants in both experimental groups was tested using *G*-test of independence. The above-ground biomass between both treatments was compared using Mann-Whitney *U* test, whereas all other plant parameters were compared using *t*-tests. The stolon lengths were (log + 0.5)-transformed to meet *t*-test assumptions.

5.2.2 Experiment 2

Hieracium pilosella rosettes grown from seeds on 14 January 2000 were individually potted in clay pots (13 cm diameter) on 17 April 2000. On 8 May, 48 *H. pilosella* plants having five to 13 rosette leaves and between zero and six stolon buds were randomly chosen for the experiment. Each 12 pots were randomly assigned to the following four treatment combinations: *M. pilosellae* present or absent (three females and three males) and grass competition present or absent (two *Festuca rubra* L. var. Echo tufts, each covering a circular area of 4.3 cm in diameter). The *F. rubra* tufts were planted next to the *H. pilosella* rosette. Grass competition was chosen to imitate field conditions and because *H. pilosella* grows extremely vigorously in the absence of neighboring plants in standard potting soil (personal observation G. Grosskopf). All pots were covered with gauze bags until evaluation of the tests. To protect the fragile insects from extreme weather conditions, all 48 pots were kept in an unheated greenhouse for one week and then embedded in a

garden bed.

On 6 and 7 July all plants were measured and the following plant parameters recorded: number of leaves, number of flower heads, number of terminal and axillary meristems, weight of dried aboveground biomass and stolon length. The data was analyzed applying two factorial ANOVAs with factors herbivory and plant competition.

5.3 Results

5.3.1 Experiment 1

Herbivory by *M. pilosellae* had a significant impact on the number of plants being dead upon evaluation of the tests. On 9 November, all 18 control plants were still alive whereas six (33.3%) of the plants exposed to gall midges had died (*G*-test of independence, $P = 0.002$). Control plants had on average 33.1% more leaves (*t*-test $P = 0.027$) and 38.9% more above-ground biomass (Mann-Whitney *U* test, $P = 0.022$) than plants attacked by *M. pilosellae* (Figure 5.1). No significant difference between the two groups was found regarding the number of flower heads (*t*-test, $P = 0.091$), weight of below-ground biomass (*t*-test, $P = 0.053$) and the total number of terminal and axillary meristems (*t*-test, $P = 0.519$). Control plants had significantly longer stolons than plants infested with *M. pilosellae*. The mean length of the longest stolon was 5.6 times greater for plants in the control group than for plants infested with *M. pilosellae* ($t = 4.72$, $P < 0.001$). The mean length of the two longest stolons was 5 times ($t = 4.35$, $P < 0.001$) and the mean length of all stolons of first order 4.4 times ($t = 3.45$, $P = 0.002$) greater in the control group. Regular measurements of the lengths indicate that *M. pilosellae* attack inhibits stolon elongation (Figure 5.2).

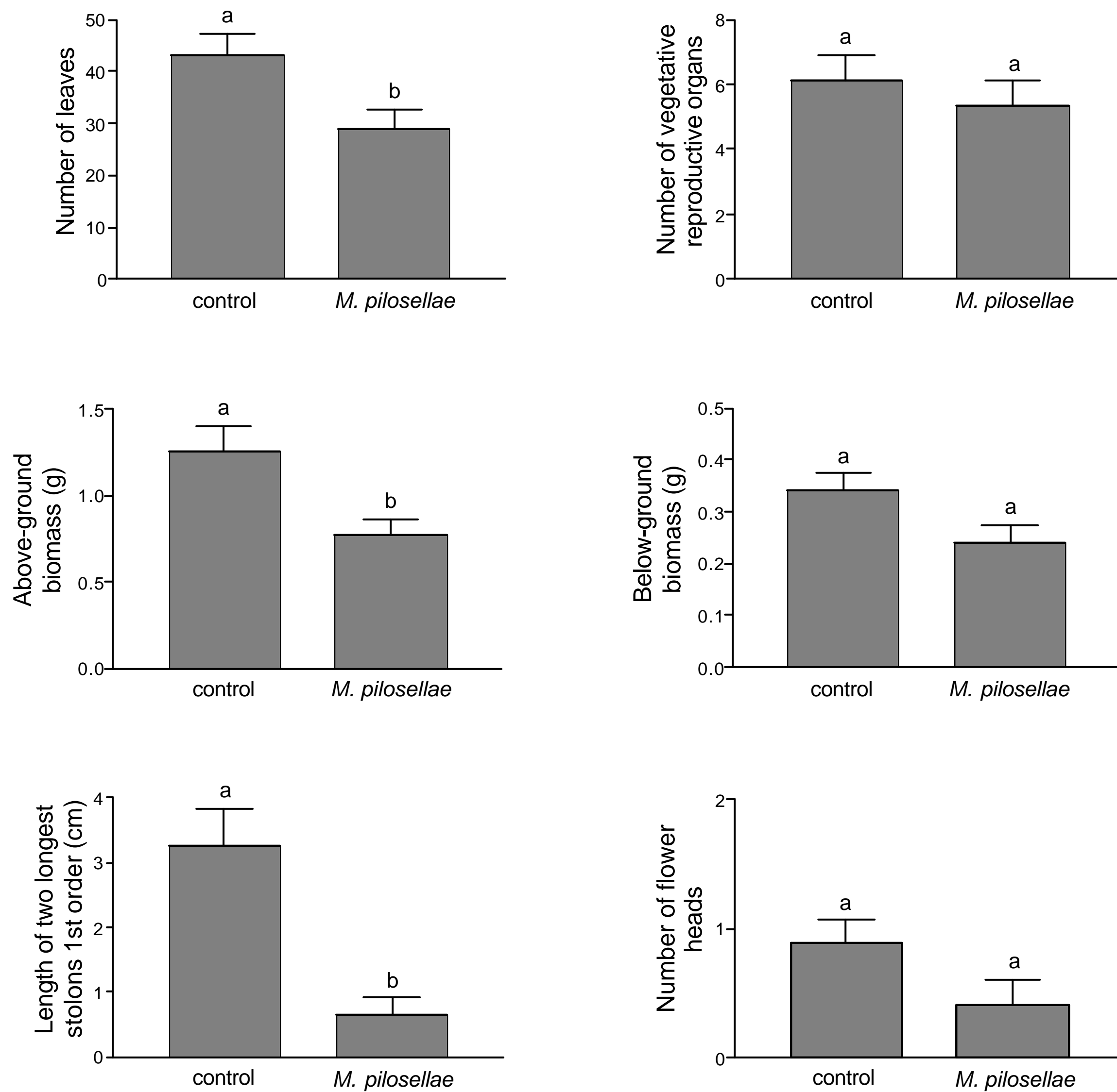

Figure 5.1. Effect of herbivory by *M. pilosellae* on different plant parameters of *H. pilosella* in comparison to control plants (in all graphs mean ± SE are given, means with the same letter are not significantly different, *t*-test or Mann-Whitney *U* test, see text for details).

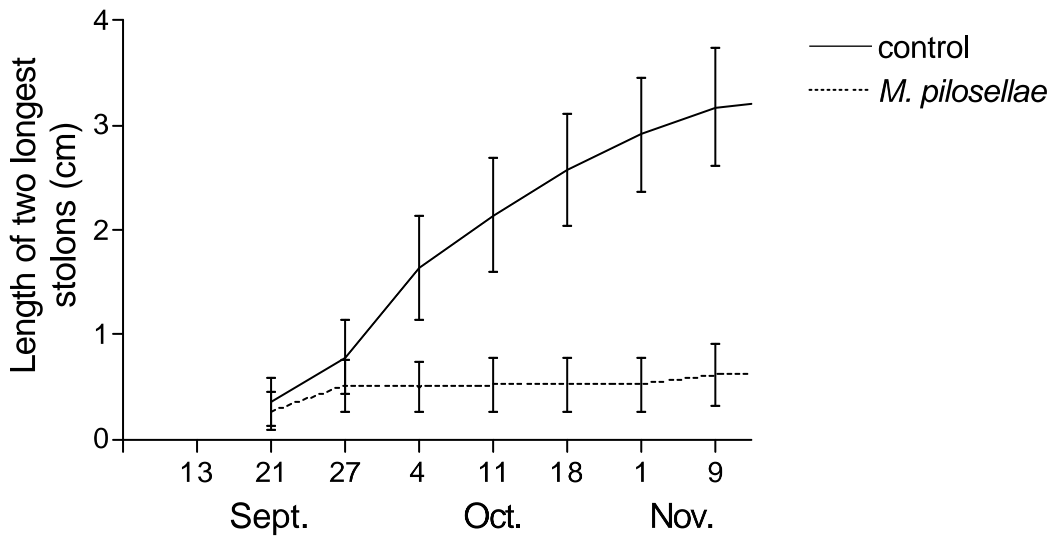

Figure 5.2. Stolon length (mean ± SE) of plants attacked by *M. pilosellae* (dotted line) and unattacked plants (solid line).

5.3.2 Experiment 2

None of the plants died until evaluation of the tests. Plant competition by *F. rubra* had a significant impact on all plant parameters of mouse-ear hawkweed recorded in this study, except the length of primary stolons (Figure 5.3, Table 5.1), while herbivory by *M. pilosellae* had a significant impact on the total number of leaves, the number of flower heads and the stolon length. No significant interactions were found between effects of herbivory and plant competition. In the absence of plant competition, attack by *M. pilosellae* reduced the number of axillary and terminal meristems by 14.9% and the above-ground biomass by 18.6% compared to plants grown without competition and herbivory but the differences were not significant. Grass competition in combination with *M. pilosellae* attack reduced the number of leaves by 61.3% in comparison to the control plants whereas *M. pilosellae* alone and plant competition alone led to a reduction of 26.8%, and 48.1%, respectively.

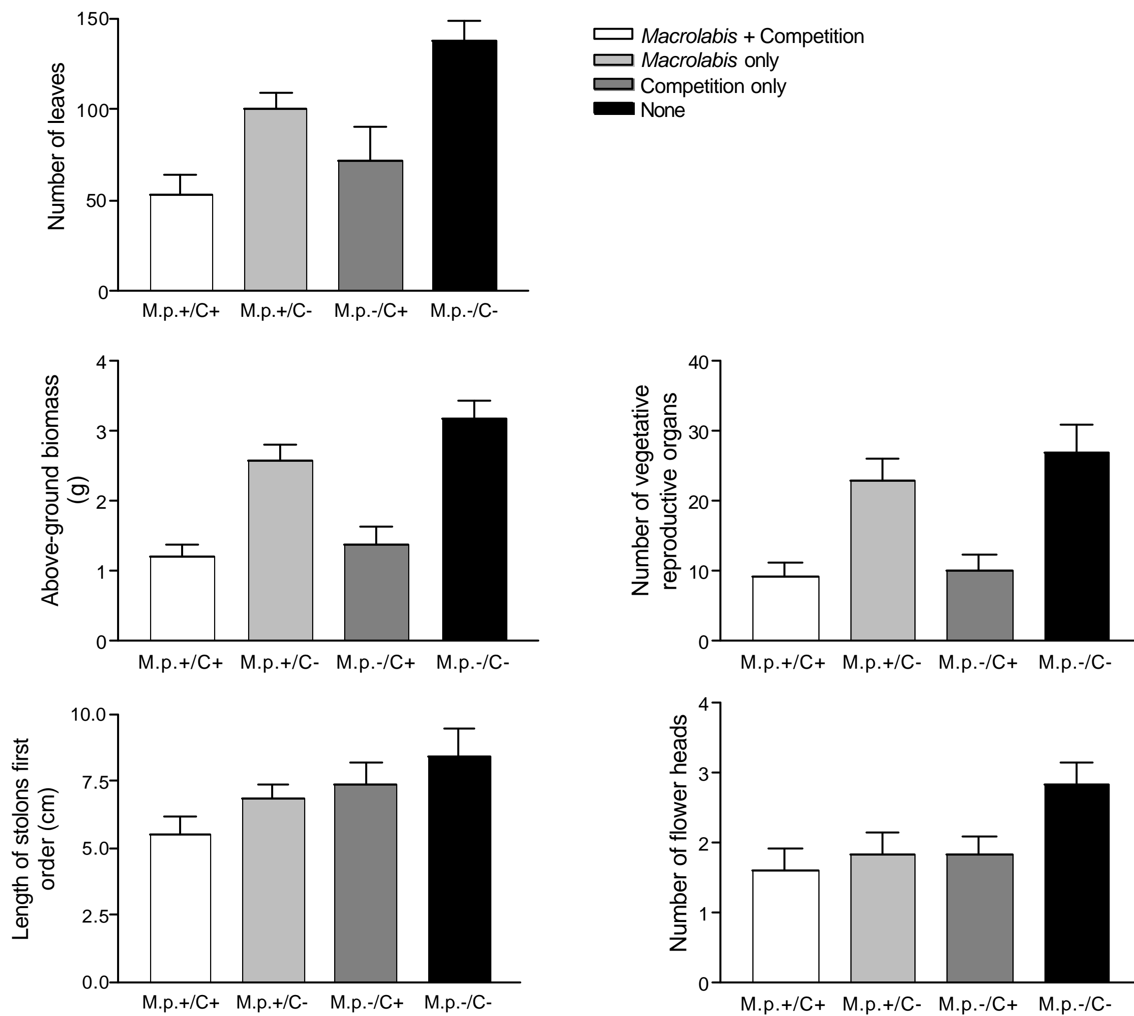

Figure 5.3. Treatment effects on *H. pilosella* plant size parameters during harvest of the plants (in all graphs mean ± SE are given, M.p.+, *M. pilosellae* herbivory; M.p.-, no *M. pilosellae* herbivory; C+, grass competition; C-, no grass competition).

Table 5.1. ANOVA table on effects of herbivory and plant competition on *H. pilosella* plant size parameters at harvest date of plants. *F*-ratios and *P*-values taken from two factorial ANOVAs.

Source of Variation		Number of leaves		Aboveground biomass		Number of terminal and axillary meristems		Length of primary stolons		Number of flower heads	
	d.f.	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Herbivory (*M.p.*)	1	4.59	0.038	2.72	0.106	0.68	0.413	4.69	0.036	4.30	0.044
Plant competition (C)	1	19.55	<0.001	45.87	<0.001	26.14	<0.001	2.24	0.142	4.30	0.044
M.p. x C	1	0.53	0.470	0.74	0.395	0.27	0.607	0.04	0.848	1.55	0.220
Error	44										

5.4 Discussion

Galls of *M. pilosellae* alter the plant architecture of mouse-ear hawkweed. Early attack of stolons inhibits or retards stolon elongation. The internodes remain shorter than the ones of *H. pilosella* plants without galls (Grosskopf, personal observation). Stunting of plants due to galling is common in gall-inducing insects and mites, e.g. *Cystiphora schmidti* (Rübsaamen) (Caresche and Wapshere, 1975) and *Eriophyes chondrillae* (G. Canestrini) (Spollen and Piper, 1995) associated with rush skeletonweed, *Chondrilla juncea* L., *Dasineura affinis* on *Viola odorata* (Birch et al., 1992), or *R. tripleurospermi* on scentless camomile (Hinz, 1999). However, the apical dominance of *H. pilosella* was generally not broken when galls were induced in the apical meristem, as it has been observed for other herbivores, e.g. *R. tripleurospermi* (Hinz, 1999). Although the total leaf area of *H. pilosella* plants used in this experiment was not determined, it is assumed that *M. pilosellae* attack decreases the area available for photosynthesis and transpiration due to the reduced number of leaves and inhibition of unfurling of leaves. Stunting of *H. pilosella* stolons may negatively affect its ability to rapidly colonize open habitats which is an advantage of clonal plants. Due to the production of stolons, clonal plants often form dense patches or can forage for resources that are heterogeneously distributed (Hutchings, 1988; Winkler and Stöcklin, 2002). *Aulacidea subterminalis* (Cynipidae), another biological control agent of mouse-ear hawkweed, reduced the stolon length of *H. pilosella* by 75% under stress-free conditions in a manipulative greenhouse experiment (Klöppel et al., 2003).

In addition to the impact on the vegetative growth of mouse-ear hawkweed, attack by *M. pilosellae* might affect propagation by seeds by significantly reducing the number of flower heads. Seeds are thought to not only play an important role for long distance spread of *H. pilosella*, but also for the persistence of populations under strong interspecific competition (Winkler and Stöcklin, 2002). In contrast to the study of Makepeace (1985a) who recorded an increased number of stolons and doubling of their lengths after artificial removal of inflorescences, the reduction of the number of flower heads due to herbivory of *M. pilosellae* did not result in a more vigorous vegetative growth.

Plant competition had a stronger effect on the performance and reproduction of *H. pilosella* than herbivory by *M. pilosellae*. However, the effect of plant competition depends on the density of the competitors. Biomass of Canada thistle decreased with increase in the density of the plant competitors seeded (Ang et al., 1994). *Hieracium pilosella* is a plant of nutrient-poor sites and favored by disturbance (Winkler and Stöcklin, 2002). However, vegetative reproduction is increased by fertilizer application (Makepeace, 1985b) but *H. pilosella* tends to be out-competed by other plants under high nutrient conditions (Scott,

1993).

It is generally challenging to realistically determine the impact of a potential biological control agent in the area of release. The influence of herbivory on plant growth and fitness depends on the study system, i.e. the life history and biology of the plant and the herbivore, and on the experimental conditions (e.g. presence of competitive plants, stress factors, density of plants or degree of herbivory). The root-mining moth *Agapeta zoegana* L. (Cochylidae), a biological control agent of spotted knapweed, *Centaurea maculosa* Lam., in North America, increased survival, shoot number and biomass/area of *C. maculosa* in field plots in Switzerland when applied in low numbers. However, under competition with *Festuca pratensis* Huds., shoot number and fecundity of knapweed decreased linearly with increasing number of herbivores (Müller-Schärer, 1991). Callaway et al. (1999) did not record a significant decrease in *Centaurea* biomass by *A. zoegana* in a common garden experiment in North America. Story et al. (2000) recorded that *A. zoegana* prefer large and usually bolted knapweed plants with large roots, which makes impact studies in the field challenging.

The results obtained for mouse-ear hawkweed in the impact experiments show that attack by *M. pilosellae* severely suppressed vegetative growth and the production of flower heads. It is therefore expected that the release of *M. pilosellae* in the field would, at least, delay growth and decrease the rate of spread of the weed. Experiments were only carried out with one generation of *M. pilosellae*. However, since the gall midge produces up to three generations per year, it is assumed that repeated attack by subsequent generations of *M. pilosellae* increases the impact on *H. pilosella,* which grows throughout the season.

6 Suitability of *Macrolabis pilosellae* for the biological control of invasive hawkweeds (*Hieracium* spp.) in North America with special regard to potential non-target effects

6.1 Introduction

Macrolabis pilosellae is a multivoltine gall midge of European origin which has been first released in New Zealand in 2002 to control alien invasive *Hieracium* spp., in particular *H. pilosella*. Host range investigations carried out prior to its release in New Zealand revealed that *M. pilosellae* is at least genus-specific (see chapter 4). Due to its ability to develop on several *Hieracium* spp. in the subgenus *Pilosella*, specifically *H. caespitosum*, and the high degree of specificity of gall-inducing insects, *M. pilosellae* was considered as a potential biological control agent of alien invasive hawkweeds in North America. However, in contrast to the situation in New Zealand where no native *Hieracium* spp. exist (Webb et al., 1988), native hawkweeds do occur in North America. They belong exclusively to the subgenera *Hieracium* and *Chionoracium* whereas most alien invasive hawkweeds belong to the subgenus *Pilosella*, and to a smaller extent, to the subgenus *Hieracium* (Fernald, 1950; Gleason and Cronquist, 1991; Scoggan, 1979). In the present study, the host spectrum of *M. pilosellae* was investigated with regard to its potential use as a biological control agent of alien invasive hawkweeds in North America. All experiments were carried out in a common garden and in the laboratory of CABI Bioscience Switzerland Centre in Delémont between 2000 and 2004.

6.2 Materials and Methods

A test plant list (Table 6.1) was complied by Dr. Linda Wilson (University of Idaho) and Jennifer Birdsall (USDA Forest Service, Rocky Mountain Research Station, Bozeman, Montana) (Wilson and Birdsall, 2001). Ornamentals and crop plants within the Asteraceae and from other families had been elaborately tested prior to release of *M. pilosellae* in New Zealand (see chapter 4). For this reason, the North American test plant list focuses on (i) target weeds from the subgenus *Pilosella*, i.e. *H. aurantiacum, H. caespitosum, H. floribundum, H. glomeratum, H. pilosella,* and *H. piloselloides* to explore whether *M. pilosellae* can develop on these plants, (ii) *Hieracium* spp. in the subgenera *Chionoracium* and *Hieracium* which are native to North America, and (iii) native and economic plants in

the family Asteraceae, especially the Lactuceae tribe (Wilson and Birdsall, 2001). Additional plant species were tested due to their availability, although they were not part of the list.

All *M. pilosellae* adults used for screening tests were obtained from a rearing colony maintained at the Centre in Delémont. The rearing was established with adults emerging from galls collected in the Black Forest (Southern Germany) and the Swiss Jura. Host range tests were set up the same day that adults emerged.

Table 6.1. Test plant list used for screening *M. pilosellae*.

	Test plant species[a]	Origin[b]	Status
Asteraceae			
Tribe Lactuceae			
Subgenus: *Pilosella*	*H. caespitosum* Dumort. ID	I	Target weed
	H. glomeratum Froel. BC	I	Target weed
	H. glomeratum EWA	I	Target weed
	H. aurantiacum L. ID	I	Target weed
	H. aurantiacum MT	I	Target weed
	H. floribundum Wimm. et Grab. MI	I	Target weed
	H. piloselloides Vill. MT	I	Target weed
Subgenus: *Hieracium*	*H. canadense* Michx.	N	Native
	H. umbellatum L.	N	Native
Subgenus: *Chionoracium*	*H. albiflorum* Hook.	N	Native
	H. argutum Nutt.	N	Native
	H. bolanderi Gray	N	Native
	H. carneum Greene	N	Native
	H. fendleri Schultz-Bip.	N	Native
	H. gracile Hook.	N	Native
	H. gronovii L.	N	Native
	H. longiberbe T. J. Howell	N	Native
	H. longipilum Torr.	N	Native
	H. parryi Zahn	N	Native
	H. scabrum Michx.	N	Native
	H. scouleri Hook.	N	Native
	H. venosum L.	N	Native
	Agoseris grandiflora (Nutt.) E. Greene	N	Native
	Catananche caerula L.	I	Ornamental
	Crepis atribarba Heller	N	Native
	Crepis intermedia Gray	N	Native
	Krigia biflora (Walt) Blake	N	Native
	Lygodesmia juncea (Pursh) D. Don	N	Native
	Microseris nutans (Hook.) Schultz-Bip.	N	Native
	Microseris troximoides (Gray) Greene	N	Native
	Prenanthes sagittata (Gray) A. Nels.	N	Native
	Stephanomeria tenuifolia (Raf.) Hall	N	Native
	[c]*Taraxacum laevigatum* (Willd.) DC	I	Same tribe
	[c]*Taraxacum lyratum* (Ledeb.) DC	N	Native
	[c]*Tragopogon dubius* Scop.	I	Same tribe
Tribe: Anthemideae	[c]*Artemisia dracunculus* L.	N	Cultivated
Tribe: Arctoteae	[c]*Gazania splendens* Lem.	I	Ornamental
Tribe: Astereae	[c]*Aster laevis* L.	I	Ornamental
Tribe: Calenduleae	[c]*Calendula officinalis* L.	I	Ornamental
Tribe: Cardueae	*Cirsium undulatum* (Nutt.) Sprengel	N	Native
Tribe: Eupatorieae	[c]*Eupatorium maculatum* L.	N	Native
Tribe: Gnaphalieae	[c]*Antennaria dioica* (L.) Gaertn.	N	Native
Tribe: Helenieae	[c]*Tagetes erecta* L.	I	Ornamental
Tribe: Inuleae	[c]*Inula helenium* L.	I	
Tribe: Mutisieae	[c]*Gerbera jamesonii* Bolus ex Hooker f.	I	Ornamental
Tribe: Vernonieae	[c] *Stokesia laevis* Greene	N	Native

[a] States or provinces where the test plant seeds originate from, ID: Idaho, BC: British Columbia, EWA: East Washington, MT: Montana; [b] origin of the test plants: I = introduced into North America, N = native to North America; [c] plants tested in addition to the actual test plant list.

6.2.1 No-choice gall formation tests with potted plants

Between 2000 and 2004, no-choice gall formation tests using potted plants were carried out with all test plant species available. In each replicate, two to three male and three female *M. pilosellae* adults were transferred onto a potted plant covered with a gauze bag. Pots were kept in a polythene-covered garden tunnel for up to one week to protect the fragile adults from extreme weather conditions. The pots were then embedded in a garden bed. Approximately four weeks after exposure to the gall midges, all plants were carefully checked for galls, feeding marks and the presence of *M. pilosellae* larvae under a stereo-microscope. Any plants with galls, feeding marks or larvae were transferred into gauze cages to record adult emergence.

6.2.2 Single-choice gall formation tests with potted plants

Single-choice gall formation tests using potted *H. albiflorum, H. argutum, H. canadense, H. carneum, H. fendleri, H. gracile, H. gronovii*, *H. scabrum, H. scouleri* and *H. umbellatum* plants (native North American *Hieracium* spp.), and *H. caespitosum* ID as a control plant, were set up between 2000 and 2004. A test plant and a control were planted together into a clay-pot (18 cm diameter) using standard potting soil. Pots were covered with gauze bags and three to six male and six female gall midges were transferred into each pot giving the females the choice to oviposit onto the test or the control plant. The plants were kept in a polythene-covered garden tunnel for approximately one week and were then planted in the garden. All plants were checked for galls, feeding marks and larvae approximately four weeks after exposure of the adults. The test and the control plant were repotted individually in smaller pots (13 cm diameter) to record adult emergence daily.

6.2.3 Single-choice oviposition tests in cups with cut plant material

Single-choice oviposition tests were carried out in plastic cups (diameter 5.5 cm, height 8 cm) offering a choice between a cut plant part of *H. caespitosum* ID and a test plant, i.e. *H. aurantiacum, H. floribundum, H. pilosella, H. canadense, H. umbellatum, H. carneum, H. gronovii, Antennaria dioica, Artemisia dracunculus* or *Tagetes erecta*, lying side by side on moist filter paper. Since females lay their eggs into the leaf axil, a piece of stem, stolon or rosette with at least one leaf was offered to the adults. One pair of gall midges was transferred into the cup for 24 hours and stored in an incubator at 20 °C and long-day conditions 16/8 (L:D). Afterwards, adults were transferred into another cup with a different

test plant species and a control plant, and the number of eggs laid onto the test and control plant parts were counted. Only cups containing eggs were included in data analysis.

6.2.4 Multiple-choice gall formation tests in field cages

Different multiple-choice gall formation tests using potted plants were carried out in field cages as described below. Approximately four weeks after incubation of the adults, all exposed plants were checked for galls, feeding marks and the presence of larvae.

Experiment 1. Multiple-choice gall formation tests using potted plants were set up between 2000 and 2002 in field cages measuring 1 x 1 x 1m. In each cage, four pots each of four different *Hieracium* spp. were embedded in sawdust using different combinations of test plants each time, and 18 to 30 males and 21 to 30 females were released into each of the cages. The following species were exposed to the adults of the gall midge: on 11 September 2000 *H. pilosella* EUR, *H. aurantiacum* ID, *H. caespitosum* ID and *H. canadense*; on 14 May 2001 *H. caespitosum* ID, *H. parryi*, *H. piloselloides* and *H. scouleri*, and *H. albiflorum*, *H. glomeratum* BC, *H. caespitosum* ID and *H. canadense*; on 29 July *H. caespitosum* ID, *H. bolanderi*, *H. canadense* and *H. scouleri* in one cage, and *H. albiflorum*, *H. caespitosum* ID, *H. glomeratum* EWA and *H. parryi* in a second cage; on 30 July 2002 *H. albiflorum*, *H. caespitosum* ID, *H. greenei*, and *H. scouleri.* A second field cage test was set up on 7 September 2002 with four potted plants each of *H. albiflorum*, *H. caespitosum* ID, *H. carneum,* and *H. fendleri*.

Experiment 2. Experiments 2 and 3 were designed according to Briese's "two-phase experiment" (Briese, 1999) but with the following modifications: (i) plots were established in field cages to protect the plants from other herbivores, and (ii) phase one and two are represented by two different cages in order to expose *M. pilosellae* to choice-minus-control and choice situations with *H. caespitosum* satellite plots to encourage emigration of *M. pilosellae*.

On 15 July 2004, two multiple-choice gall development tests were set up in field cages (2m x 2m x 1.6m) containing the same combination of control (*H. caespitosum*, $n = 4$) and native North American test plants ($n = 21$ pots). Plants were interspersed in the centre of the cage, with an additional *H. caespitosum* plant in all four corners of the two cages. The soil of the *H. caespitosum* plants in the centre contained immature *M. pilosellae* stages since they

were exposed to gall midges in May. In one of the two cages, all above-ground plant parts were removed from the pots located in the centre which contained larvae and pupae of *M. pilosellae* (cage treatment 1). This forced the insects to use either the native North American *Hieracium* spp. in the centre or to emigrate to the *H. caespitosum* plants in the corners. In contrast, the *H. caespitosum* plants exposed in the center of the other cage were not defoliated (cage treatment 2). All plants were checked for galls, feeding marks and larvae on 9 and 10 August 2004.

Experiment 3. On 26 August 2004, two multiple-choice gall development tests were set up in field cages measuring 2m x 2m x 1.6m (Figure 6.1). In both cages, four potted *H. caespitosum* plants, the soil of which contained mature larvae and pupae of *M. pilosellae,* were placed between 21 pots of eight native North American *Hieracium* spp. The above-ground plant parts of the *H. caespitosum* plants in the centre were removed to oblige freshly emerged *M. pilosellae* adults to move within the cage. Potted *H. caespitosum* plants with foliage were exposed in the four corners of only one of the cages as target plants (TP) (see Figure 6.1).

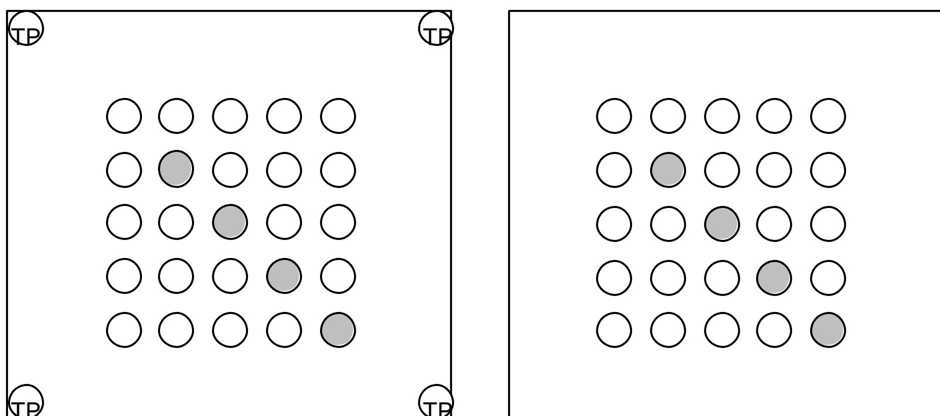

Figure 6.1. Experimental set up of field cage experiment 3 (TP = target plant; filled dots = *H. caespitosum* without foliage but soil containing immature *M. pilosellae* stages).

6.2.5 Open-field gall formation tests

On 28 August 2004, two open-field plots were set up containing nine embedded potted plants, of which three pots contained *H. caespitosum* with immature stages in the soil, and the remaining six contained test plants of either *H. scouleri* or *H. carneum*. In one of the plots, all above-ground plant parts of the *H. caespitosum* plants were removed (Figure 6.2) displaying a choice minus control situation. The two plots were located five meters from

each other and were separated by shrubs as a natural barrier. One month later all plants were checked for galls, larvae and feeding marks.

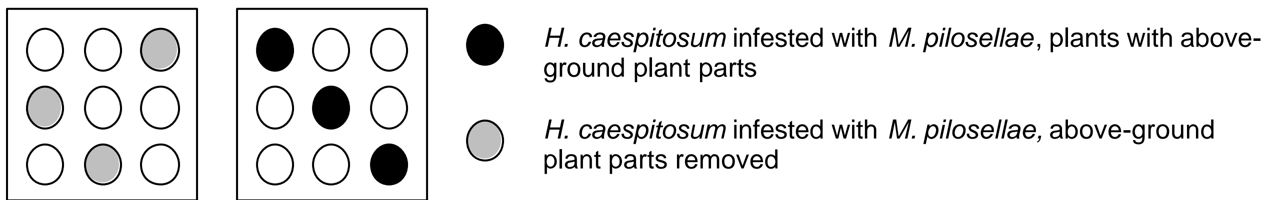

Figure 6.2. Experimental set up of open-field tests (the empty circles are the test plants).

6.3 Results

6.3.1 No-choice gall formation tests with potted plants

Galls were exclusively recorded on plants within the genus *Hieracium* but not on plants of any of the other plant genera. *Hieracium* spp. from all three subgenera supported gall formation and development to adulthood. The highest attack rate was recorded on *H. caespitosum* with 83.9% of the exposed plants attacked (Table 6.2). The highest survival rate to adulthood was recorded on *H. caespitosum* with 14 adults per pot. In the subgenus *Pilosella*, *H. aurantiacum* was the less suitable species with adult emergence rates of 0 for *H. aurantiacum* ID and 1.2 for *H. aurantiacum* MT and gall formation rates of 4.8% and 50%. *Hieracium umbellatum* and *H. canadense* in the subgenus *Hieracium* were also only attacked to a limited extent. Some of the test plants, especially in the subgenus *Chionoracium,* were difficult to rear and their quality decreased in the course of the study. Therfore, survival rates to adulthood and gall development on certain hawkweed species in the subgenus *Chionoracium* are probably higher than recorded in this study.

6.3.2 Single-choice gall formation tests with potted plants

All *H. caespitosum* plants exposed to the gall midges were galled. No galls, feeding marks or larvae were observed on *H. argutum, H. carneum*, or *H. gracile* (Table 6.3). About 10% of the *H. canadense* and *H. umbellatum* plants were galled when offered simultaneously with the control plants. *Hieracium albiflorum, H. gronovii, H. scabrum,* and *H. scouleri* were also attacked. However, the number of adults emerged from test plants was in all cases low in comparison to the number of *M. pilosellae* adults emerged from the control plants.

Table 6.2. No-choice gall formation tests *M. pilosellae,* 2000-2004.

Test plant species	No. plants tested	Plants with galls		Mean No. galls per plant	Plants with feeding marks		Plants with larvae		Adults[a]
		No.	%		No.	%	No.	%	
Subgenus *Pilosella*									
Hieracium aurantiacum ID	21	1	4.8	0	5	23.8	1	4.8	0
H. aurantiacum MT	6	3	50.0	1.2	6	100.0	4	66.7	1.2
H. caespitosum ID	62	52	83.9	2.8	54	87.1	50	80.6	14
H. floribundum	15	9	60.0	2.3	9	60.0	8	53.3	4.7
H. glomeratum BC	12	8	66.7	0.8	8	66.7	7	58.3	1.3
H. glomeratum EWA	9	4	44.4	2.0	8	88.9	5	55.6	4.2
H. pilosella EUR	6	4	66.7	1.5	4	66.7	4	66.7	6.8
H. pilosella WA	12	10	83.3	2.9	8	66.7	8	66.7	7.5
H. piloselloides	15	8	53.3	2.1	8	53.3	8	53.3	1.8
Subgenus *Hieracium*									
**H. canadense*	21	3	14.3	0.3	5	23.8	5	23.8	0.8
**H. umbellatum*	30	6	20.0	0.3	9	30.0	9	30.0	0.3
Subgenus *Chionoracium*									
**H. argutum*	1	0	0	0	0	0	0	0	0
**H. albiflorum*	17	1	5.9	0.1	2	11.8	2	11.8	0
**H. bolanderi*	6	4	66.7	0.7	4	66.7	3	50.0	0
**H. carneum*	17	8	47.1	0.5	9	52.9	8	47.1	13.6
**H. fendleri*	7	1	14.3	0.1	2	28.6	2	28.6	0.1
**H. gracile*	12	3	25.0	0.4	3	25.0	3	25.0	2.3
**H. greenei*	10	1	10.0	0.1	2	20.0	2	20.0	0
**H. gronovii*	17	0	0	0	2	11.8	2	11.8	0
**H. longiberbe*	6	1	16.7	0.2	1	16.7	0	0	0
**H. longipilum*	3	0	0	0	0	0	0	0	0
**H. parryi*	11	3	27.3	0.3	2	18.2	4	36.4	0.4
**H. scabrum*	16	6	37.5	0.5	9	56.3	4	25.0	0.1
**H. scouleri* var. *cynoglossoides*	3	0	0	0	0	0	0	0	0
**H. scouleri* var. *albertinum*	13	4	30.8	0.4	8	61.5	6	46.2	1.7

Table 6.2. (continued)

Test plant species	No. plants tested	Plants with galls		Mean No. galls per plant	Plants with feeding marks		Plants with larvae		Adults[a]
		No.	%		No.	%	No.	%	
**H. venosum*	3	0	-	0	0	-	0	-	0
Agoseris grandiflora	12	0	-	0	0	-	0	-	0
Antennaria dioica	12	0	-	0	0	-	0	-	0
Aster laevis	9	0	-	0	0	-	0	-	0
Calendula officinalis	12	0	-	0	0	-	0	-	0
Catananche caerula	15	0	-	0	0	-	0	-	0
Cirsium undulatum	9	0	-	0	0	-	0	-	0
Crepis intermedia	11	0	-	0	0	-	0	-	0
Crepis sp. (Applegut)	6	0	-	0	0	-	0	-	0
Eupatorium maculatum	6	0	-	0	0	-	0	-	0
Gazania splendens	9	0	-	0	0	-	0	-	0
Gerbera jamesonii	12	0	-	0	0	-	0	-	0
Inula helenium	12	0	-	0	0	-	0	-	0
Krigia biflora	14	0	-	0	0	-	0	-	0
Lygodesmia juncea	9	0	-	0	0	-	0	-	0
Microseris troximoides	6	0	-	0	0	-	0	-	0
Prenanthes sagittata	15	0	-	0	0	-	0	-	0
Stephanomeria tenuifolia	9	0	-	0	0	-	0	-	0
Stokesia laevis	12	0	-	0	0	-	0	-	0
Tagetes erecta	12	0	-	0	0	-	0	-	0
Taraxacum laevigatum	13	0	-	0	0	-	0	-	0
Taraxacum lyratum	15	0	-	0	0	-	0	-	0
Tragopogon dubius	12	0	-	0	0	-	0	-	0

[a] Total No. of adults emerged divided through the number of replicates; **Hieracium* spp. indigenous to North America.

Table 6.3. Single-choice gall formation tests with *M. pilosellae* using potted plants 2000-2004.

Test plant [a]	*n*	% plants galled		% plants with feeding marks		% plants with larvae		Mean No. adults per plant	
		test	control	test	control	test	control	test	control
<u>Subg. *Hieracium*</u>									
H. canadense	10	10	100	10	90	10	90	0.2	15.6
H. umbellatum	9	11.1	100	11.1	100	11.1	100	0	9.6
<u>Subg. *Chionoracium*</u>									
H. albiflorum	2	50	100	50	100	50	100	0.5	4.5
H. argutum	3	0	100	0	100	0	100	0	8.7
H. carneum	7	0	100	0	100	0	100	0	10.4
H. fendleri	3	0	100	0	100	3	100	0	21
H. gracile	2	0	100	0	100	0	100	0	0
H. gronovii	10	30	100	30	100	20	100	0.1	5.6
H. scabrum	11	27.3	100	18.2	100	18.2	100	0.7	27.4
H. scouleri	8	37.5	100	50	100	50	100	0	14.1

[a] All *Hieracium* spp. tested are indigenous to North America.

6.3.3 Single-choice oviposition tests in cups with cut plant material

An oviposition ratio below one, i.e. more eggs were laid onto *H. caespitosum* than onto the corresponding test plant, was recorded for all *Hieracium* spp. tested except *H. floribundum* (subgenus *Pilosella*) which received about 72% of the eggs compared to 38% on *H. caespitosum* (Table 6.4). The lowest preference was observed on *H. gronovii* with an acceptance factor of 0.04. Almost 20% of the eggs were laid onto *H. aurantiacum* ID, a plant with a very low attack rate in no-choice gall formation tests. In contrast, not a single egg was recorded on any of the plant species tested outside the genus *Hieracium*, i.e. *Antennaria dioica, Artemisia dracunculus,* and *Tagetes erecta*.

Table 6.4. Single-choice oviposition tests in cups with *M. pilosellae,* 2000-2003 with *H. caespitosum* as control plant.

Test plant	*n*	No. eggs laid on		Proportion (%)		Oviposition ratio[a]
		test	control	test	control	(test/control)
Subgenus *Pilosella*						
H. aurantiacum ID	28	97	406	19.3	80.7	0.24
H. floribundum	22	316	125	71.7	38.3	2.53
H. pilosella EUR	19	115	201	36.4	63.6	0.57
Subgenus *Hieracium*						
**H. canadense*	25	67	386	14.8	85.2	0.17
**H. umbellatum*	52	46	789	5.5	94.5	0.06
Subgenus *Chionoracium*						
**H. carneum*	21	74	265	21.8	78.2	0.28
**H. gronovii*	14	9	237	3.7	96.3	0.04
Antennaria dioica	11	0	115	0	100	0
Artemisia dracunculus	11	0	173	0	100	0
Tagetes erecta	20	0	377	0	100	0

[a] Number of eggs laid on test plant divided by the number of eggs on control plant; **Hieracium* spp. indigenous to North America.

6.3.4 Multiple-choice gall formation tests in field cages

Experiment 1. Since galls were found on plants in all field cages set up, they were all included in the analysis. All *Hieracium* spp. tested in the subgenus *Pilosella* were attacked except *H. aurantiacum* ID which had neither galls nor larvae (Table 6.5). The hightest gall formation rates were recorded on *H. pilosella* EUR, *H. glomeratum* BC and *H. caespitosum* with 100%, 75%, and 71.4% of the plants galled. Attack rates in the subgenera *Hieracium* and *Chionoracium* were much lower. Only one out of twelve *H. canadense* plants exposed in the tests had a gall. Two *Hieracium* spp. in the subgenus

Chionoracium, *H. carneum* and *H. parryi*, had galls or slight deformations, whereas all other native North American *Hieracium* spp. did not show any signs of attack.

Table 6.5. Multiple-choice gall formation tests with *M. pilosellae* in field cages 2000-2002.[a]

Test plant	No. plants offered [b]	Plants with galls		No. plants slightly deformed	Mean No. galls per plant	No. plants with feeding marks	No. plants with larvae
		No.	%				
<u>Pilosella</u>							
H. aurantiacum ID	4	0	0	0	0	0	0
H. caespitosum ID	28	20	71.4	0	1.9 ± 0.38	20 (71.4%)	21 (75%)
H. glomeratum BC	4	3	75.0	0	1 ± 0.41	3 (75%)	3 (75%)
H. glomeratum EWA	4	1	25.0	0	1	1 (25%)	1 (25%)
H. pilosella EUR	4	4	100.0	0	5.3 ± 1.55	4 (100%)	4 (100%)
H. piloselloides MT	4	1	25.0	0	0.3 ± 0.11	1 (25%)	1 (25%)
<u>Hieracium</u>							
**H. canadense*	12	1	8.3	0	0.1 ± 0.06	1 (8.3%)	0
<u>Chionoracium</u>							
**H. albiflorum*	8	0	0	0	0	0	0
**H. bolanderi*	4	0	0	0	0	0	0
**H. carneum*	4	1	25.0	0	0.5 ± 0.5	0	2 (50%)
**H. fendleri*	3	0	0	0	0	0	0
**H. greenei*	3	0	0	0	0	0	0
**H. parryi*	5	0	0	2 (40%)	0	0	0
**H. scouleri*	11	0	0	0	0	0	0

[a] Data from different cages and years were pooled; [b] only plants which were still alive four weeks after incubation of the adults were included in the analysis; * *Hieracium* spp. indigenous to North America.

Experiment 2. One test plant died in cage 1 and four in cage 2 before evaluation of the experiments. None of the plants in cage 1 were galled (Table 6.6). In the second cage, all four *H. caespitosum* plants in the centre, two of the *H. caespitosum* plants in the corners and one *H. carneum* plant from the centre were galled (Table 6.7).

Table 6.6. Multiple-choice gall formation tests with *M. pilosellae* under field cage conditions with target plants in the corners and defoliated *H. caespitosum* plants in the centre (Experiment 2, cage treatment 1).

Test plant	No. plants alive	No. plants with galls	Mean No. Galls	No. plants with larvae	No. plants with feeding
H. caespitosum ID in the corners	4	0	0	0	0
H. caespitosum ID in the centre	cut!	-	-	-	-
H. albiflorum	2	0	0	0	0
H. argutum	3	0	0	0	0
H. carneum	2	0	0	0	0
H. gronovii	3	0	0	0	0
H. longipilum	2	0	0	0	0
H. scabrum	2	0	0	0	0
H. scouleri	4	0	0	0	0
H. venosum	2	0	0	0	0

Table 6.7. Multiple-choice gall formation tests with *M. pilosellae* under field cage conditions with target plants in the corners and *H. caespitosum* plants in the centre (Experiment 2, cage treatment 2).

Test plant	No. plants alive	No. plants with galls	Mean No. Galls	No. plants with larvae	No. plants with feeding
H. caespitosum ID in the corners	4	2	1.3	0	0
H. caespitosum ID in the centre	4	4	4	4	4
H. albiflorum	1	0	0	0	0
H. argutum	3	0	0	0	0
H. carneum	2	1	1	1	0
H. gracile	1	0	0	0	0
H. gronovii	3	0	0	0	0
H. scabrum	2	0	0	0	0
H. scouleri	4	0	0	0	0
H. venosum	1	0	0	0	0

Experiment 3. Six of the 21 test plants in the cage with the four target plants (cage treatment 1) died before evaluation of the experiment. Three target plants (*H. caespitosum*) were galled and had between two and six galls (Table 6.8). One *H. carneum* and one *H. gronovii*

plant were deformed and contained mature larvae. In the cage without above-ground *H. caespitosum* foliage (cage treatment 2), four of the 21 test plants died, and two *H. carneum*, one *H. gronovii,* and one *H. longipilum* plant were galled (Table 6.9).

Table 6.8. Multiple-choice gall formation tests with *M. pilosellae* under field cage conditions (Experiment 3, cage treatment 1).

Test plant	No. plants alive	No. plants with galls	Mean No. Galls	No. plants with larvae	No. plants with feeding
H. caespitosum ID in the centre	cut!	-	-	-	-
H. caespitosum in the 4 corners	4	3	3.3	3	3
H. argutum	3	0	0	0	0
H. carneum	2	1	0.5	1	1
H. gronovii	4	1	0.3	1	1
H. scabrum	2	0	0	0	0
H. scouleri	4	0	0	0	0
H. venosum	1	0	0	0	0

Table 6.9. Multiple-choice gall formation tests with *M. pilosellae* under field cage conditions (Experiment 3, cage treatment 2).

Test plant	No. plants alive	No. plants with galls	Mean No. Galls	No. plants with larvae	No. plants with feeding
H. caespitosum ID in the centre	cut!	-	-	-	-
H. argutum	1	0	0	0	0
H. carneum	2	2	1.5	2	2
H. gronovii	3	1	0.7	1	1
H. longipilum	2	1	0.5	1	1
H. scabrum	2	0	0	0	0
H. scouleri	4	0	0	0	0
H. venosum	2	0	0	0	0

6.3.5 Open-field gall formation tests

In plot 1 (containing the *H. caespitosum* plants with foliage), one leaf of a *H. carneum* and all three *H. caespitosum* plants (4, 6 and 3 galls) were galled (Table 6.10). In contrast, three test plants, one *H. scouleri* plant and two *H. carneum* plants, in plot 2 (containing *H. caespitosum* without foliage) had galls.

Table 6.10. Open-field gall formation tests with *M. pilosellae* in 2004.

Plot	Test plant	No. plants alive	No. plants with galls	Mean No. galls	No. plants with larvae	No. plants with feeding
1	*H. caespitosum*	3	3	4.3	3	3
	H. carneum	3	1	0.3	1	1
	H. scouleri	3	0	0	0	0
2	*H. caespitosum*	plants cut!	-	-	-	-
	H. carneum	3	2	1.3	2	2
	H. scouleri	3	1	0.7	1	1

6.4 Discussion

Since the release of exotic biological control agents is irreversible, results obtained in host-specificity investigations have to be carefully assessed before releasing an agent to avoid feeding on plant species other than the target weed (Harris and Zwölfer, 1968; Wapshere, 1974). It is recommended to either select target weeds that have few or no native congeners and/or to introduce biological control organisms with suitably narrow diets exclusively (Pemberton, 2000). Published literature about agents which are not sufficiently host-specific and which were therefore rejected, is relatively rare, e.g. McFadyen and Weggler-Beaton (2000), McFadyen and Marohasy (1990), and Hinz (1999).

Macrolabis pilosellae was first selected as a biological control agent of alien invasive *Hieracium* spp. in New Zealand. Hawkweeds have no native congeners in New Zealand. Therefore, host-specificity investigations carried out prior to its release in New Zealand were aimed at exploring whether plants outside the genus *Hieracium* were at risk, and at determining which of the ten naturalized species were potential host plants (chapter 4). However, it was beyond the scope of that study to define the host range within the genus *Hieracium*. In contrast, the test plant list used for North America focused mainly on *Hieracium* spp., i.e. native *Hieracium* spp. in the subgenera *Hieracium* and *Chionoracium* and the target weeds, i.e. alien invasive *Hieracium* spp. in the subgenus *Pilosella.* Testing of plant species that are closely related to the target weed allows a far more precise prediction of the host range of *M. pilosellae*. Buhr (1964) stated that the host range of *M. pilosellae* comprises species in the subgenus *Pilosella*. Results of host range tests carried out for North America confirm and also expand this observation. In summary it can be ascertained that *M. pilosellae* is host-specific to species within the genus *Hieracium* with a

preference for hawkweed species in the subgenus *Pilosella*. No-choice gall formation tests carried out to explore the physiological host range of *M. pilosellae* demonstrated that this gall midge has not only the potential to attack the major target weed *H. caespitosum*, but also other weedy *Hieracium* spp. in the subgenus *Pilosella*, e.g. *H. floribundum*, *H. glomeratum*, *H. piloselloides*, and *H. pilosella*. However, no-choice gall development tests revealed also that *M. pilosellae* has the potential to attack several native North American *Hieracium* spp. belonging to the subgenera *Hieracium* and *Chionoracium*, e.g. *H. scouleri*, *H. carneum* and *H. gracile*. The physiological host range of *M. pilosellae* is possibly wider than indicated in Table 6.2 since some of the native North American hawkweed species were only tested with a few replicates due to difficulties in cultivating the plants.

Feeding on test plant species in no-choice conditions can be tolerated if additional tests or field observations demonstrate that the ecological host range of the insect is sufficiently narrow. Under these conditions an agent may nonetheless be approved for release, e.g. *Gratiana spadicea* (Klug), a leaf-feeding tortoise beetle for the biological control of *Solanum sisymbriifolium* Lamarck in South Africa (Hill and Hulley, 1995). If, as in our study, indigenous North American *Hieracium* spp. are attacked under choice conditions, including open-field tests (e.g. *H. carneum*, a native occurring in Arizona) it is possible that *M. pilosellae* will utilize these species as host plants, especially when the agent reaches outbreak densities. The exposure to a choice minus control situation (Briese et al., 2002) in field cage and open-field tests mimics the circumstance of a food shortage, e.g. due to outbreak densities of the agent. Although gall midges are active flyers, they have limited mobility compared to other insect species due to their small size and fragileness. Several gall midge species are known to have drifted by wind, e.g. *Dasineura brassicae* (Jacobs and Renner, 1988), and it is therefore imaginable that *M. pilosellae* can also potentially drift onto non-target plants without having actively chosen the plant. We therefore do not recommend field release of this gall midge, even though it shows a strong preference towards *H. caespitosum* and *H. pilosella*. Like the weevil *Rhinocyllus conicus* Fröl., *M. pilosellae* is an example of a biological control agent which is sufficiently host-specific for one area but does not show the required level of specificity for another region due to the absence or presence of congeners. In North America, *R. conicus* threatens native *Cirsium* thistles because its host range is too broad (Arnett and Louda, 2002; Pemberton, 2000), but

in Argentina or New Zealand, where no native *Cirsium* spp. occur, the weevil's host range is suitably narrow, enabling its use without risk to native plants (Aeschlimann, 1999; Pemberton, 2000).

In summary, it can be stated that the potential risk to native North American *Hieracium* spp. is considered too high. *Macrolabis pilosellae* was therefore removed from the list of potential agents for control of alien invasive hawkweeds in North America. However, if *M. pilosellae* proves to be a successful biological control agent of North American target *Hieracium* spp. in New Zealand, a reconsideration of the risk-benefit assessment for this agent might be worthwhile. Apart from chemical control, there are currently no satisfactory means to effectively control hawkweeds in North America. Herbicides give good control, but hawkweeds re-infest meadows from forest margins, roadsides and unmanaged meadows (Wilson and Callihan, 1999). Biological control might offer the possibility to permanently control this weed in these stable habitats.

7 General discussion

In this chapter, the outcomes of the thesis are integrated and discussed in a broader context. Factors facilitating the invasiveness of exotic plants, hawkweeds in particular, are assessed and the suitability and potential effectiveness of the three herbivores evaluated.

7.1 Factors facilitating the invasiveness of non-indigenous plants

When introduced into new habitats by humans, either deliberately or accidentally, plants have to overcome numerous obstacles to become established, comprising abiotic factors such as temperature, light, soil, disturbance (fire, flooding), precipitation, and biotic interactions such as competition, disturbance, trampling, and herbivory (Crawley, 1983; Elton 1958; Tscharntke, 1991). The range of genetic variability in an exotic plant species may be lower in the introduced range than in native communities as a result of the small size of the initial introductions (Crawley, 1989a). However, a small proportion of plant species become much more competitive in the introduced range than in the native one, and some of them cause enormous environmental and economic costs (Pimentel et al., 2000; Williamson and Fitter, 1996; Wittenberg and Cock, 2001). Several hypotheses have been put forward to explain plant invasions, some of which are briefly presented below.

The “enemy-release-hypothesis” posits that natural enemies (e.g. insect herbivores and fungal pathogens) can limit growth or survival of plants in their native area (Keane and Crawley, 2002). The absence of specialized natural enemies in the exotic range leads to a competitive advantage over native, co-occurring plants that are attacked by specialists and generalists, e.g. *Clidemia hirta* (L.) D. Don (Melastomataceae) from Costa Rica, which invaded tropical forests in Hawaii (DeWalt et al., 2004). Classical biological control is based on the assumption that herbivores can have a negative impact on their host plant, which in turn impairs the competitiveness of the invader (DeBach 1964). The “EICA hypothesis” (evolution of increased competitive ability) argues that exotics liberated from their specialist enemies loose costly traits that confer resistance to their native specialist enemies. In the exotic range, selection favors vigorously growing (but relatively poorly defended) individuals, leading to the development of relatively vigorous genotypes which reallocate these resources to traits such as size or fecundity (Blossey and Nötzold, 1995).

There is evidence for the EICA hypothesis (Leger and Rice, 2003; Willis and Blossey, 1999) and against it (Bossdorf et al., 2004; Vilà et al., 2003). The "species richness hypothesis" predicts that more diverse communities might be more resistant to invasion than species-poor communities (Elton, 1958; Hierro et al., 2005 and references therein). Dukes (2001) found that high functional diversity reduced the success of *Centaurea solstitialis* L. (Asteraceae) in North America by reducing resource availability for the invader. According to the "novel weapons hypothesis" some exotic plants may succeed because they bring novel ways of interaction to natural plant communities by exuding allelochemicals that are relatively ineffective against well-adapted neighbours in origin communities, but highly inhibitory to naive plants in the exotic range of the plant, e.g. *Centaurea diffusa* Lam. (Callaway and Ridenour, 2004).

In the case of hawkweeds, the absence of specialized herbivores in New Zealand was documented by Syrett and Smith (1998) based on field surveys. In addition, past and current land management practices such as deforestation, burning, and overgrazing are responsible for favoring the establishment of *Hieracium* in New Zealand (Hunter, 1991). However, field experiments, i.e. insect exclosure experiments, in both the native and the introduced range, are needed to confirm if enemy release is a possible reason for the invasiveness of hawkweeds.

7.2 Benefits and challenges of biological control

7.2.1 Successes in biological control

In a review, Cruttwell McFadyen (2000) lists 44 weed species, which have been successfully controlled by applying classical biological control with insects and pathogens. The savings to agriculture and the environment are enormous. Successful control of skeleton weed, *Chondrilla juncea*, resulted in a cost-benefit ratio of 1:112, 1:15 for tansy ragwort, *Senecio jacobaea* L., and a spectacular 1:1675 for the water weed *Salvinia molesta* Mitchell in Sri Lanka (Cruttwell McFadyen, 2000 and references therein). Yet, success comprises not only economic benefits, but also substantial non-economic benefits, namely the re-establishment of native plants and fauna, sustainability, low risk of non-target effects, and affordability, i.e. factors that are very difficult to quantify in monetary terms. Pimentel et al. (2000) give an overview on environmental and economic costs caused by nonindigenous species in the United States.

7.2.2 Direct and indirect non-target effects of biological control agents

When a biological control agent is introduced into a new environment, it will inevitably encounter novel plant species which do not occur in its native range. Therefore, it is essential that the host range of a candidate is adequately described prior to its introduction so that the risks of non-target effects can be realistically evaluated. The perceived importance of non-target effects of biological control agents has increased in recent years following the documentation of damage to non-target plants (Cruttwell McFadyen, 1998; Pemberton, 2000; Wittenberg and Cock, 2001). However, the risk to native flora can be judged reliably before introduction (Pemberton, 2000). Cruttwell McFadyen (1998) lists only eight weed biological control insect species that fed on non-target hosts when introduced. For five of these the host range was already known at the time of release, but attack on native plants of no economic value was not then seen as a problem. Probably the best-known example is *Rhinocyllus conicus*, a weevil introduced into Canada for the biological control of exotic thistles in 1968 and into the United States in 1969 (Gassmann and Louda, 2001; Julien and Griffiths, 1998). In Canada, the weevil attacks 70% of the *C. nutans* plants over the weed's range and reduces plant density by up to 95% on well-managed pastures (Julien and Griffiths, 1998). However, after field release, *R. conicus* established self-sustaining populations on native North American thistle species in the absence of its host plant (Gassmann and Louda, 2001). A review of the screening program indicates that no native North American *Cirsium* spp. were tested, and that feeding on native thistles was expected; however, feeding on indigenous thistles was not considered a matter for concern at that time (Gassmann and Louda, 2001). Today, *R. conicus* would not have been tested in the same way, and it would not have been approved for release in North America.

Indirect non-target effects are much more challenging to predict. Unintended indirect effects of host-specific biological control agents include: (i) ecological replacement, (ii) compensatory responses, and (iii) food-web interactions (Pearson and Callaway, 2003). Efficient host-specific biological control agents pose a small risk of indirect non-target effects, e.g. food-web interactions, because the density of the target weed will be reduced and the population of the biological control agent will subsequently crash (Pearson and Callaway, 2004). However, it has to be taken into account that biological control agents represent only a small proportion of exotic organisms. For example, in New Zealand, up to 1999, only about 1% of the exotic insect fauna is represented by insects, which have been

intentionally introduced for biological weed control (Emberson, 1999). These insects are well-studied, host-specific insects whereas 99% of the exotic insects have been accidentally or deliberately introduced without any study or assessment of risks.

7.3 Suitability and potential effectiveness of the herbivores investigated

7.3.1 Host-specificity

A prerequisite for any introduction of an exotic natural enemy to control an alien invasive weed is a restricted host range (chapter 1). The two sympatric hoverflies *C. urbana* and *C. psilophthalma* proved to be at least genus-specific, thus sufficiently host-specific for release in New Zealand, where no native *Hieracium* spp. exist (chapter 3). It is likely that both species will attack not only *H. pilosella*, but also other weedy *Hieracium* spp. in the subgenus *Pilosella* in New Zealand, i.e. *H. praealtum* and *H. caespitosum*. Yet the suitability of *C. urbana* and *C. psilophthalma* as biological control agents in North America, where native *Hieracium* spp. exist, has still to be evaluated. First results of no-choice larval transfer tests indicate that *C. urbana* and *C. psilophthalma* can develop on several native North American species in the subgenera *Hieracium* and *Chionoracium* (Grosskopf et al., 2004). Open-field tests are being carried out to explore the ecological host range of both hoverflies.

Some biological control agents are introduced even though it was known that they could develop on non-target plants, even in multiple-choice tests. In these cases, it was assessed whether the risks were acceptable to most people in return for the probable gains, i.e. the prospective benefits of controlling the weed. Releases were either approved because (i) attack of the non-target species was not considered important, (ii) attack of the non-target species was low in host range tests, (iii) because of the urgent need for the control of the target weed, or a combination of the these reasons (Harris and McEvoy, 1995; Hill and Hulley, 1995; Olckers et al., 1995). It has been argued that temporary “spill-over” events, i.e. high population densities of the biological control agent due to outbreaks and shortage of the host plant, short term non-target feeding should be tolerated if the population cannot persist on the non-target plants (Blossey et al., 2001).

Host range investigations with *M. pilosellae* showed that the gall midge is at least genus-specific with a preference for *Hieracium* spp. in the subgenus *Pilosella*. Based on test

results, *H. pilosella*, *H. caespitosum* and *H. praealtum*, all target weeds in New Zealand, are expected to be host plants in the field (chapter 4). However, additional tests revealed the potential use of native North American *Hieracium* spp. in the subgenus *Chionoracium* (chapter 6). The risk for native North American *Hieracium* spp. is considered too high, and therefore release of *M. pilosellae* in North America is not recommended at this stage.

7.3.2 Life cycle and biology

Of the three herbivores studied, the gall midge *M. pilosellae* is the only multivoltine herbivore species with three generations per year at Delémont. A high intrinsic rate of increase that may lead to outbreak densities is considered one of the most important characteristics of a potentially successful biological control agent. This is considered more likely for insects with small body size and high fecundity (Crawley, 1989b; Gassmann, 1996). In this regard, due to their univoltine life cycle, *C. urbana* and *C. psilophthalma* may be less effective than *M. pilosellae*. In contrast, *C. urbana* and *C. psilophthalma* are very active flyers, and thus may have much higher dispersal abilities than *M. pilosellae* adults which are very small and fragile insects. Although *M. pilosellae* adults also fly actively, it is very likely that midges are easily borne by strong winds with the risk to be carried away from hawkweed infestations and to encounter non-target hawkweed species. However, it is also possible that midges are carried onto new infestations of alien invasive *Hieracium* spp. which would be beneficial.

Macrolabis pilosellae is expected to establish well in proximity to other plants where the fragile insects are protected against desiccation whereas *C. urbana* is likely to do well under drier conditions. However, there are numerous field sites where all three insect species co-occur (Grosskopf, personal observation).

Many herbivores are controlled by a complex of parasitoids in their native range, which is seen as a reason for their rarity (Harris, 1991). Released from those, e.g. by introduction into other parts of the world as biological control agents, these insects can reach outbreak densities. Whether *C. urbana*, *C. psilophthalma* and *M. pilosellae* will be attacked by parasitoids in New Zealand has to be evaluated in the course of post-release studies. Immature stages of *C. psilophthalma* and *M. pilosellae* are probably more exposed to predators and parasitoids than *C. urbana* larvae and puparia, which are concealed in the

soil.

7.3.3 Impact

In this study, the gall midge *M. pilosellae* had the highest impact on growth of *H. pilosella* reducing stolon length, number of leaves, and the number of flower heads (chapter 5). In addition, larvae that feed in young flower buds damage the unripe seeds (Grosskopf, personal observation) and rosettes attacked by the gall midge had a higher mortality rate than the control plants. Due to its multivoltine life cycle, *M. pilosellae* has the potential to attack target weeds throughout the growing season until late autumn. Insect feeding is much more likely to increase the death rate of established plants when these plants are growing in dense stands and are subject to intense inter- or intraspecific competition (Crawley, 1989a). The aim of classical biological control is not necessarily to kill the plant but to reduce the competitive edge of the target weed (Harris, 1991). However, the effect of an agent on individual plants needs to be clearly distinguished from the potential it may have on the population level (Crawley, 1989a). *Macrolabis pilosellae* is already established in New Zealand, and landowners are encouraged to redistribute infested rosettes in autumn to accelerate its spread (Hayes, 2005). However, it is too early to evaluate the impact of this agent or the project. It usually takes several years before this can be done for a biological control project because the insects need time to build up sufficient densities to achieve control, and to disperse to their limits. Cruttwell McFadyen (2000) is of the opinion that no program should be classified as a success or a failure until at least 10 years after release of the last agent. Nonetheless, rapid successes have been recorded in some programs, e.g. purple loosestrife in North America (Lindgren et al., 2002).

In comparison with *M. pilosellae*, the two hoverflies had a lower impact on the growth of *H. pilosella*, which may have been due to the absence of indirect effects as there were no competitive plants in the experimental design (chapter 2). However, below-ground herbivory by *C. urbana* reduced above-ground biomass significantly, and feeding by *C. psilophthalma* significantly reduced seed production. Because of the enormous reproductive capacity of hawkweeds, a single insect species will most likely not be able to successfully control the weed. Instead, a complex of agents attacking different plant parts at different times of the year should be released. It could be argued that the gall midge alone can do this, but this species may not establish equally well in all habitats or climatic regions,

and native parasitoids in the area of introduction may compromise its impact over time.

Taking into consideration the possible reasons for the invasiveness of hawkweeds, i.e. the lack of specialized phytophagous insects and unsustainable land management practices, biological control should be combined with other cultural practices, e.g. a sound grazing management and sowing of competitive pasture plants. An advantage of hawkweed infestations on meadows and in conservation areas as a target for biological control is that they represent relatively stable habitats where biological control agents have a greater chance to become established and build up to high population levels that can have a significant impact on the target weeds.

8 Summary

Several hawkweed species, *Hieracium* spp. (Asteraceae), of Eurasian origin have become noxious weeds in New Zealand (e.g. *H. pilosella, H. praealtum, H. cæspitosum, H. lepidulum*) and in North America (e.g. *H. aurantiacum, H. caespitosum*). Traditional management techniques to control these plants proved to be either uneconomic and/or ineffective. Furthermore, field surveys carried out in New Zealand demonstrated the absence of specialized herbivores and pathogens associated with hawkweeds, indicating the potential benefits of a biological control program which was intiated in 1993. In this thesis, investigations on three species of Diptera studied for their potential as biological control agents of hawkweeds in New Zealand and North America are presented: *Cheilosia urbana* (Syrphidae), *Cheilosia psilophthalma*, and *Macrolabis pilosellae* (Cecidomyiidae). The research was carried out at the CABI Bioscience Switzerland Centre at Delémont, and at field sites in Switzerland and the Black Forest, Germany. A few test plant species which could not be grown at Delémont were tested by project partners in quarantine facilities in New Zealand.

Cheilosia urbana and *C. psilophthalma* are sympatric, univoltine hoverfly species. Both species co-occur in large areas in Europe. Females of both species lay their eggs into the leaf axils of *Hieracium* rosettes during April and May. Freshly hatched *C. urbana* larvae move into the soil and feed externally on the roots, whereas *C. psilophthalma* larvae stay on the above-ground plant parts and bore into leaf axils, the rosette centre, and stolon tips, and they can move from one rosette to another. Mating in captivity could not be obtained for *C. urbana* and *C. psilophthalma*. Therefore, fecundity was estimated by keeping gravid, field-collected females in small vials provided with food. *Cheilosia urbana* females laid on average 74 eggs with a maximum of 184 eggs per female, *C. psilophthalma* females laid 54.3 eggs with a maximum of 158 eggs. Kept at 20 °C, *C. urbana* eggs need five days until the larvae hatch and *C. psilophthalma* eggs need four days. Both species have three larval instars and pupate in autumn. *Cheilosia urbana* puparia overwinter in the soil close to the surface and *C. psilophthalma* pupates on the soil surface. Adults emerge between April and early May in the following year with a protandric emergence pattern. Two *Phygadeuon* spp. (Hymenoptera, Ichneumonidae) were reared from puparia of *C. urbana* and *C.*

psilophthalma, and a braconid from mummified *C. psilophthalma* larvae. In an impact experiment carried out with potted *H. pilosella* plants, above-ground herbivory by *C. psilophthalma* significantly reduced the number of seeds, and below-ground herbivory by *C. urbana* reduced above-ground biomass.

Apart from a literature record of a *C. urbana* female ovipositing onto mouse-ear hawkweed, *H. pilosella*, neither the feeding niches of *C. urbana* and *C. psilophthalma* were known, nor their host plants. Host range investigations demonstrated that both species are at least genus-specific, thus safe for release in New Zealand, where no native hawkweed species exist. Based on results obtained in host range investigations, it is expected that *C. urbana* and *C. psilophthalma* will not only attack *H. pilosella*, but also other weedy *Hieracium* spp. in the subgenus *Pilosella*. However, due to the existence of native North American *Hieracium* spp. in the subgenera *Hieracium* and *Chionoracium*, both flies have to undergo additional host range investigations to evaluate if they are sufficiently host-specific for release in North America.

Macrolabis pilosellae is a multivoltine gall midge, which has three generations at Delémont. Oocytes of *M. pilosellae* are mature at emergence and there is no further oogenesis. The abdomen of freshly emerged females contained an average of 67.7 eggs. Females lay their eggs near meristematic tissue in rosette centres, leaf axils and stolon tips. Larval feeding leads to coalescence of young leaves in rosette centres and stolon tips. The enrolled, furled leaves enclose the larvae that feed in the cavities among the leaves. Field-collected galls contained on average 4.7 immature stages with a maximum number of 24. Mature larvae move into the soil and pupate in a cocoon. Occasionally, cocoons can also be found in the galls, but this was an exception. Overwintering occurs in the larval stage in the soil. *Aprostocetus* sp. (Hymenoptera, Eulophidae), probably a not yet described larval endoparasitoid, was reared from mummified *M. pilosellae* larvae that remained within the galls, whereas unparasitized *M. pilosellae* larvae moved into the soil. Of the three herbivores studied, *M. pilosellae* has the most significant impact on the growth of *H. pilosella*. Galled plants had significantly shorter stolons, reduced above-ground biomass, and a reduced number of leaves and flower heads. Plants attacked by the gall midge also had a higher mortality rate. Host range studies showed that most weedy hawkweed species in New Zealand, i.e. *H. pilosella, H. praealtum* and *H. caespitosum*, are likely to be

attacked in the field, whereas *Hieracium* spp. in the subgenus *Hieracium* are less suitable host plants, and plants outside the genus *Hieracium* remained unattacked. *Macrolabis pilosellae* is thus sufficiently host-specific for New Zealand. In contrast, host-specificity investigations for North America revealed that development to adulthood is possible on several native North American *Hieracium* spp., some of which were also attacked in multiple-choice field cage and open-field situations. Its use as a biological control agent in North America is therefore not recommended at this stage. However, if *M. pilosellae* proves to be a successful biological control agent of North American target *Hieracium* spp. in New Zealand, a reconsideration of the risk-benefit assessment for this agent might be worthwhile.

A petition for approval of release of *C. urbana*, *C. psilophthalma* and *M. pilosellae* in New Zealand was granted by ERMA (Environmental Risk Management Authority) in June 2001.

9 Zusammenfassung

Mehrere Habichtskräuter, *Hieracium* spp. (Asteraceae), eurasischer Herkunft sind nach Neuseeland (z.B. *H. pilosella, H. praealtum, H. caespitosum*, *H. lepidulum*) und Nordamerika (z.B. *H. aurantiacum*, *H. caespitosum*) eingeschleppt worden, wo sie sich stark ausbreiten konnten und heutzutage als Unkräuter einstuft werden. Da konventionelle Kontrollmethoden sich entweder als unwirtschaftlich oder als ineffizient erwiesen, und zudem Feldstudien in Neuseeland gezeigt haben, dass dort keine spezialisierten phytophagen Insekten mit Habichtskräutern assoziiert sind, wurde 1993 ein Projekt zur klassischen biologischen Kontrolle von Habichtskräutern ins Leben gerufen. Im Rahmen dieser Arbeit werden Untersuchungen an drei Dipteren-Arten vorgestellt, welche als potenzielle natürliche Gegenspieler für die Kontrolle von invasiven *Hieracium*-Arten ausgewählt wurden: *Cheilosia urbana* (Syrphidae), *Cheilosia psilophthalma* sowie *Macrolabis pilosellae* (Cecidomyiidae). Sämtliche Untersuchungen wurden am CABI Bioscience Switzerland Centre in Delémont, Schweiz, sowie an verschiedenen Standorten im Schwarzwald (Südwestdeutschland) und in der Schweiz durchgeführt. Einige Wirtsspezifitätsuntersuchungen mit Pflanzen, die in der Schweiz nicht keimten, wurden von Projektpartnern in Neuseeland durchgeführt.

Cheilosia urbana und *C. psilophthalma* sind sympatrische, univoltine Schwebfliegenarten, deren Verbreitung sich in weiten Teilen Europas überschneidet. Die Weibchen beider Arten legen ihre Eier im April und Mai in den Blattachseln der Rosettenblätter verschiedener *Hieracium*-Arten ab. Frischgeschlüpfte *C. urbana*-Larven wandern in den Boden und fressen äusserlich an den Wurzeln ihrer Wirtspflanze, während *C. psilophthalma*-Larven sich in die Rosettenmitte, Stolonenspitze sowie Blattachseln bohren und bei Bedarf von einer Rosette zur nächsten wechseln können. Da die Fliegen während dieser Untersuchungen in Gefangenschaft nicht kopulierten, wurde die Fertilität begatteter, feldgesammelter Weibchen ermittelt. *Cheilosia urbana* Weibchen legten im Durchschnitt 74 Eier, mit einer maximalen Anzahl von 184, *C. psilophthalma*-Weibchen legten duchschnittlich 54,3 Eier (Maximum 158). Bei 20 °C dauert es fünf Tage, bis aus frischgelegten *C. urbana*-Eiern Junglarven schlüpfen, bei *C. psilophthalma* lediglich vier Tage. Beide Arten durchlaufen drei Larvenstadien, und die Verpuppung erfolgt im Herbst.

Puparien von *C. urbana* überwintern im Boden nahe der Oberfläche, während Puparien von *C. psilophthalma* auf der Erdoberfläche überwintern. Die Imagines schlüpfen zwischen April und Anfang Mai des darauffolgenden Jahres, wobei ein protandrischer Schlupfverlauf beobachtet wurde. Zwei *Phygadeuon*-Arten (Hym., Ichneumonidae) wurden aus Puparien von *C. urbana* und *C. psilophthalma* gezogen. Eine weitere Parasitoidenart aus der Familie der Brackwespen (Braconidae) schlüpfte aus mumifizierten *C. psilophthalma*-Larven. In einem kontrollierten Versuch mit getopften *H. pilosella* Pflanzen wurde der Einfluss der Herbivorie von *C. urbana* und *C. psilophthalma* auf das Pflanzenwachstum untersucht. Befall mit *C. psilophthalma* hat die Anzahl der Samen signifikant reduziert, während unterirdische Herbivorie duch *C. urbana* eine signifikante Abnahme der oberirdischen Biomasse zur Folge hatte.

Mit Ausnahme eines Literaturzitats, in dem von einer Eibablage eines *C. urbana*-Weibchens an *H. pilosella* die Rede ist, waren bis zu dieser Studie weder die Frassnischen der beiden Schwebfliegen-Arten noch deren Wirtspflanzen bekannt. Untersuchungen zur Wirtsspezifität beider Fliegen für ihren potentiellen Einsatz in Neuseeland zeigten, dass nicht nur *H. pilosella,* sondern mehrere *Hieracium*-Arten aus der Untergattung *Pilosella* ihrem Wirtskreis zuzuordnen sind, während Pflanzen aus anderen Pflanzengattungen unbefallen blieben. Da es in Neuseeland keine einheimischen Habichtskräuter gibt, können *C. urbana* und *C. psilophthalma* als natürliche Gegenspieler eingesetzt werden, ohne dass ein Befall von Nicht-Zielpflanzen zu erwarten ist. Anders sieht die Situation in Nordamerika aus, wo es einheimische *Hieracium*-Arten in den Untergattungen *Hieracium* und *Chionoracium* gibt. Beide *Cheilosia*-Arten müssten demnach an einheimischen, nordamerikanischen Habichtskräutern getestet werden.

Die dritte Diptere, die Gallmücke *M. pilosellae,* hat drei Generationen in Delémont. Nach dem Schlupf der Imago sind sämtliche Eier ausgereift. Das Abdomen frisch geschlüpfter Weibchen enthielt durchschnittlich 67,7 Eier. Weibchen legen ihre Eier an meristematisches Gewebe in der Rosettenmitte, Blattachseln sowie Stolonenspitzen. Herbivorie durch *M. pilosellae*-Larven führt zum blasigen Auftreiben der jungen Blätter, welche sich nicht entfalten. Die Larven leben in den so entstehenden Hohlräumen zwischen den befallenen Blättern. Im Freiland gesammelte Gallen enthielten durchschnittlich 4,7 Individuen der Gallmücke, aber es konnten bis zu 24 Larven in einer Galle beobachtet werden.

Verpuppungsreife Larven verlassen die Galle und wandern in den Boden ab, wo sie sich in einem Kokon verpuppen. Vereinzelt konnten auch Kokons in den Gallen beobachtet werden, deren Zahl jedoch vernachlässigbar klein war. Die Überwinterung erfolgt als Altlarve in einem Kokon in der Erde. *Aprostocetus* sp., eine vermutlich noch unbeschriebene, parasitische Erzwespen-Art (Eulophidae), wurde aus mumifizierten *M. pilosellae*-Larven gezogen. Während unparasitierte Gallmückenlarven in der Regel in den Boden abwandern, verweilen parasitierte *M. pilosellae* Larven in den Gallen.

Von den drei untersuchten Dipteren-Arten hat *M. pilosellae* den bedeutendsten Einfluss auf das Wachstum von *H. pilosella*. Von *M. pilosellae* befallene Pflanzen hatten signifikant kürzere Stolone, eine geringere oberirdische Biomasse sowie eine reduzierte Anzahl von Blättern und Blütenköpfen. Zudem führte *M. pilosellae* zu einer höheren Sterblichkeitsrate bei *H. pilosella*-Pflanzen. Wirtsspezifitätsuntersuchungen haben gezeigt, dass die Mehrzahl der in Neuseeland problematischen Habichtskräuter dem ökologischen Wirtskreis von *M. pilosellae* zugerechnet werden können (*H. pilosella*, *H. praealtum* sowie *H. caespitosum*), während Habichtskräuter der Untergattung *Hieracium* weniger geeignete Wirtspflanzen darstellen. In zusätzlichen Untersuchungen für einen eventuellen Einsatz der Gallmücke in Nordamerika wurden nicht nur die Unkräuter aus der Untergattung *Pilosella* befallen, sondern auch einheimische, nordamerikanische Arten der Untergattungen *Hieracium* und *Chionoracium*. Einige nordamerikanische Arten wurden auch in Feldkäfigen sowie Freilandversuchen befallen. Aus diesem Grund wird zum jetzigen Zeitpunkt von einem Einsatz der Gallmücke als natürlicher Gegenspieler in Nordamerika abgeraten. Falls sich *M. pilosellae* jedoch in Neuseeland als erfolgreicher Kontrollorganismus von invasiven *Hieracium*-Arten herausstellen sollte, könnte eine Risiken-Nutzen-Analyse eine weitere Entscheidungshilfe darstellen.

Einem Freilassungsgesuch für *C. urbana, C. psilophthalma* und *M. pilosellae* in Neuseeland wurde von ERMA (Environmental Risk Management Authority) im Juni 2001 zugestimmt.

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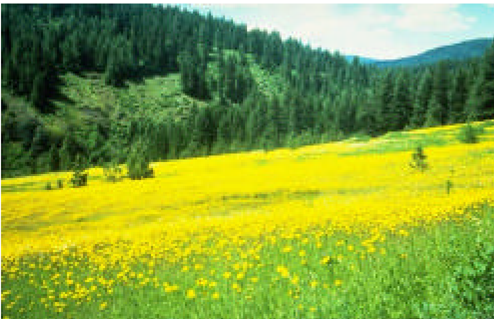
Annex

H. aurantiacum infestation in Washington State, USA (Photo: G. Großkopf)

Larva and feeding marks of *C. psilophthalma* (Photo: G. Großkopf)

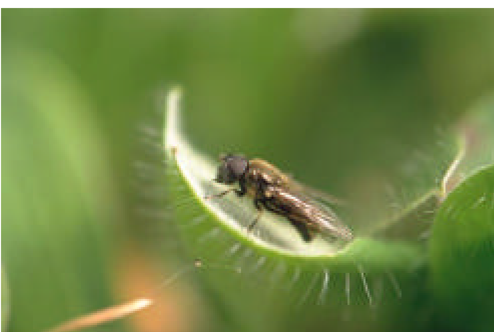


H. caespitosum infestation in Northern Idaho, USA (Photo: L. Wilson)

Larva and feeding marks of *C. urbana* (Photo: G. Großkopf)

H. pilosella patch on a meadow in New Zealand (Photo: H. Harman)

Ovipositing *M. pilosellae* female (Photo: G. Großkopf)



C. psilophthalma resting on *H. pilosella* (Photo: G. Großkopf)

H. pilosella plant attacked by *M. pilosellae* (left) and plant without galls (right) (photo: G. Großkopf)

Curriculum vitae

Personal data

Name:	Gitta Großkopf
Born:	31 March 1970 in Eckernförde, Germany
Nationality:	German
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Education and professional experience

1976 - 1980	Primary school in Osdorf
1980 - 1986	Secondary school in Eckernförde, qualification: Mittlere Reife
1986 - 1989	Secondary school in Eckernförde, qualification: Allgemeine Hochschulreife (Abitur)
1989-1996	Studies in Biology at the Christian-Albrechts-University at Kiel
April – Sept. 1992 Aug. – Sept. 1993	Technical assistant at the International Institute of Biological Control in Delémont, Switzerland
1994 - 1995	Diploma student at IIBC Delémont. Title of the thesis: "Investigations on selected herbivores associated with mouse-ear hawkweed, *Hieracium pilosella*"
1996 – present	PhD student at the Christian-Albrechts-University at Kiel
1996 - 1999	PhD training at the CABI Bioscience Switzerland Centre (former IIBC), Delémont. Responsible for the biological control project against *Hieracium* spp.
2000 - present	Weed scientist at the CABI Bioscience Switzerland Centre.

Erklärung

Hiermit versichere ich, dass diese Abhandlung – abgesehen von der Beratung durch meine akademischen Lehrer – nach Inhalt und Form meine eigene Arbeit ist, und dass ich keine anderen als die angegebenen Hilfsmittel und Quellen verwendet habe. Die Arbeit hat bisher weder ganz noch zum Teil an anderer Stelle im Rahmen eines Prüfungsverfahrens vorgelegen.

Teile dieser Arbeit wurden als Manuskripte bei Zeitschriften bzw. als Beitrag eines Kompendiums veröffentlicht: Auszüge aus Kapitel 1 wurden im Crop Protection Compendium integriert, Kapitel 2 wurde in Biological Control veröffentlicht, ebenso Kapitel 3 mit Lindsay Smith und Pauline Syrett als Koautoren. Kapitel 4 befindet sich in Vorbereitung für eine Publikation mit Lindsay Smith und Pauline Syrett als Koautoren, Kapitel 6 mit Linda Wilson als Koautorin.

Delémont, den 14.3.2006