

United States Department of Agriculture

Marketing and Regulatory Programs Field release of the hoverfly *Cheilosia urbana* (Diptera: Syrphidae) for biological control of invasive *Pilosella* species hawkweeds (Asteraceae) in the contiguous United States.

Environmental Assessment, July 2019 Field release of the hoverfly *Cheilosia urbana* (Diptera: Syrphidae) for biological control of invasive *Pilosella* species hawkweeds (Asteraceae) in the contiguous United States.

# Environmental Assessment, July 2019

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# I. Purpose and Need for the Proposed Action

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ), Pests, Pathogens, and Biocontrol Permits (PPBP) is proposing to issue permits for environmental release of the hoverfly *Cheilosia urbana* (Diptera: Syrphidae). The agent would be used by the applicant for the classical biological control invasive hawkweeds, *Pilosella* species (Asteraceae), in the contiguous United States.

Classical biological control of weeds is a weed control method where natural enemies from a foreign country are used to reduce exotic weed infestations that have become established in the United States. Several different kinds of organisms have been used as biological control agents of weeds: insects, mites, nematodes, and plant pathogens. Efforts to develop a weed biological control agent consist of the following steps (TAG, 2016):

- 1. Foreign exploration in the weed's area of origin.
- 2. Host specificity studies.
- 3. Approval of the exotic agent by PPBP.
- 4. Release and establishment in areas of the United States invaded by the target weed.
- 5. Post-release monitoring.

This environmental assessment<sup>1</sup> (EA) has been prepared, consistent with USDA, APHIS' National Environmental Policy Act of 1969 (NEPA) implementing procedures (Title 7 of the Code of Federal Regulations (CFR), part 372). It examines the potential effects on the quality of the human environment that may be associated with the release of *C. urbana* to control infestations of invasive hawkweeds within the contiguous United States. This EA considers the potential effects of the proposed action and its alternatives, including no action. Notice of this EA was made available in the Federal Register on May 28, 2019 for a 30-day public comment period. APHIS received 8 comments on the EA by the close of the comment period. Six comments were in favor of the release of the biological control agents. One comment was a general comment against APHIS but raised no substantive issues. One comment raised a question regarding impacts on native hawkweeds. This comment is addressed in appendix 6 of this document.

<sup>&</sup>lt;sup>1</sup> Regulations implementing the National Environmental Policy Act of 1969 (42 United States Code 4321 et seq.) provide that an environmental assessment "shall include brief discussions of the need for the proposal, of alternatives as required by section 102(2)(E), of the environmental impacts of the proposed action and alternatives, and a listing of agencies and persons consulted." 40 CFR § 1508.9.

APHIS has the authority to regulate biological control organisms under the Plant Protection Act of 2000 (Title IV of Pub. L. 106–224). Applicants who wish to study and release biological control organisms into the United States must receive PPQ Form 526 permits for such activities. The PPBP received a permit application requesting environmental release of the hoverfly, *Cheilosia urbana,* from Europe, and PPBP is proposing to issue permits for this action. Before permits are issued, PPBP must analyze the potential impacts of the release of this agent into the contigous United States.

The applicant's purpose for releasing C. urbana is to reduce the severity of infestations of invasive hawkweeds in the contiguous United States. The following hawkweeds are considered noxious in many western states and are currently targets for biological control: *Pilosella aurantiaca* (L.) F.W. Schultz & Sch. Bip. (orange hawkweed), Pilosella caespitosa (Dumort.) P.D. Sell & C. West (syn. *Hieracium pratense* Tausch) (meadow hawkweed), Pilosella flagellaris (Willd.) P.D. Sell. & C. West (whiplash hawkweed), *Pilosella floribunda* Wimm. & Grab. (= *P. prealta*) (king devil hawkweed), P. glomerata (Froel.) Fr. (queen- or yellowdevil hawkweed), Pilosella officinarum Vaill. (syn. Hieracium pilosella L.) (mouseear hawkweed), and Pilosella piloselloides Vill. (tall hawkweed). In North America, invasive hawkweeds are primarily weeds of moist pastures and forest meadows, and mesic (containing a moderate amount of moisture), montane rangelands. Habitats most vulnerable to initial invasion include human-disturbed sites, such as roadsides, permanent pastures, hayfields, cleared timber units, and abandoned farmland where the soil is well drained, coarse textured, and moderately low in organic matter. However, invasive hawkweeds are highly competitive with native vegetation (Makepeace, 1985) and can quickly invade and achieve dominance within intact, natural sites (Reader, 1978). Consequently, wildlife habitat, recreation areas, and pristine mountain meadows in areas that have a climate similar to that in their native range are particularly susceptible, making invasive hawkweeds a major threat to native biodiversity, vegetation integrity, and associated ecosystem services within sensitive, upland habitats.

Existing management options for management of hawkweeds are expensive, temporary, ineffective, and can have nontarget impacts. For these reasons, the applicant has a need to release *C. urbana*, a host-specific, biological control organism for the control of invasive hawkweeds, into the environment.

# II. Alternatives

This section will explain the two alternatives available to PPBP-no

action and issuance of permits for environmental release of *C. urbana*. Although PPBP's alternatives are limited to a decision on whether to issue permits for release of *C. urbana*, other methods available for control of invasive hawkweeds are also described. These control methods are not decisions to be made by PPBP, and their use is likely to continue whether or not permits are issued for environmental release of *C. urbana*, depending on the efficacy of *C. urbana* to control invasive hawkweeds. These are methods presently being used to control invasive hawkweeds by public and private concerns.

A third alternative was considered, but will not be analyzed further. Under this third alternative, PPBP would have issued permits for the field release of *C. urbana*; however, the permits would contain special provisions or requirements concerning release procedures or mitigating measures. No issues have been raised that would indicate special provisions or requirements are necessary.

## A. No Action

Under the no action alternative, PPBP would not issue permits for the field release of *C. urbana* for the control of invasive hawkweeds. The release of this biological control agent would not take place. The following methods are presently being used to control invasive hawkweeds; these methods will continue under the "No Action" alternative and will likely continue even if permits are issued for release of *C. urbana*, depending on the efficacy of the organism to control invasive hawkweeds.

1. Chemical Control Herbicides such as 2,4-D, clopyralid, and picloram have resulted in the greatest degree of control when applied at the normally recommended rate for perennial pasture weeds (Noel et al., 1979; Lass and Callihan, 1992a). Studies conducted at the University of Idaho showed that over 50 percent control was achieved for 6 years following treatment with a 0.47 liter rate of clopyralid (Lass and Callihan, 1992a). Similar results were achieved using picloram at the rate of 0.42–0.56 kilograms of active ingredient per hectare.

# 2. Mechanical Mechanical control of invasive hawkweeds includes digging the plants, but all parts of the plant must be removed. Mowing of plants prevents seed production by removing flowering stems.

**3. Cultural Control** When perennial grasses, legumes, and other beneficial forbs are present in the plant community, fertilizers can help control hawkweeds by increasing the competitive ability of the more desirable species. Marked reductions of hawkweed density and vigor have been obtained by fertilizer treatments in the United States (Reader and Watt, 1981), Canada (Hay and Ouellette, 1959), and New Zealand (Scott et al., 1990). Depending on soil productivity and grass condition, a single nitrogen application may be sufficient for grasses to competitively suppress hawkweed growth for 3–5 years (Reader, 1990). Good grazing management can extend this period.

# 4. Biological<br/>ControlFive species of hawkweed-specific insects were tested and released for<br/>the biological control of hawkweeds in New Zealand. They are:

1. *Aulacidea subterminalis* Niblett (Hymenoptera: Cynipidae) – hawkweed gall wasp

2. *Cheilosia psilophthalma* (Becker) (Diptera: Syrphidae) – hawkweed crown-feeding hoverfly

3. *Cheilosia urbana* (Meigen) (Diptera: Syrphidae) – hawkweed root-feeding hoverfly

4. *Macrolabis pilosellae* Binnie (Diptera: Cecidomyiidae) – hawkweed stem gall fly

5. *Oxyptilus pilosellae* Zeller (Lepidoptera: Pterophoridae) – hawkweed plume moth

These insect species were selected for testing in North America because preliminary screening tests conducted between 1994 and 1999 revealed that the weedy hawkweeds in North America were suitable hosts. Additional screening of these insect species against indigenous North American hawkweeds and their close relatives was proposed before surveying for additional candidate biocontrol species. *Oxyptilus pilosellae* was dropped from consideration due to rearing problems and its utilization of several native hawkweed species. *Macrolabis pilosellae* is not currently under consideration for release in North America because it will also infest two non-target species in open-field tests.

Aulacidea subterminalis, a stolon-attacking gall wasp which has one generation per year, was approved for field release in North America in 2011. Aulacidea subterminalis mainly develops on Pilosella officinarum but P. aurantiaca, P. floribunda, and P. flagellaris are considered alternative target species based on pre-release host specificity test results. First field-releases of this wasp were conducted in summer 2011 with subsequent recoveries being made in British Columbia from the targeted hosts P. flagellaris and P. aurantiaca, although from the latter to a limited extent.

Two additional *Aulacidea* species wasps, *A. pilosellae* (Kieffer) and *A. hieracii* (Bouché) have been considered as biological control candidates. *Aulacidea pilosellae* forms galls on stems, leaves, and stolons. Two populations exist: *A. pilosellae* collected in eastern Germany, Poland, and the Czech Republic is univoltine (single generation) and can be found on *P. caespitosa*, *P. glomerata*, and *P. piloselloides*, while the population from southern Germany and Switzerland is bivoltine (two generations) and shows a preference for *P. officinarum*. In host-range tests conducted

so far, only a few native North American hawkweed species were attacked under no-choice conditions, and only one in multiple-choice cage tests. Impact of *A. pilosellae* on invasive hawkweeds still needs to be demonstrated.

Aulacidea hieracii induces multi-chambered stem galls on flowering stems. Galls were collected from *Hieracium robustum* Fr. and *Pilosella procera* (Fr.) F. W. Schultz & Sch. Bip. These are probably two different species because females reared from *P. procera* galls did not induce galls on *H. robustum* and females reared from *H. robustum* did not induce galls on *P. procera*. Wasps reared from *P. procera* attacked *P. caespitosa* plants in no-choice tests conducted in 2008, and oviposition (egg laying) behavior was also observed on *P. caespitosa* in multiple-choice tests. However, during additional host range tests with *A. hieracii* in 2009 and 2010, gall development on *P. caespitosa* was a rare event and work on this species was therefore discontinued. An adventive population of the gall wasp species also was found in Ontario, Canada galling the native hawkweed species, *Hieracium umbellatum* L. (Sliva and Shorthouse 2006).

Compared to *C. urbana*, the testing of a second *Cheilosia* species, *C. psilophthalma* is less advanced against North American plant species. Larvae of *C. psilophthalma* survived on three native North American hawkweed species in no-choice larval transfer tests so far. Because *C. psilophthalma* is less common in the field, it is difficult to obtain valid results of open-field tests, which would be necessary to describe its ecological host range. Work with *C. psilophthalma* has therefore been postponed.

Because there are currently so few prospective European insect candidates for invasive *Pilosella* species in North America, early investigations into the potential use of fungal pathogens began in 2006. One rust, *Puccinia hieracii* (Röhl) H. Mart occurs on *Pilosella* species and other related Asteraceae genera in Europe (Farr and Rossman, 2014), and the rust (*P. hieracii* var. *piloselloidarum* (Probst) Jorst.) was introduced to New Zealand in the 1990's to control mouseear hawkweed, *P. officinarum* (Jenkins, 1995; Barton, 2012).

# B. Issue Permits for Environmental Release of *Cheilosia urbana* (Preferred Alternative)

Under this alternative, PPBP would issue permits for the field release of the hoverfly, *C. urbana*, for the control of invasive hawkweeds. These permits would contain no special provisions or requirements concerning release procedures or mitigating measures.

#### **Biological Control Agent Information**

1. Taxonomy	Phylum	Arthropoda
	Class	Insecta
	Order	Diptera
	Family	Syrphidae
	Genus	Cheilosia
	Species	C. urbana (Meigen, 1822)
		synonym: Cheilosia praecox (Zetterstedt, 1843)

Other synonyms: *Cheilosia fulvipes* (Wiedemann in Meigen, 1822); *Cheilosia globulipes* Becker, 1894; *Cheilosia nevadensis* Strobl, 1909 (as variation of *praecox*); *Cheilosia punctigenis* Hellén, 1914.

Common name(s): Hawkweed root-feeding hoverfly

2. Description of *C. urbana* Like all *Cheilosia* species, *C. urbana* is a dark hoverfly species. Adults of *C. urbana* were distinguished from co-occurring *C. psilophthalma* adults using the key from Claussen and Kassebeer (1993).

### 3. Geographical a. Native Range

# Range of C. urbana

*Cheilosia urbana* is a common hoverfly in Europe. It has a wide distribution which reflects the occurrence of *Pilosella* species in Europe. The hoverfly occurs throughout Europe from Scandinavia to Spain and Italy (Peck, 1988) and Northern Asia (Lundbeck, 1916) including alpine regions (Claussen and Kassebeer, 1993). *Cheilosia urbana* can be found in the north towards southern Sweden and in Finland.

### b. Other Areas of Introduction

*Cheilosia urbana* is one of a suite of five insect biological control agents introduced to New Zealand for suppression of *P. officinarum* (Grosskopf, 2006). Between five and 78 adults of *C. urbana* have been released at 13 sites in New Zealand since February 2002 but the hoverfly has not been recovered thus far (Littlefield et al., 2014).

### c. Expected Attainable Range of C. urbana in North America

*Cheilosia urbana* is very common and widespread on *Pilosella* species in Europe, including Northern Europe. The fly's potential range in North America is expected to match much of the distributions of the targeted *Pilosella* species that occur in the northwestern United States, southwestern Canada, and in the northeastern United States and southeastern Canada. None of the weedy hawkweed species occur in much of the Great Plains, the southwest, or the southeastern United States.

# 3. Life History of a. Adult Emergence and Longevity

### C. urbana

*Cheilosia urbana* has one generation per year. Kept under semi-natural conditions, adults emerge in April and males emerge before females. At 20°C, *C. urbana* males live  $9.5 \pm 0.47$  days (mean  $\pm$  SE, n = 43) and females live  $13.0 \pm 0.94$  days when kept in small vials and provided with food (Grosskopf, 2006). Marked *C. urbana* females that were released into the field after emergence, caught on *Pilosella* patches during oviposition and kept in small vials under semi-natural temperature conditions until they died, lived on average  $27.0 \pm 1.14$  days (SE, n = 62).

# b. Mating, Oviposition Period, and Fecundity

Freshly emerged *C. urbana* females contain no fully developed eggs. All eggs found in the abdomen were very small and transparent. In contrast, provided with food, 5-day-old females contained fully developed eggs (Grosskopf, 2006). Eggs are laid at the base of the rosette leaves of the plant. Females carrying eggs (gravid) land on the rosette and walk on the rosette with an extended ovipositor (egg laying organ). Once a suitable place is found, they turn around and walk backwards to lay an egg close to the stem of the plant.

After being transferred into plastic vials, most *C. urbana* females caught on hawkweed plants started laying eggs immediately. The oviposition period of *C. urbana* started with capture of the first gravid female in April and lasted until mid- to the end of May. Between 96.8 percent and 100 percent of the *C. urbana* females laid eggs in tests conducted in 1997– 1999. They laid on average 74.0  $\pm$  2.68 SE eggs (n = 161 females, range: 0–184) (Grosskopf, 2005). The maximum number of eggs laid by a *C. urbana* female was 184.

# c. Egg, Larval Development and Pupation

Kept at 20°C, *C. urbana* eggs need 5 days to eclose (hatch). The larvae move immediately into the soil to feed externally on the roots. *Cheilosia urbana* has three larval instars (stages of immature development). Mature larvae pupate in the soil very close to the surface from late September onwards.

# **III. Affected Environment**

# A. Taxonomy of Hawkweeds

Phyllum: Magnoliophyta Class: Magnoliopsida Order: Asterales
Family: Asteraceae
Subfamily: Cichorioideae
Tribe: Lactuceae
Subtribe: Hieraciinae Dumort.
Genus: *Pilosella*Species: *P. aurantiaca* (L.) F.W. Schultz & Schultz Bip.
Species: *P. caespitosa* (Dumort.) P. D. Sell & C. West
Species: *P. flagellaris* (Willd.) P.D. Sell. & C. West
Species: *P. floribunda* (Wimm. & Grab.)
Species: *P. officinarum* Vaill.
Species: *P. piloselloides* (Vill.) Soják

The hawkweeds (*Pilosella* and *Hieracium* species) are members of the tribe Lactuceae (= Cichorieae) in the family Asteraceae. The name *Hieracium* is derived from the Greek 'hierax', meaning hawk; allegedly keen-sighted hawks of yore ate the sap of the brightly colored plants to sharpen their eyesight (Fernald, 1950). Hawkweeds are an extremely diverse group morphologically, and include a large number of taxa in which apomixes (asexual seed production), hybridization, and polyploidy (more than two paired sets of chromosomes) are all common (Stebbins, 1950). Species of European origin reproduce asexually by apomixis and vegetatively. Apomixis enables highly adapted and competitive genotypes to persist in a population without change, allowing them to spread quickly throughout an available area. Moreover, these reproductive strategies generate enormous variation within and among species, making *Hieracium* and *Pilosella* difficult plant genera taxonomically.

Problems with *Pilosella* taxonomy are worsened by the lack of clearly defined, stable characters that are important in classifying species within the genus. For example, species are broadly distinguished by the character and amount of hairiness, stem height, presence/absence of stolons, stolon structure, and flower structure (Bailey and Bailey, 1976; Voss and Böhlke, 1978). The result has been the description of a huge number of species, subspecies, varieties, and forms that differ slightly (Sell, 1974). Zahn (1922) conducted the most comprehensive treatment of the genus in which he described 756 species worldwide. It is estimated that while over 10,000 Hieracium and Pilosella species, subspecies, varieties, and forms have been described worldwide, only about 700 species are valid, with the remainder being genetic forms of a "related" species (Hitchcock et al., 1955). The difficulty of differentiating invasive *Pilosella* species due to morphological similarities was recently highlighted in a study in British Columbia, where it was determined that approximately 40 percent of the yellow-flowered species recorded for monitoring and management purposes had been mis-identified by trained weed surveyors (Ensing et al.,

2013). Such taxonomic uncertainty was then found to have a significant effect on the reliability of results from an ecological niche model being developed using these data for management purposes to predict the potential limits of spread of different invasive *Pilosella* species (Ensing et al., 2013).

Recently, phylogenetic analysis of introduced and native hawkweeds of North America was conducted by Gaskin and Wilson (2007) to test morphologically-based infrageneric classification and to determine the phylogenetic relationships of native hawkweeds with exotic species. From this analysis, the current morphologically-based infrageneric classification of *Hieracium* is supported, with the exception of *H. canadense* being placed within the subgenus *Chionoracium*. The earlier morphologicallybased conclusion that the species targeted for biological control are in a separate lineage from native North American species is also supported.

#### B. Areas Affected by Hawkweeds

### 1. Native and Introduced Range of Invasive Hawkweeds

There are many species of non-native, invasive hawkweeds in North America (see Table 1). These species originated from Europe, which is the native range of a complex of species, subspecies, and varieties of hawkweeds in the genera *Pilosella* and *Hieracium*. The majority currently problematic in North America belong to the genus, Pilosella. Invasive hawkweeds were probably introduced into the eastern United States and Canada during the 19<sup>th</sup> century. Species in both genera vary widely, both in their appearance and in their invasion history and distribution. The following hawkweeds are considered noxious in many western states and are currently targets for biological control: Pilosella aurantiaca (orange hawkweed), Pilosella caespitosa (synonym Hieracium pratense Tausch) (meadow hawkweed), Pilosella flagellaris (whiplash hawkweed), Pilosella floribunda (= P. prealta) (king devil hawkweed), P. glomerata (queen- or yellowdevil hawkweed), Pilosella officinarum (synonym Hieracium pilosella) (mouseear hawkweed), and Pilosella piloselloides (tall hawkweed).

The current main distribution of these species includes the northeastern and northwestern United States. (USDA-NRCS, 2014). In Canada, invasive hawkweeds are present across much of the southern portions of the country (Kartesz and Meacham, 1999), but are particularly problematic in British Columbia (Wilson et al., 2006; Wilson 2007).

*Pilosella caespitosa* (meadow hawkweed) was likely introduced into the United States in 1828 (Britton and Brown, 1970), and is now commonly found from Québec and Ontario (Frankton and Mulligan, 1970) southward to Georgia and Tennessee (Rickett, 1973). The first record of *P. caespitosa* in the western United States was in Pend Oreille County,

Washington, in 1969. *Pilosella caespitosa* is now recorded in the western states of Idaho, Montana, Oregon, Washington, and Wyoming; and is widespread throughout most of southern British Columbia and has begun encroachment into southern Alberta (Table 1).

Pilosella aurantiaca (orange hawkweed) was introduced into Vermont in 1875 as an ornamental, and within 25 years spread throughout much of the northeastern United States and southeastern Canada (Voss and Böhlke, 1978). During this period, it had spread throughout much of New England, west to Michigan (Voss and Böhlke, 1978) and into Canada from New Brunswick to Ontario (Britton and Brown, 1970). It now occurs throughout the eastern seaboard and into the Midwest, extending west to Minnesota and Iowa, and south to Virginia and North Carolina (Johnson, 1977). First recorded in Spokane, Washington, in 1945, P. aurantiaca has been collected from gardens in Nevada County, California (Munz and Keck, 1959), on the east slope of the Rocky Mountains in Colorado (Weber, 1990), from coastal Oregon and Washington (Rickett, 1973), and in southwestern British Columbia (Guppy, 1976). In the western United States, populations of orange hawkweed were first reported from coastal Washington and Oregon (Peck, 1941; Abrams and Ferris, 1960). It has also been reported from the Lower Mainland of British Columbia (Guppy, 1976) where it infests pastures, old fields and roadsides. Orange hawkweed is now also recorded in Idaho, Montana, and Wyoming (USDA-NRCS, 2014), and as common in Alberta (Posey, 2013). Pilosella *aurantiaca* is more widespread than other hawkweed species primarily because it is planted as an ornamental and often escapes cultivation (Table 1).

Both P. *floribunda* (king devil hawkweed) and *P. piloselloides* (tall hawkweed) were first reported in northern New York in 1879 (Voss and Böhlke, 1978) and 1900, respectively (Kennedy, 1902). Recently, infestations of *P. floribunda* have been reported in western Washington, northern Idaho, and Montana (USDA-NRCS, 2014) and is reportedly expanding in range (Table 1).

*Pilosella piloselloides* has been recorded in the western states of Montana and Washington (USDA-NRCS, 2014) and is also reportedly expanding in range (Table 1). Suspected potential for hybridization among *Pilosella* species, including *P. caespitosa*, *P. floribunda*, and *P. piloselloides*, and the potential threat of these species in western habitats is the rationale for including them as target weeds in the biological control program.

*Pilosella officinarum* is widespread in the eastern United States and Canada. In the west, it occurs in western Washington, western Oregon, Alaska, and to a limited area in south-central British Columbia (Table 1). *Pilosella flagellaris* (whiplash hawkweed) occurs primarily in the northeastern United States and eastern Canada, but also occurs in British Columbia and Wyoming (Table 1).

The first records of *Pilosella glomerata* in North America were recently confirmed in 2001 for southwestern British Columbia and eastern Washington State, where the species was likely previously confused with *P. caespitosa* (Wilson et al., 2006). The finding has increased the number of invasive *Pilosella* species reported as occurring in Canada and the United States to 15. Molecular analyses using both North American and European material and the narrow distribution of the species where it was found in North America suggest that *P. glomerata* is a recent introduction from Europe (Wilson et al., 2006). The species has been reported in a mountain pass of southwestern Alberta (Posey, 2013), so appears to be spreading east from the newly infested regions of the Pacific Northwest.

**Table 1.** Non-native *Pilosella* and *Hieracium* species, in the United States and Canada (modified from Wilson et al., 2006; USDA-NRCS, 2014).

#### Species

#### Distribution

Pilosella arvicola (Naeg. & Peter) Soják	CAN: NB
P. aurantiaca (L.) F. W. Schultz & Sch. Bip.	CAN: AB, BC, NB, NL, NS, ON, PE, QC, SK
	USA: AK, AR, CA, CO, CT, FL, ID, IN, IA, MA, MD, ME,
	MI, MN, MT, NC, NH, NJ, NY, OH, OR, PA, RI,
	SD, VA, VT, WA, WV, WI, WY
P. piloselloides subsp. bauhinii (Schult.) S. Bräut.	CAN: BC;
& Greuter	USA: CT, ID, MA, MN, NH, NY, VT, WA
<i>P. brachiata</i> Sch.Bip.fratt.	CAN: QC; USA: NY
P. caespitosa (Dumort.) P. D. Sell & C. West	CAN: AB, BC, NB, NL, NS, ON, PE, QC;
	USA: CT, DE, GA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MT, NC, NH, NJ, NY, OH, OR, PA, RI, SC, TN, VT, VA, WA, WV, WI, WY
P. derubella (Gottschl. et Schuhw.) S. Bräut.	CAN: QC;
& Greuter	USA: MI, NY, WI
P. flagellaris (Willd.) ArvTouv.	CAN: BC, NB, NL, NS, ON, PE, QC;
	USA: CT, ID, MA, ME, MI, NH, NY, PA, VA, VT
<i>P. floribunda</i> (Wimm. & Grab.) Fr. = <i>P. prealta</i>	CAN: BC, NB, NL, NS, ON, PE, QC;
(Gochnat) S. Bräut. & Greuter	USA: CT, ID, MA, MD, ME, MN, MT, NH, NJ, NY, OH,
	OR, RI, VA, VT, WA, WV
P. fuscoatra (Nägeli & Peter) Soják	USA: CT, NY, RI
P. glomerata (Froel.) Fr.	CAN: BC
	USA: ID, WA
P. lactucella (Wallr.) P. D. Sell & C. West	CAN: NS
P. officinarum Vaill.	CAN: BC, NB, NL, NS, ON, PE, QC;
	USA: CT, DE, GA, MA, MD, ME, MI, MN, NC, NH, NJ,
	NY, OH, OR, PA, RI, TN, VA, VT, WA, WV
P. piloselliflora (Naeg. & Peter) Soják	CAN: NB, NL, NS, ON, PE, QC
P. piloselloides (Vill.) Soják	CAN: BC, NB, NL, NS, ON, PE, QC;
	USA: CT, DE, GA, IA, IL, IN, KY, MA, ME, MI, MN, MT,
	NC, NH, NJ, NY, OH, PA, RI, SC, VA, VT, WA,
D. stolonifloug (Woldst. at Kit) E. W. Sahultz	WI, WV
P. stoloniflora (Waldst. et Kit.) F. W. Schultz	CAN: QC
& Sch. Bip.	
Hieracium atratum Fries.	USA: WA
<i>H. lachenalii</i> C. C. Gmel.	CAN: BC, NB, NL, NS, ON, PE, QC;
	USA: CT, DE, MA, ME, MI, MN, NH, NJ, NY, OR, PA, RI,
	VT, WA, WI
<i>H. laevigatum</i> Willd. [excl. H. canadense Michx.]	CAN: BC, NB, NS, ON, QC
	USA: WA, NE (USA precise distribution unknown)
H. maculatum Schrank	USA: MI
H. murorum L.	CAN: BC, NB, NL, NS, ON, QC
	USA: AK, CT, IL, MA, ME, MI, NH, NJ, NY, PA, VT
H. sabaudum L.	CAN: BC, NS, QC;
	USA: CT, MA, NJ, NY, PA, WI, WA

#### 2. Habitats Where Hawkweeds are Found in North America

In North America, invasive hawkweeds are primarily weeds of moist pastures, forest meadows, and other mountain or rangeland habitats with a moderate amount of moisture. Pilosella species occurrences in North America during the last century and their original distribution in northern and central Europe, suggest that they pose the greatest threat to cooler, sub-humid to humid sites in the northern regions of the United States and Canada. This prediction appears to be supported by a niche model developed for P. glomerata in British Columbia using both European and North American distribution and associated geoclimatic data (Ensing et al., 2013). Based on current infestations, habitats most susceptible to invasion occur at elevations of 450 meters (m) to over 1,500 m within the northern Pacific coastal montane region, with the largest infestations concentrated at about 1,000 m (Wilson et al., 1997). Habitats most vulnerable to initial invasion include human-disturbed sites, such as roadsides, permanent pastures, hayfields, cleared timber units, and abandoned farmland where the soil is well drained, coarse textured, and moderately low in organic matter. However, the invasive hawkweeds are highly competitive with native vegetation (Makepeace, 1985) and can quickly invade and achieve dominance within intact, natural sites (Reader, 1978). Consequently, wildlife habitat, recreation areas, and pristine mountain meadows in areas that have a climate similar to that in their native range are particularly susceptible, making this complex of invasive plants a major threat to native biodiversity, vegetation integrity and associated ecosystem services within sensitive, upland habitats.

Across the United States and Canada, introduced hawkweeds are closely associated with habitats used for livestock grazing that also support oxeye daisy (Leucanthemum vulgare), sulfur cinquefoil (Potentilla recta), spotted knapweed (Centaurea maculosa Lam.), gray goldenrod (Solidago nemoralis), wild carrot (Daucus carota), dandelion (Taraxacum officinale), and Kentucky bluegrass (Poa pratensis) (Thomas and Dale, 1974; Maycock and Guzikowa, 1984). Currently, none of the introduced Pilosella species are found in the natural grasslands or shrub-steppe of the northern Intermountain West, and are not expected to become problem weeds in the dry, lower elevation habitats associated with the western prairies. Neither meadow nor orange hawkweed appear to survive in annually tilled cropland. However, in a niche modelling study to predict the future spread of P. aurantiaca and P. officinarum in Australia using global distribution and associated climatic data, it was determined that the invasive hawkweeds can occur under different climatic conditions in the invaded range compared to what they experience within their European home range (Beaumont et al., 2009). Thus, there may be some surprises as the realized niches of *Pilosella* species become fully expressed within North America. There are also some differences in invasiveness among the *Pilosella* species that are beginning to become prevalent on the North

American landscape.

# C. Plants Related to Invasive Hawkweeds and Their Distribution

Plants related taxonomically to invasive hawkweeds would be the most likely to be attacked by the proposed biological control organism *C. urbana*. Plants related to the target hawkweeds in North America are discussed below.

Distribution of native *Hieracium* species ranges from the east coast to west coast, from sea level to over 2,725 m in elevation, and from northern to southern latitudes. As with the European invasive hawkweeds, the number of native species has long been disputed. To understand species relationships in *Hieracium*, a brief outline of the generic divisions is provided.

There are generally considered to be about 36 species of hawkweeds belonging to the genera Pilosella and Hieracium in North America north of Mexico (Strother, 2006) (Appendix 1). In North America, Hieracium species fall into two subgenera: Hieracium and Chionoracium. Subgenus *Hieracium* is circumboreal in distribution (a range that circles the boreal (north) portions of the earth) and is represented in North America by H. umbellatum, H. robinsonii, and H. canadense (considered part of Chionoracium by Gaskin and Wilson, 2007). Species in this subgenus reproduce sexually and apomictically and all are polyploid. Subgenus Chionoracium is restricted to the New World (western hemisphere; especially North and South America) and contains most of North America's native taxa. Species in this subgenus are strictly sexual and most are diploid (nucleus containing two sets of chromosomes). Conflicting characters used to distinguish species have generated considerable debate regarding classification. For example, Guppy (1978) suggested that H. scouleri, H. albertinum, and H. cynoglossoides are too closely correlated in characters to be distinguished into separate species and instead form a complex of species. Guppy (1978) also suggested that H. umbellatum and its close relative, H. canadense, may be two forms of the same species. Deardorff (1977) added H. longiberbe and H. nudicale to the *H. scouleri* complex. Kartesz and Meacham (1999) provided a comprehensive synthesis of Hieracium species in North America north of Mexico, in which they list 108 species, subspecies, and hybrids, clearly giving evidence of the myriad of described taxa in the genus.

Twenty-one additional hawkweed species found in the United States and Canada are introduced from Europe (Wilson et al., 2006) and belong to the genera *Hieracium* and *Pilosella* (Table 1). Six species belong to the genus *Hieracium*. Plants in this genus have not become weedy in North America. The remaining 15 species belong to the genus *Pilosella* and include the species targeted for biological control.

# **IV. Environmental Consequences**

#### A. No Action

# 1. Impact of a. Native Plants

#### Invasive Hawkweeds

Hawkweed invasiveness can be attributed to a high success in establishment, whether vegetatively or by seed, and then quickly becomes dominant in disturbed habitats such as native grasslands stressed by burning, heavy grazing, and/or climatic events such as drought (Hunter, 1991). The Pilosella invaders aggressively out-compete native species on disturbed sites by forming either dense, mono-specific patches or multihawkweed species stands (Makepeace, 1985; Lass and Callihan, 1997; Wilson et al., 1997). In North America, as in New Zealand, there is serious concern about the loss of native plant biodiversity and forage species in pastures due to hawkweed invasions (Wilson and Callihan, 1999). However, compared to the dry, thin soil, montane habitats inhabited in New Zealand, in North America, *Pilosella* species appear to prefer the more mesic habitats of the Pacific Northwest based on niche modelling using climatic data (Ensing et al., 2013) and are not expected to be problematic in the dry prairie regions of Canada and the United States (Wilson and Callihan, 1999; Giroday and Baker, 2006). However, they are very cold tolerant and easily persist at higher elevations, making sensitive upland habitats throughout the Rocky Mountains and other western mountain ranges, and the boreal areas of northern Canada susceptible to invasion (Wilson and Callihan, 1999). There also is concern in Canada that as climate change increases the outbreaks of forest pests in its boreal zones, so will the incursions of invasive Pilosella species into new regions (Ensing et al., 2013).

Monocultures of hawkweeds compete for soil moisture and nutrients, thereby posing risks to native species. As hawkweed monocultures invade species-rich range and mountain habitats, ecosystem functions and ecological relationships are affected. While no studies that specifically address the effects of hawkweed on nutrient cycling and disturbance regimes are known, hawkweed's ability to dominate a community suggests that these species do affect habitat function. Hawkweeds have been reported to have allelopathic effects on neighboring vegetation by exuding toxic chemicals into the soil (Makepeace, 1976; Dawes and Maravolo, 1973).

### **b. Economic Impact**

It is estimated that primarily four invasive hawkweed species (Hunter, 1991) have reduced the value of high country agricultural production in New Zealand by between \$1.1 and \$4.4 million (NZ dollars) annually (Grundy, 1989). Similarly in North America hawkweeds have had a major impacts. In the United States, an estimated \$58 million is lost annually to reduced resource revenues and expenses related to hawkweed control (Wilson, 2002; Duncan, 2005; Frid et al., 2009). In an economic analysis conducted for the province of British Columbia, invasive hawkweeds were predicted to have eventual economic impacts in the tens of millions of dollars if left to spread at current rates (Frid et al., 2012).

## c. Human health

Meadow hawkweed (*Hieracium caespitosum*) causes severe allergenic reactions. Reactions in people working closely with meadow hawkweed range from minor skin rashes, sneezing, congestion, and difficulty in breathing (Duncan, 2005). It is not known what portion of the plant causes this allergenic reaction.

Impact from Use of Other Control Methods
 The continued use of chemical herbicides, and mechanical, cultural, and biological controls at current levels would be a result if the "no action" alternative is chosen. These environmental consequences may occur even with the implementation of the biological control alternative, depending on the efficacy of *C. urbana* to reduce invasive hawkweed populations in the contiguous United States.

# a. Chemical Control

The use of herbicides, while effective, is limited to relatively accessible sites. Herbicides such as 2,4-D, clopyralid, and picloram have resulted in the greatest degree of control when applied at the normally recommended rate for perennial pasture weeds (Noel et al., 1979; Lass and Callihan, 1992a). Other herbicides either failed to control meadow hawkweed or suppression was for less than three years (Lass and Callihan, 1992b; Miller et al., 1998). In New Zealand, hawkweeds are especially abundant in low productivity areas, where herbicide applications are often not economical (Grundy, 1989).

# b. Mechanical Control

Mechanical control of invasive hawkweeds has had limited success. Digging the plants or otherwise disturbing the stolons, rhizomes, or roots only serves to spread the weed because plants can grow from buds on small root, stolon, and rhizome fragments. Disturbance by machinery spreads the weeds across fields. Local disturbances caused by grazing livestock, ungulates, and rodents also enhance the rate of spread of hawkweeds. In lawns, mowing does not kill invasive hawkweeds because the low-lying stolons and rhizomes are missed by the mower blades. Repeated mowing encourages faster vegetative spread (Wilson and Callihan, 1999).

## c. Cultural Control

When perennial grasses, legumes, and other beneficial forbs are present in the plant community, fertilizers can help control hawkweeds by increasing the competitive ability of the more desirable species. However, Reader and Watt (1981) found that repeated fertilizer treatments had no effect on dense patches of hawkweed that contained few grasses or other forbs. A combination of herbicides and fertilizers may contain the spread of invasive hawkweeds once they becomes established in a field. However, this is neither feasible nor desired in remote areas, native grasslands, and nature reserves where access is limited or herbicides are forbidden.

## d. Biological Control

The only biological control agent approved for release in the contiguous United States is *Aulacidea subterminalis*, a stolon-attacking gall wasp which has one generation per year. *Aulacidea subterminalis* mainly develops on *Pilosella officinarum* but *P. aurantiaca*, *P. floribunda*, and *P. flagellaris* are considered alternative target species based on pre-release host specificity test results. However, this insect alone is not expected to control invasive hawkweeds.

# B. Issue Permits for Environmental Release of *Cheilosia urbana*

 Impact of C. urbana on Nontarget Plants
 Host specificity of C. urbana to invasive hawkweeds has been demonstrated through field observations and host specificity testing. If an insect species only attacks one or a few closely related plant species, the insect is considered to be very host-specific. Host specificity is an essential trait for a biological control organism proposed for environmental release.

### a. Field Observations

Host records of *C. urbana* were limited to field observations of females ovipositing on *P. officinarum* (mouseear hawkweed) rosettes (Doczkal, 1996). Egg-laying behavior of a *C. urbana* female on *Filipendula ulmaria* (meadowsweet or queen-of-the-meadow) (Rosaceae) was reported by Kassebeer (1993), but the fly may have been confused with *Cheilosia vernalis* (Fallén, 1817); a species of similar size but which has a broad host range (Kassebeer, 2000). However, *F. ulmaria* was included in nochoice larval transfer tests and also exposed to gravid *C. urbana* females in single-choice oviposition tests. None of the 30 neonate larvae transferred onto *F. ulmaria* survived whereas 62 percent of the larvae transferred onto *P. officinarum* were retrieved in late summer. In addition, not a single egg was laid onto *F. ulmaria* in six single-choice oviposition tests carried out with gravid females whereas 77 eggs were laid onto the control, *P. officinarum* (Grosskopf, 2006).

#### b. Host Specificity Testing

Host specificity tests are tests to determine how many plant species *C*. *urbana* attacks, and whether nontarget species may be at risk. See appendix 3 for information regarding host specificity testing methods and results. From host specificity testing, the researchers determined that *C*. *urbana* is restricted to species in the genera *Pilosella* and *Hieracium*. Tests indicated that *C. urbana* might attack native North American *Hieracium* species in the subgenera *Hieracium* and *Chionoracium* in the field. However, plants in these subgenera have a low suitability to support larval development.

#### (1) Site of Quarantine Studies

Host specificity tests were carried out at the Centre for Agriculture and Biosciences International Europe-Switzerland Centre (CABI EU- CH) in Delèmont, Switzerland, in the quarantine facilities at Montana State University at Bozeman, and at Landcare Research Ltd., Lincoln, New Zealand.

#### (2) Test Plant List

The list of plant species used for host specificity testing of *C. urbana* is shown in appendix 2. The strategy used for selecting plants for testing is based on the phylogenetic approach, where closely related species are theorized to be at greater risk of attack than are distantly related species (Wapshere, 1974).

Plants for testing the host range of *C. urbana* were selected from seven possible categories. Test categories consisted of the following:

#### **CATEGORY 1:**

# Genetic types of invasive *Pilosella* species (varieties, races, forms, genotypes, apomicts, etc.)

For North American testing, six invasive *Pilosella* species were included: *P. aurantiaca* (2 populations), *P. caespitosa*, *P. floribunda*, *P. glomerata* (2 populations), *P. officinarum* (2 populations) and *P. piloselloides*. *Pilosella stoloniflora* was also tested for New Zealand.

All invasive species of this genus were attacked by the fly. However *C*. *urbana* prefers certain *Pilosella* species for egg laying and plants differ in their suitability for larval development.

#### **CATEGORY 2:**

# North American species in the same genus as *Pilosella*, divided by subgenera, including economically and environmentally important plants.

The genus *Pilosella* is not represented in the native flora of North America – all members of this genus are European and are listed under category 1.

#### CATEGORY 3:

#### North American species in other genera in the Asteraceae family, divided by subtribe, tribe, and subfamily, including economically and environmentally important plants.

The Asteraceae is the largest plant family worldwide and is divided into 3 subfamilies, 17 tribes, and numerous subtribes.

#### 3a. Species in the same subtribe (Hieraciinae) as Pilosella

Other genera in the Hieraciinae subtribe include *Andryala, Arnoseris, Hieracium, Hispidella, Hololeion*, and *Tolpis*. Native North American hawkweeds are in the genus *Hieracium* and belong to the subgenera *Hieracium* (three species) and *Chionoracium* (22 species). Representatives of these two subgenera were an important component of the test plant list.

No plant species from the remaining five genera of the Hieraciinae subtribe were tested because they are not native to North America, nor do they contain any economically important plants in North America (Bremer, 1994). Two species, *Arnoseris minima* and *Tolpis barbata*, have been introduced to North America (Flora of North America North of Mexico, 2008; USDA-NRCS Plants Database, 2014).

In total, 22 species in category 3a were tested and half of them supported development to mature larvae. However, their overall acceptance and suitability was much lower than of species in the subgenus *Pilosella*.

# **3.b.** Species in different subtribes of the same tribe (Lactuceae) as *Pilosella*

The Lactuceae is one of the best known tribes of the Asteraceae and is well supported as a distinct monophyletic group. Subtribal and generic classifications are more defined in this tribe than in most other tribes (Bremer, 1994). Besides the Hieraciinae, there are five other subtribes within the Lactuceae that contain species native to North America (Crepidinae, Lactucinae, Malcothrinae, Microseridinae, and Stephanomeriinae); four subtribes with introduced species (Catananchinae, Hypochaeridinae = Leontodontinae, Scorzonerinae, and Sonchinae); and two introduced genera (*Cichorium* and *Scolymus*) that have not been assigned to a subtribe (Bremer, 1994).

Two genera in the Crepidinae subtribe (*Crepis* and *Taraxacum*) contain species native to North America (Bremer, 1994). The two native *Crepis* species tested were *Crepis atribarba* and *Crepis intermedia*. The genus *Taraxacum* contains native species in western North America (USDA-NCRS, 2014). The native North American species *T. lyratum* and the naturalized species *T. laevigatum* were tested. For New Zealand, the cosmopolitan species *Taraxacum officinale* was tested.

Within the Lactucinae, two genera contain species native to North America: *Lactuca* and *Prenanthes* (Bremer, 1994; USDA-NRCS, 2014). *Lactuca sativa* (lettuce) was included because of its economic importance. In addition, *Lactuca serriola* and *L. virosa* were tested because they are widespread weeds. *Prenanthes sagittata* was tested because it is a native.

The Malcothrinae contains the following genera native to North America: *Anisocoma, Atrichoseris, Calycoseris, Glyptopleura, Malacothrix,* and *Pinaropappus* (Bremer, 1994; Flora of North America North of Mexico, 2008; USDA-NRCS, 2014). Most are native to the southwestern United States, except *Glyptopleura* (western annuals) and *Malacothrix* (western annuals and perennials). No species from this subtribe were tested because it was not possible for the researchers to obtain viable seeds and/or plant material.

The Microseridinae contains the following genera native to North America: Agoseris, Krigia, Microseris, Nothocalais, Phalacroseris, Pyrrhopappus, Stebbinsoseris, and Uropappus (Bremer, 1994; USDA-NRCS, 2014). Microseris bigelovii and Uropappus lindleyi are both listed as endangered in Canada, where small endemic populations occur on the islands off of the British Columbia coast. Four native species: Microseris nutans, M. troximoides (syn. Nothocalais troximoides (A. Gray) Greene), Agoseris grandiflora, and Krigia biflora were included in tests. For New Zealand, Microseris scapigera was tested.

The Stephanomeriinae contains the following genera native to North America: *Chaetadelpha*, *Lygodesmia*, *Prenanthella*, *Rafinesquia*, *Shinnersoseris*, and *Stephanomeria* (Bremer, 1994; USDA-NCRS, 2008). Three native species with a western distribution were tested: *Lygodesmia juncea*, *Stephanomeria cichoriacea*, and *S. virgata*.

Of the four subtribes containing only introduced species, *Catanache caerula*, an introduced ornamental from the Catananchinae was included. For the subtribe Sonchinae, *Sonchus arvensis* and *S. asper* were tested for

North America, and *S. oleraceus* plus *S. kirkii* were tested for New Zealand. *Kirkianella novae-zelandiae* and *Embergeria grandifolia* are native to New Zealand and were used in the New Zealand testing program.

For the subtribe Hypochaeridinae, *Hypochoeris radicata, Leontodon taraxacoides,* and *Picris hieracioides* were included in the New Zealand testing program because they are widespread species in Europe, often co-occurring with *Pilosella* species.

No species from the Scorzonerinae were included for North American testing, as this subtribe is not native to North America and contains no economically important introduced species (Bremer, 1994; USDA-NCRS, 2008). For New Zealand *Tragopogon porrifolius* was tested. This species belongs to the subtribe Scorzonerinae which is restricted to Eurasia and North Africa.

Among the introduced genera unassigned to a subtribe, *Cichorium intybus* (chicory) was used in New Zealand tests because of its economic importance.

In summary 29 species belonging to other subtribes within the tribe Lactuceae were tested for North America and New Zealand. None were used by *C. urbana* as a host.

**3.c.** Species in different tribes in the same subfamily (Cichorioideae) According to Bremer (1994), the subfamily Cichorioideae contains three tribes apart from Lactuceae with natives in North America (Cardueae, Mutisieae, and Vernonieae) plus a tribe with some introduced ornamentals (Arctoteae). The Mutisieae and the Vernonieae are believed to be closely related to the Lactuceae (Mañez et al., 1994; Tomb, 1977). *Stokesia laevis*, a native ornamental in the Veronieae, *Gerbera jamesonii* in the Mutisieae, and *Gazania rigens* in the Arctoteae were included in the testing. From the Cardueae, a native North American thistle was tested, *Cirsium undulatum*. *Carduus acanthoides, Carduus nutans, Cirsium palustre*, and *Cirsium vulgare* were tested for New Zealand because they are host plants of other *Cheilosia* species. In addition, the commercially grown *Cynara scolymus* (globe artichoke) and *Carthamus tinctorius* (safflower) were tested. No larval development was recorded on any of the ten species tested.

**3.d. Species in different subfamilies in the same family (Asteraceae)** Besides the Cichorioideae, there are two additional subfamilies (Barnadesioideae and Asteroideae) in the Asteraceae family (Bremer, 1994).

The Barnadesioideae contains nine genera that occur exclusively in South America. Because there are no species native to North America (Bremer, 1994), no Barnadesioideae were included in testing.

The Asteroideae include the majority of the Asteraceae and contains eight tribes with species native to the United States (Anthemideae, Astereae, Eupatorieae, Gnaphalieae, Helenieae, Heliantheae, Plucheeae, and Senecioneae), two additional tribes with ornamental species (Calenduleae and Inuleae), and eight genera unassigned to a tribe (Bremer, 1994). In combined North American and New Zealand tests, 20 species were used representing the eight native subtribes and a non-native subtribe of the Asteraceae. Larvae of *C. urbana* were not able to develop on any of these species in no-choice larval feeding tests.

In summary, a total of 81 species were tested in Category 3. Results showed that the physiological host range of *C. urbana* larvae is restricted to the genera *Hieracium* and *Pilosella*.

#### CATEGORY 4: Threatened and endangered species in the Asteraceae family, divided by subgenus, genus, tribe and subfamily.

#### 4.a. Species in the same genus as *Pilosella*

There are no *Pilosella* species listed as threatened, endangered, or sensitive. All *Pilosella* species present in North or South America are introduced.

#### 4.b. Species in the same genus as *Hieracium*

While there are no Federally-listed threatened or endangered species of *Hieracium* in the United States or Canada, several U.S. States identify one or more *Hieracium* species as being restricted in distribution (often at the edge of their ecological range).

Five of the State-listed *Hieracium* species (*H. canadense, H. fendleri, H. longipilum, H. scabrum,* and *H. umbellatum*) were included in tests and four supported adult development to a certain degree. However, their overall acceptance and suitability only ranged from 0.3 (*H. longipilum*) to 1.7 (*H. canadense*). No eggs were laid in open-field tests on a total of 60 *H. scabrum* plants.

#### 4.c. Species in the same subtribe (Hieraciinae) as Pilosella

There are no threatened, endangered, or sensitive species within the same subtribe as *Pilosella*.

# 4.d. Species in different subtribes of the same tribe (Lactuceae) as *Pilosella*

Four of the Lactuceae subtribes (besides Hieraciinae) have threatened, endangered, or sensitive species in the contiguous United States (USDA-NRCS 2014). Because obtaining plant material of protected species can be difficult and can further decimate populations, a more common member from each genus was tested.

Two Crepidinae species, Taraxacum californicum (federally listed as endangered) and T. officinale ssp. ceratophorum (sensitive species), occur in the contiguous United States. The more common *Taraxacum officinale* (listed in Category 3.b.) was tested. The Lactucinae has two species of Prenanthes considered sensitive (P. barbata and P. boottii). The more common native species, Prenanthes sagittata, was tested instead (listed in Category 3.b.). The Microseridinae has two species of Microseris listed as sensitive (*M. decipens* and *M. howellii*) in the United States, and Microseris bigelovii is listed as endangered (endemic population on Vancouver Island, BC). The more common native species, Microseris nutans and Microseris troximoides (listed in Category 3.b.), were tested. The Stephanomeriinae contains one federally endangered Stephanomeria (S. malheurensis Gottlieb), one sensitive Stephanomeria (S. blairii Munz. & Johnston) and one sensitive Lygodesmia species (L. doloresensis S. Tomb) in the United States. Rather than test these species, Stephanomeria *minor* (= *S. tenuifolia*), *S. cichoriacea*, and *Lygodesmia juncea* were tested instead (see Category 3.b.)

#### 4.e. Species in different tribes in the same subfamily (Cichorioideae)

The Cardueae contains several *Cirsium* species listed as threatened, endangered, or sensitive in the contiguous United States and Canada: *Cirsium fontinale* var. *fontinale* (fountain thistle), *Cirsium fontinale* var. *obispoense* (Chorro Creek bog thistle), *Cirsium hydrophilum* var. *hydrophilum* (Suisun thistle), *Cirsium loncholepis* (La Graciosa thistle), and *Cirsium pitcheri* (Pitcher's thistle). In Canada, *Cirsium hillii*, another Great Lakes endemic, is also listed as endangered. Because obtaining achenes and/or plant material of protected species can be difficult and can further decimate populations, *Cirsium undulatum*, a more common member from this genus was tested.

#### 4.f. Species in different subfamilies in the same family (Asteraceae):

At this level, representatives from genera with threatened, endangered, or sensitive individuals were not directly tested. With over 20 genera with federally listed threatened or endangered species and over 70 additional genera with sensitive species in the contiguous United States, the number of species to test was prohibitively large and unnecessary given the narrow host range that was delineated. Testing was conducted on representatives of the various subtribes of Asteraceae (Category 3.d). Results showed that *C. urbana* is restricted in its host range to the genera *Hieracium* and *Pilosella*.

#### **CATEGORY 5:**

#### North American species in other families in the Asterales order that have some phylogenetic, morphological, or biochemical relationship to the target weed, including economically and environmentally important plants.

Neither Cronquist nor Dahlgren list any families other than the Asteraceae in the Asterales order; however, Thorne lists the Calyceraceae as belonging in the Asterales. Cronquist places the Calyceraceae in the Calycerales while Dahlgren places it in the Dipsacales. Because two out of three systems list the Calyceraceae in an order other than Asterales, this family is discussed in Category 6.

#### **CATEGORY 6:**

#### North American species in other orders that have some morphological or biochemical relationship to the target weed, including economically and environmentally important plants.

# 6.a. Species in other orders that are phylogenetically related to the Asteraceae

The Asteraceae form such a well-defined group that they are sometimes considered systematically isolated. The most closely related families are generally considered to be the Calyceraceae, Campanulaceae sensu lato, and Goodeniaceae (Bremer, 1994). The Calyceraceae contains six genera with about 60 species in southern South America (Bremer, 1994). One species, Acicarpha tribuloides, is listed as present in North America (Kartesz and Kartesz, 1980). The Campanulaceae sensu lato contains about 85 genera and more than 2,200 species (Bremer, 1994). There are about 23 genera and 290 species in the United States and Canada (Zomlefer, 1994). The Campanulaceae have been shown to be chemotaxonomically linked to the Asteraceae (Mabry and Bohlmann, 1977). The Goodeniaceae contain 12 genera and 400 predominately Australian species (Bremer, 1994). One genus, Scaevola, has two species listed as present in the central and southern United States (USDA-NRCS, 2014). Due to the restricted physiological host range of C. urbana larvae, plants within these orders were not tested.

# 6.b. Species in other orders that are biochemically similar to the Asteraceae.

Species in the Lactuceae are characterized, in part, by their milky latex. However, more than 12,500 species in 900 genera and 20 families have been identified worldwide that produce latex (Metcalfe, 1967). Over 300 species in the United States have been shown to contain latex (Buchanan et al., 1978). The following four latex-producing species from different families were selected for testing: the commercially grown *Allium cepa*  (onions), the two native North American species *Asclepias syriaca* and *Monarda fistulosa*, and *Papavar nudicaule*. None of these latex-producing species were attacked by *C. urbana*.

The Lactuceae also differ phytochemically in that their sesquiterpene lactones are predominately guaianolides whereas the other Asteraceae subfamilies contain plants with considerable portions of other sesquiterpene lactones (Wapshere, 1983). Unlike most other Asteraceae, the Lactuceae possess either no or a low level of polyacetylene compounds (Sørensen, 1977). The Apiaceae (= Umbellifereae) have been shown to be chemotaxonomically linked to the Asteraceae (Mabry and Bohlmann, 1977). *Petroselinum crispum* (parsley), which is economically important and chemically similar to the Asteraceae was not infested during tests conducted for New Zealand.

6.c. Selected cultivated species in other orders

No cultivated species in other plant orders were tested.

#### **CATEGORY 7:**

#### Any plant on which the biological control agent or its close relatives (within the same genus) have been previously recorded to feed and/or reproduce.

Species in the genus *Cheilosia* are often restricted to a single plant genus or species. So far, species of *Cheilosia* have been found on plant species in 11 families, and two *Cheilosia* species develop on mushrooms. A number of species in the genera *Carduus, Cirsium, Cynara, Taraxacum, Primula, Petasites, Senecio, Cynoglossum,* and *Sempervivum* were tested with *C. urbana*. Mature larvae were only retrieved from species in the genera *Hieracium* and *Pilosella*.

#### (3) Discussion of Host Specificity Testing

In no-choice larval transfer tests, *C. urbana* is able to develop on all major non-native, invasive, hawkweed species in the genus *Pilosella*, (*P. aurantiaca*, orange hawkweed; *P. caespitosa*, meadow hawkweed; *P. floribunda*, king devil; *P. glomerata*, queen-devil; *P. officinarum*, mouseear hawkweed; and *P. piloselloides*, yellow devil hawkweed). The survival rate to adulthood ranged from 7.1 percent on a *P. officinarum* population from Europe to 52.4 percent on a *P. aurantiaca* population from Idaho. In tests carried out for New Zealand, the survival rate reached even 80 percent on *P. caespitosa* from Idaho. There are no native North American hawkweeds in the genus *Pilosella*. Therefore, attack of all *Pilosella* species tested is desired.

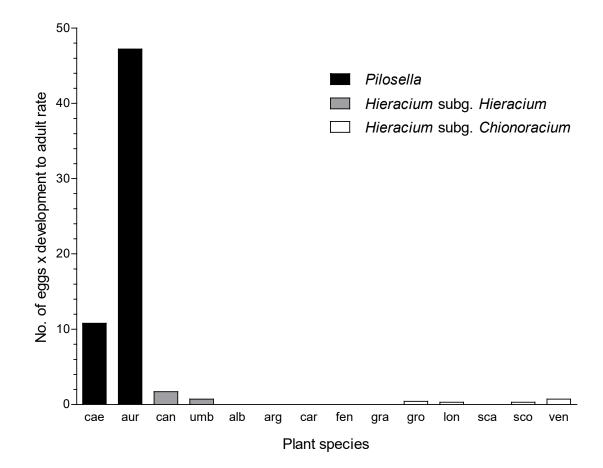
*Cheilosia urbana* also developed to mature larvae on five out of seven tested *Hieracium* species in the subgenus *Hieracium*. Two of the species tested are native to North America (*H. umbellatum*, narrowleaf hawkweed

and *H. canadense*, Canadian hawkweed), with survival rates to mature larva of 4.8 percent and 16.7 percent, respectively. Development to adulthood was 1.2 percent and 8.3 percent, respectively. In contrast, only five out of 15 native North American *Hieracium* species in the subgenus *Chionoracium* supported development to mature larvae with development rates ranging from 0.6 percent to 6.1 percent. Adults developed on four species (*H. gronovii, H. longipilum, H. scabrum,* and *H. venosum*).

When given the choice between a native North American *Hieracium* species and *P. caespitosa* in single-choice oviposition tests, *C. urbana* females laid less than 10 percent of their eggs on *H. umbellatum*, *H. gracile*, *H. longipilum* and *H. scouleri*. Because females preferred *P. aurantiaca*, *P. floribunda* and *P. piloselloides* over *P. caespitosa*, the preference would likely have been even stronger when offering these species.

Open-field oviposition tests with *C. urbana* carried out between 2006 and 2008 showed that *C. urbana* mostly prefers *P. caespitosa* over native North American *Hieracium* species. However, *C. urbana* also laid eggs onto ten of the 12 native hawkweed species tested; however, oviposition was highly variable between years and test plots and sometimes occurred in absence of the target hawkweed.

To better interpret test results, the data of open-field oviposition tests obtained between 2006 and 2008 were pooled and the overall acceptance and suitability of target and test plant species for C. urbana was calculated by taking the average number of eggs laid onto the different test plants exposed in open-field tests multiplied by the average survival rate to adulthood (from neonate larva to adult fly). The suitability value was below two for native North American *Hieracium* species in the subgenus *Hieracium* (range: 0.7–1.7) and between zero and 0.7 for species in the subgenus Chionoracium, while the target weeds P. caespitosa and P. aurantiaca had an overall acceptance and suitability of almost 11 and 47, respectively (Figure 1). The low overall suitability of native *Hieracium* species is confirmed by results of the open-field test established in 2005, where subsequent larval development was also monitored. While nearly 90 percent of control plants (P. caespitosa) were attacked and had a maximum of 11 larvae, only 10-33 percent of exposed native Hieracium species were attacked and a maximum of only one C. urbana larva was found.



**Figure 1.** Overall acceptance and suitability of target and test plant species for *Cheilosia urbana*, calculated as the average number of eggs laid in open-field tests carried out between 2006 and 2008, multiplied by the average survival rate from neonate larva to adulthood in no-choice larval transfer tests. The letters indicate the first letters of the *Hieracium* and *Pilosella* species exposed: *P. caespitosa* (cae) and *P. aurantiaca* (aur) in the genus *Pilosella*, *H. canadense* (can) and *H. umbellatum* (umb) in the subgenus *Hieracium*, and the remaining *Hieracium* species in the subgenus *Chionoracium* (*H. albiflorum* (alb), *H. argutum* (arg), *H. carneum* (car), *H. fendleri* (fen), *H. gracile* (gra), *H. gronovii* (gro), *H. longipilum* (lon), *Hieracium scabrum* (sca), *H. scouleri* (sco), and *H. venosum* (ven)) (Littlefield et al., 2014).

In an impact experiment using *P. caespitosa* and the native North American *H. gronovii*, larval feeding did not significantly affect growth of *H. gronovii*. This result is most probably due to high initial mortality of *C. urbana* larvae transferred onto the non-target plant. The survival rate of *C. urbana* larvae transferred onto *H. gronovii* was 3.6 percent in no-choice larval transfer tests, whereas an average of 21.7 percent of transferred larvae survived on *P. caespitosa*. The researchers conclude that it is very unlikely that *C. urbana* will have a measurable impact on native *Hieracium* species considering their low suitability for *C. urbana* larval survival.

Feeding by *C. urbana* had a significant negative impact on several parameters of *P. caespitosa*. Biomass was reduced by 51 percent, the number of flower heads produced was reduced by 89 percent, and the number of stolons was reduced by 81 percent. *Cheilosia urbana* is therefore expected to significantly reduce plant vigor, vegetative reproduction by stolons and generative reproduction by seeds of invasive hawkweed species (Littlefield et al., 2014).

Host specificity tests indicated that *C. urbana* may attack native North American *Hieracium* species in the subgenera *Hieracium* and *Chionoracium* in the field. However, these plants have low suitability to support larval development.

2. Impact of C. urbana on invasive hawkweeds
The root-feeding hoverfly C. urbana has one generation per year. The adults emerge in spring and females lay their eggs into the leaf axils of Pilosella plants. Neonate larvae move into the soil and feed externally on the roots, creating small cavities. The larvae feed until late September or early October and pupate in the soil, close to the surface, where they overwinter. An impact experiment showed that feeding by C. urbana can significantly reduce the numbers of bolting plants, flower heads with ripe seeds, stolons, and the above-ground biomass of P. caespitosa.

3. Uncertainties Once a biological control agent such as C. urbana is released into the Regarding the environment and becomes established, there is a possibility that it could Environmove from the target plants (invasive hawkweeds) to attack nontarget mental plants, such as *Hieracium* species in the subgenera *Hieracium* and Release of C. Chionoracium. Host shifts by introduced weed biological control agents to unrelated plants are rare (Pemberton, 2000). Native species that are closely urbana related to the target species are the most likely to be attacked (Louda et al., 2003). If other plant species were to be attacked by C. urbana, the resulting effects could be environmental impacts that may not be easily reversed. Biological control agents such as C. urbana generally spread without intervention by man. In principle, therefore, release of this biological control agent at even one site must be considered equivalent to release over the entire area in which potential hosts occur, and in which the climate is suitable for reproduction and survival.

> In addition, this agent may not be successful in reducing invasive hawkweed populations in the contiguous United States. Worldwide, biological weed control programs have had an overall success rate of 33 percent; success rates have been considerably higher for programs in individual countries (Culliney, 2005). Actual impacts on invasive hawkweeds by *C. urbana* will not be known until after release occurs and

post-release monitoring has been conducted (appendix 4). However, it is expected that *C. urbana* will significantly reduce plant vigor, vegetative reproduction by stolons, and generative reproduction by seeds of invasive hawkweed species (Littlefield et al., 2014).

#### **4. Cumulative Impacts** "Cumulative impacts are defined as the impact on the environment which results from the incremental impact of the action when added to other past, present and reasonably foreseeable future actions regardless of what agencies or person undertakes such other actions" (40 CFR 1508.7).

Other private and public concerns work to control hawkweeds in invaded areas using available chemical, mechanical, cultural, and biological control methods, as described in this document. In North America, invasive hawkweeds are primarily weeds of moist pastures and forest meadows, and mesic, montane rangelands. Other invasive weeds such as spotted knapweed are also targeted in habitats invaded by hawkweeds.

Release of *C. urbana* is not expected to have any negative cumulative impacts in the contiguous United States because of its host specificity to *Hieracium* and *Pilosella* species. Effective biological control of invasive hawkweeds will have beneficial effects for weed management programs, and may result in a long-term, non-damaging method to assist in the control of hawkweeds, particularly in natural or environmentally sensitive areas.

#### **5. Endangered Species Act** Species Act Section 7 of the Endangered Species Act (ESA) and ESA's implementing regulations require Federal agencies to ensure that their actions are not likely to jeopardize the continued existence of federally listed threatened and endangered species or result in the destruction or adverse modification of critical habitat.

#### a. Critical Habitat

APHIS has determined that release of *C. urbana* will have no effect on any designated critical habitat in the United States. Invasive hawkweeds are not part of the primary constituent elements of any listed species. Invasive hawkweeds are adversely affecting golden paintbrush (*Castilleja levisecta*) but this species does not have designated critical habitat.

#### b. Animals

Release of *C. urbana* will have no effect on any listed vertebrate animals including mammals, birds, reptiles, amphibians, or fishes. Orange hawkweed (*Pilosella aurantiaca*) was identified as a nectar species for the Karner blue butterfly (*Lycaeides melissa samuelis*) in Wisconsin (Kleintjes et al., 2003). However, Karner blue adults obtain nectar from a

wide variety of flowering plants, including both native and exotic species (Haack, 1993). Therefore, release of *C. urbana* may affect, but is not likely to adversely affect the Karner blue butterfly.

### c. Plants

There are 56 listed plants in the contiguous United States that belong to the family Asteraceae, the same family as the target weed. Closely related plants to the target weed are those that would most likely be affected by a biocontrol agent. However, based on the host specificity of *C. urbana* demonstrated in studies, APHIS has determined that release of *C. urbana* may affect, but is not likely to adversely affect listed plants in the Asteraceae or their critical habitats (Appendix 5). In addition, APHIS has determined that release of *C. urbana* may affect beneficially the golden paintbrush because mouseear hawkweeds (*Pilosella officinarum*) are encroaching on its habitat in Thurston County, Washington (Appendix 5)

A biological assessment was prepared and submitted to the U.S. Fish and Wildlife Service (FWS) and is part of the administrative record for this EA (prepared by T.A. Willard, April 3, 2017). APHIS requested concurrence with these determinations from the FWS and received a concurrence letter dated July 10, 2018.

# V. Other Issues

Consistent with Executive Order (EO) 12898, "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations," APHIS considered the potential for disproportionately high and adverse human health or environmental effects on any minority populations and low-income populations. There are no adverse environmental or human health effects from the field release of *C. urbana* and will not have disproportionate adverse effects to any minority or lowincome populations.

Consistent with EO 13045, "Protection of Children from Environmental Health Risks and Safety Risks," APHIS considered the potential for disproportionately high and adverse environmental health and safety risks to children. No circumstances that would trigger the need for special environmental reviews are involved in implementing the preferred alternative. Therefore, it is expected that no disproportionate effects on children are anticipated as a consequence of the field release of *C. urbana*.

EO 13175, "Consultation and Coordination with Indian Tribal Governments," was issued to ensure that there would be "meaningful consultation and collaboration with tribal officials in the development of Federal policies that have tribal implications...." APHIS is consulting and collaborating with Indian tribal officials to ensure that they are well-informed and represented in policy and program decisions that may impact their agricultural interests in accordance with EO 13175.

# VI. Agencies, Organizations, and Individuals Consulted

The Technical Advisory Group for the Biological Control Agents of Weeds (TAG) recommended the release of *C. urbana* on May 26, 2016. TAG members that reviewed the release petition (Littlefield et al., 2014) included USDA representatives from Forest Service, National Institute of Food and Agriculture, and Plant Protection and Quarantine; U.S. Department of Interior's U.S. Fish and Wildlife Service and Bureau of Land Management; Environmental Protection Agency; U.S. Army Corps of Engineers; and representatives from California Department of Food and Agriculture (National Plant Board), SAGARPA-Mexico, and Agriculture and Agri-Food Canada.

This EA was prepared by personnel at APHIS, Montana State University, Agriculture and Agri-Food Canada, and CABI Europe-Switzerland Centre. The addresses of participating APHIS units, cooperators, and consultants follow.

U.S. Department of Agriculture Animal and Plant Health Inspection Service Policy and Program Development Environmental and Risk Analysis Services 4700 River Road, Unit 149 Riverdale, MD 20737

U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine Regulations, Permits, and Manuals 4700 River Road, Unit 133 Riverdale, MD 20737

Montana State University Department of Land Resources and Environmental Sciences P.O. Box 173120 Bozeman, MT 59717-3120 Agriculture and Agri-Food Canada Lethbridge Research Centre 5403 1 Ave S Lethbridge Alberta, Canada, T1J 4B1

CABI Europe-Switzerland Centre Rue des Grillons 1 CH-2800 Delémont, Switzerland

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# **Appendix 1.** Comprehensive list of Hieracium taxa in North America north of Mexico (as per Kartesz and Meacham, 1999; Strother, 2006).

Hieracium acranthophorum Om.	
Hieracium acranthophorum var. isortoquense Böcher	
Hieracium acuminatum Jord.	
Hieracium albertinum Farr	W/hite Element Handlerer d
<i>Hieracium albiflorum</i> Hook.	White-Flower Hawkweed
Syn. <i>Chlorocrepis albiflora</i> (Hook.) W.A. Weber Syn. <i>Hieracium helleri</i> Gandog.	
<i>Hieracium ×alleghaniense</i> Britt. (pro sp.)	
Hieracium alpinum L.	Alpine Hawkweed
Hieracium angmagssalikense Om.	Black-Style Hawkweed
Hieracium argutum Nutt.	Southern Hawkweed
Syn. Hieracium argutum var. parishii (Gray) Jepso	on
Hieracium × atramentarium (Naegeli & Peter) Zahn ex Engl. (pr	ro sp.)
Hieracium atratum Fries	Polar Hawkweed
Hieracium aurantiacum L.	Devil's-Paintbrush
Hieracium bolanderi Gray	Bolander's Hawkweed
Syn. Hieracium siskiyouense M.E. Peck	
<i>Hieracium</i> × <i>brachiatum</i> Berth. ex DC.	
Hieracium caespitosum Dumort.	Meadow Hawkweed
Syn. Hieracium pratense Tausch	
Hieracium canadense Michx.	Canadian Hawkweed
Hieracium canadense var. canadense	
Syn. <i>Hieracium columbianum</i> auct. non Rydb.	
Syn. Hieracium kalmii var. canadense (Michx.) Re	eveal
Syn. Hieracium scabriusculum var. columbianum a Lepage	auct. non (Rydb.)
Syn. <i>Hieracium umbellatum</i> var. <i>canadense</i> (Mich	y) Breitung
Syn. Hieracium umbellatum var. scabriusculum Fa	· •
Hieracium canadense var. divaricatum Lepage	11 vv .
Hieracium carneum Greene	Huachuca Hawkweed
Hieracium cynoglossoides ArvTouv.	Hound-Tongue Hawkweed
Syn. <i>Hieracium albertinum</i> Farr	Hound-Toligue Hawkweed
Syn. <i>Hieracium cusickii</i> Gandog.	
Syn. <i>Hieracium flettii</i> St. John & Warren	
Syn. Hieracium scouleri var. albertinum (Farr) G.V	V Douglas & G A Allen
Syn. Hieracium scouleri var. griseum (Rydb.) A. N	-
Hieracium ×dutillyanum Lepage	NC13.
Hieracium ×fassettii Lepage	
Hieracium × fassettii Lepage Hieracium × fassettii var. fassettii	
Hieracium × fassettii var. mendicum Lepage (pro nm.) Hieracium × fassettii var. wisconsinense Lepage (pro nm.)	
merucium ~jusseum var. wisconsmense Lepage (pro min.)	

Hieracium fendleri Schultz-Bip. Yellow Hawkweed Hieracium fendleri var. discolor Gray Hieracium fendleri var. fendleri Syn. Chlorocrepis fendleri (Schultz-Bip.) W.A. Weber *Hieracium fendleri* var. *mogollense* Gray Hieracium × fernaldii Lepage Syn. Hieracium canadense var. hirtirameum Fern. *Hieracium* ×*flagellare* Willd. (pro sp.) *Hieracium* ×*flagellare* var. *amauracron* (Missbach & Zahn) Lepage (pro nm.) Syn. Hieracium × flagellare ssp. amauracron Missbach & Zahn *Hieracium* ×*flagellare* var. *cernuiforme* (Naegeli & Peter) Lepage (pro nm.) Syn. *Hieracium* ×*duplex* Peter Syn. *Hieracium flagellare* ssp. *cernuiforme* Naegeli & Peter Syn. Hieracium × macrostolonum Schneid. *Hieracium* ×*flagellare* var. *flagellare Hieracium* ×*flagellare* var. *pilosius* Lepage *Hieracium* ×*floribundum* Wimmer & Grab. (pro sp.) Syn. Hieracium auricula L. p.p. Syn. *Hieracium* ×*dorei* Lepage *Hieracium* ×*fuscatrum* Naegeli & Peter (pro sp.) *Hieracium gracile* Hook. Low Alpine Hawkweed Hieracium gracile var. alaskanum Zahn *Hieracium gracile* var. *detonsum* (Gray) Gray *Hieracium gracile* var. *gracile* Syn. Chlorocrepis tristis ssp. gracilis (Hook.) W.A. Weber Syn. *Hieracium gracile* var. *densifloccosum* (Zahn) Cronq. Syn. Hieracium triste ssp. gracile (Hook.) Calder & Taylor Syn. Hieracium triste var. gracile (Hook.) Gray Hieracium gracile var. yukonense Porsild Hieracium greenei Gray Greene's Hawkweed Hieracium greenii Porter & Britt. Green's Hawkweed Syn. Hieracium traillii Greene Hieracium groenlandicum (Arv.-Touv.) Almquist Greenland Hawkweed *Hieracium* ×*grohii* Lepage *Hieracium* ×*grohii* var. *farwellii* Lepage *Hieracium* ×*grohii* var. *grohii* Hieracium gronovii L. Queendevil Syn. Hieracium gronovii var. foliosum Michx. Hieracium horridum Fries Rayed Shaggy Hawkweed Hieracium inuloides Tausch Butterfly Hawkweed *Hieracium kalmii* L. Kalm's Hawkweed Hieracium kalmii var. fasciculatum (Pursh) Lepage Syn. Hieracium canadense var. fasciculatum (Pursh) Fern. Hieracium kalmii var. kalmii Syn. Hieracium canadense var. kalmii (L.) Scoggan Hieracium kalmii var. subintegrum (Lepage) Lepage

Syn. Hieracium canadense var. subintegrum Lepa	ge
Hieracium lachenalii K.C. Gmel.	Common Hawkweed
Syn. Hieracium acuminatum Jord.	
Syn. Hieracium vulgatum Fries	
Hieracium laevigatum Willd.	Smooth Hawkweed
Syn. Hieracium acranthophorum Om.	
Syn. Hieracium acranthophorum var. isortoquense	e Böcher
Syn. <i>Hieracium devoldii</i> Om.	
Syn. Hieracium eugenii Om.	
Syn. Hieracium musartutense Om.	
Syn. Hieracium nepiocratum Om.	
Syn. Hieracium rigorosum (Laestad.) Almquist	
Syn. <i>Hieracium rigorosum</i> var. nanusekense Om.	
Syn. <i>Hieracium rigorosum</i> var. sermilikense Om.	
Syn. Hieracium rigorosum var. unanakense Om.	
Syn. Hieracium stiptocaule Om.	
Hieracium lemmonii Gray	Lemmon's Hawkweed
Hieracium longiberbe T.J. Howell	Long-Beard Hawkweed
Hieracium longipilum Torr.	Hairy Hawkweed
Hieracium maculatum Sm.	Spotted Hawkweed
Hieracium marianum Willd.	Maryland Hawkweed
Syn. Hieracium pennsylvanicum Fries	-
Hieracium megacephalon Nash	Coastal-Plain Hawkweed
Syn. <i>Hieracium argyraeum</i> Small	
Hieracium murorum L.	Wall Hawkweed
Hieracium nigrescens Willd.	Arctic Hawkweed
Syn. Hieracium hyparcticum Almquist	
Syn. Hieracium lividorubens (Almquist) Elfstr.	
Syn. Hieracium lividorubens var. pseudostylum Os	skarss.
Syn. Hieracium lividorubens var. subnudulum Dal	ılst.
Hieracium paniculatum L.	Allegheny Hawkweed
Hieracium pilosella L.	Mouse-Ear Hawkweed
Hieracium pilosella var. niveum MuellArg.	
Hieracium pilosella var. pilosella	
Hieracium piloselloides Vill.	Tall Hawkweed
Syn. Hieracium florentinum All.	
Hieracium plicatum Lindb.	Boreal Hawkweed
Hieracium praealtum Vill. ex Gochnat Kingdevil	
Hieracium praealtum var. decipiens W.D.J. Koch	
Hieracium prenanthoides Vill.	Prenanth Hawkweed
Syn. Hieracium amitsokense (Almquist) Dahlst.	
Syn. Hieracium ivigtutense (Almquist) Om.	
Syn. Hieracium scholanderi Om.	
Syn. Hieracium sylowii Om.	
Syn. Hieracium sylowii var. norwagorum Om.	
Hieracium pringlei Gray	Pringle's Hawkweed

Hieracium robinsonii (Zahn) Fern.	Robinson's Hawkweed
Syn. Hieracium ungavense Lepage	
Hieracium rusbyi Greene	Rusby's Hawkweed
Hieracium sabaudum L.	New England Hawkweed
Syn. <i>Hieracium vagum</i> Jord.	
Hieracium scabrum Michx.	Rough Hawkweed
Hieracium scabrum var. intonsum Fern. & St. John	
Hieracium scabrum var. leucocaule Fern. & St. John	
Hieracium scabrum var. scabrum	
Hieracium scabrum var. tonsum Fern. & St. John	
<i>Hieracium scouleri</i> Hook.	Woollyweed
Hieracium scouleri var. nudicaule (Gray) Cronq.	
Syn. Hieracium cynoglossoides var. nudicaule Gray	7
Hieracium scouleri var. scouleri	
Syn. <i>Hieracium chapacanum</i> Zahn	
Syn. <i>Hieracium parryi</i> Zahn	
Hieracium scribneri Small	Scribner's Hawkweed
<i>Hieracium stelechodes</i> Om.	Dal Hawkweed
Hieracium ×stoloniflorum Waldst. & Kit. (pro sp.)	
Hieracium ×stoloniflorum var. cayouetteanum Lepage (pro	nm.)
Hieracium ×stoloniflorum var. laurentianum Lepage (pro n	m.)
Hieracium strictum Fries	Stiff Hawkweed
Hieracium tridentatum (Fries) Fries	Three-Tooth Hawkweed
Hieracium ×trigonophorum Oskarss. (pro sp.)	
Hieracium triste Willd. ex Spreng.	Woolly Hawkweed
Hieracium triste var. fulvum Hultén	
Hieracium triste var. triste	
Syn. Chlorocrepis tristis (Willd. ex Spreng.) A.& D	). Löve
Syn. Hieracium triste var. tritiforme Zahn	
Hieracium umbellatum L.	Narrow-Leaf Hawkweed
Syn. <i>Hieracium columbianum</i> Rydb.	
Syn. Hieracium scabriusculum Schwein.	
Syn. Hieracium scabriusculum var. columbianum (1	Rydb.) Lepage
Syn. Hieracium scabriusculum var. perhirsutum Le	page
Syn. Hieracium scabriusculum var. saximontanum	Lepage
Syn. Hieracium scabriusculum var. scabrum (Schw	ein.) Lepage
Hieracium venosum L.	Rattlesnake-Weed
Syn. Hieracium venosum var. nudicaule (Michx.) F	arw.
Hieracium wrightii (Gray) Robins. & Greenm.	Rough-Stem Hawkweed

**Appendix 2.** Plant species tested during *C. urbana* host specificity trials for North America. Asteraceae species are listed by family, subfamily, tribe, and subtribe, with species listed alphabetically within tribes. Common names, state distributions, growth habits, durations, and U.S. nativities are from the USDA-NRCS Plants Database (2014) and Canadian nativities from Flora of North America North of Mexico (1993+).

Family Subfamily Tribe Subtribe	Scientific Name	Common Name	U.S State Distribution Canada - Province Distribution	Growth Habit	Duration	USA Nativity	Test Category <sup>1</sup>
Asteraceae Cichorioideae Lactuceae Microseridinae	Agoseris grandiflora	bigflower agoseris	CA, ID, MT, NV, OR, UT, WA BC	Forb/herb	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae Catananchinae	Catananche caerulea	Cupid's Dart	Cultivated	Forb/herb	Perennial	Cultivated	3b Ornamental
Asteraceae Cichorioideae Lactuceae unassigned	Cichorium intybus	chicory	AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY AB, BC, MB, NB, NF, NS, ON, PE, PQ, SK	Forb/herb	Biennial Perennial	Introduced	3b Economic
Asteraceae Cichorioideae Lactuceae Crepidinae	Crepis atribarba	slender hawksbeard	CO, ID, MT, NE, NV, OR, UT, WA, WY AB, BC, SK	Forb/herb	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae	Crepis intermedia	limestone hawksbeard	AZ, CA, CO, ID, MT, NM, NV, OR, UT, WA, WY	Forb/herb	Perennial	Native	3b

Crepidinae			AB, BC, SK				
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium albertinum	houndstongue hawkweed	ID, MT, OR, WA, WY AB, BC	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium albiflorum	white hawkweed	AK, CA, CO, ID, MT, NV, OR, SD, UT, WA, WI, WY AB, BC, NT, PQ, SK	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium argutum	southern hawkweed	CA	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Pilosella aurantiaca	orange hawkweed	AK, AR, CA, CO, CT, FL, GA, IA, ID, IL, IN, MA, MD, ME, MI, MN, MT, NC, NH, NJ, NY, OH, OR, PA, RI, SD, TN, VA, VA, VT, WA, WI, WV, WY AB, BC, LB, MB, NB, NF, NS, ON, PE, PQ, SK	Forb/herb	Perennial	Introduced	1
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium bolanderi	Bolander's hawkweed	CA, OR	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Pilosella caespitosa	meadow hawkweed	CT, DC, GA, ID, IL, IN, KY, MA, MD, ME, MI, MN, MT, NC, NH, NJ, NY, OH, OR, PA, RI, SC, TN, VA, VT, WA, WI, WV, WY BC, MB, NB, NF, NS, ON, PE, PQ	Forb/herb	Perennial	Introduced	1
Asteraceae Cichorioideae	Hieracium canadense	Canadian hawkweed	MI, ME	Forb/herb	Perennial	Native	2

Lactuceae Hieraciinae			AB, BC, LB, MB, NB, NF, NS, NT, ON, PE, PQ, SK, YT				
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium carneum	Huachuca hawkweed	AZ, NM, TX	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium fendleri	yellow hawkweed	AZ, CO, NM, TX, VT, WY	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium flagellare	large mouseeared	CT, DE, IN, MA, ME, MI, NJ, NH, NY, OH, PA, VA, VT, WY BC, NB, NS, PE, PQ	Forb/herb	Perennial	Introduced	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Pilosella floribunda	king devil	CT, ID, MA, MD, ME, MI, MN, MT, NH, NJ, NY, OH, RI, VA, VT, WA, WV, WY BC	Forb/herb	Perennial	Introduced	1
Asteraceae Cichorioideae Lactuceae Hieraciinae	Pilosella glomerata	queen-devil	WA, ID BC	Forb/herb	Perennial	Introduced	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium gracile	slender hawkweed	AK, CA, CO, ID, MT, NM, OR, UT, WA, WY AB, BC, NT, YT	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae	Hieracium greenei	Greene's hawkweed	CA, OR	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae	Hieracium gronovii	queen devil	AL, AR, CT, DC, DE, FL, GA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MO, MS,	Forb/herb	Perennial	Native	2

Hieraciinae			NC, NJ, NY, OH, OK, PA, RI, SC, TN, TX, VA, WY ON				
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium horridum	prickly hawkweed	CA, NV, OR	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium Iongiberbe	longbeard hawkweed	OR, WA	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium Iongipilum	hairy hawkweed	AR, IA, IL, IN, KS, KY, LA, MI, MN, MO, NE, OH, OK, TN, TX, WI ON	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium parryi	Scouler's woollyweed	CA, ID, MT, NV, OR, WA, WY	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Pilosella officinarum	mouseear hawkweed	CT, GA, MA, ME, MI, MN, NC, NH, NJ, NY, OH, OR, PA, RI, TN, VA, VT, WA BC, NB, NF, NS, ON, PQ	Forb/herb	Perennial	Introduced	1
Asteraceae Cichorioideae Lactuceae Hieraciinae	Pilosella piloselloides	yellow devil hawkweed	CT, DE, GA, IA, IL, IN, MA, ME, MI, MN, MT, NC, NH, NJ, NY, OH, PA, RI, VA, VT, WA BC, NB, NF, NS, ON, PE, PQ	Forb/herb	Perennial	Introduced	1
Asteraceae Cichorioideae Lactuceae	Hieracium scabrum	rough hawkweed	AR, CT, DC, DE, GA, IA, IL, IN, KY, MA, MD, ME, MI, MN, MO, NC, NH, NJ,	Forb/herb	Perennial	Native	2

Hieraciinae			NY, OH, OK, PA, RI, SC, TN, VA, VT, WI NB, NS, ON, PE, PQ				
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium scouleri var. albertinum	western hawkweed	ID, MT, OR, WA, WY BC	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium scouleri var. cynoglossoides	houndstongue hawkweed	CA, ID, OR, WA BC	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium scouleri var. scouleri	Scouler's woollyweed	CA, ID, MT, NV, OR, WA, WY	Forb/herb	Perennial	Native	2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Pilosella stoloniflora		Not Present in USA or Canada				2
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium umbellatum	narrowleaf hawkweed	AK, CO, IA, ID, IL, IN, MI, MN, MO, MT, ND, NE, NH, OR, SD, WA, WI, WV, WY	Forb/herb	Perennial	Native	2
			AB, BC, LB, MB, NB, NF, NS, NT, ON, PE, PQ, SK, YT				
Asteraceae Cichorioideae Lactuceae Hieraciinae	Hieracium venosum	rattlesnakeweed	AL, CT, DC, DE, FL, GA, IN, KY, MA, MD, ME, MI, MO, MS, NC, NH, NJ, NY, OH, PA, RI, SC, TN, VA, VT, WY ON	Forb/herb	Perennial	Native	2
Asteraceae	Hypochaeris	hairy catsear	AK, AL, AR, CA, CO, CT,	Forb/herb	Perennial	Introduced	3b

Cichorioideae Lactuceae Hypochaeridinae	radicata		DC, DE, FL, GA, HI, ID, IL, IN, KY, LA, MA, MD, ME, MI, MO, MS, MT, NC, NH, NM, NJ, NV, NY, OH, OR, PA, RI, SC, TN, TX, UT, VA, VT, WA, WI, WV BC, LB, NF				
Asteraceae Cichorioideae Lactuceae Microseridinae	Krigia biflora	twoflower dwarfdandelion	AL, AR, AZ, CO, CT, DE, GA, IA, IL, IN, KS, KY, MA, MD, MI, MN, MO, MS, NC, NJ, NM, NY, OH, OK, PA, TN, VA, WI, WV ON	Forb/herb	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae Lactucinae	Lactuca sativa	garden lettuce	AL, CA, DC, DE, ID, IL, IN, MA, ME, MI, MO, ND, NM, NY, OH, OK, OR, PA, WA, WV, Puerto Rico, Virgin Islands	Forb/herb	Annual Biennial Perennial	Introduced	3b Economic
Asteraceae Cichorioideae Lactuceae Lactucinae	Lactuca serriola	prickly lettuce	AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, HI, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY AB, BC, MB, NB, NS, ON, PE, PQ, SK	Forb/herb	Annual Biennial	Introduced	3b
Asteraceae Cichorioideae Lactuceae Lactucinae	Lactuca virosa	bitter lettuce	AL, CĂ, DC	Forb/herb	Annual Biennial	Introduced	3b

Asteraceae Cichorioideae Lactuceae Stephanomeriinae	Lygodesmia juncea	rush skeletonplant	AR, AZ, CO, IA, ID, IN, KS, MN, MO, MT, ND, NE, NM, NV, OK, OR, SD, TX, UT, WA	Forb/herb	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae Microseridinae	Microseris nutans	nodding microseris	AB, BC, MB, SK CA, CO, ID, MT, NV, OR, SD, UT, WA, WY AB, BC	Forb/herb	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae Lactucinae	Prenanthes racemosa	purple rattlesnakeroot	CO, IA, IL, IN, KY, ME, MI, MN, MO, MT, ND, NE, NJ, NY, OH, PA, SD, WI, WY AB, BC, MB, NB, NF, NS, ON, PE, PQ, SK	Forb/herb	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae Sonchinae	Sonchus asper	spiny sowthistle	AK, AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, HI, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY, Puerto Rico AB, BC, LB, MB, NB, NF, NS, ON, PE, PQ, SK, YT	Forb/herb	Annual	Introduced	3b
Asteraceae Cichorioideae Lactuceae Sonchinae	Sonchus arvensis	field sowthistle	AK, CA, CO, CT, DC, DE, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OR, PA, RI, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	Forb/herb	Perennial	Introduced	3b

			AB, BC, MB, NB, NF, NS, NT, ON, PE, PQ, SK, YT				
Asteraceae Cichorioideae Lactuceae Sonchinae	Sonchus oleraceus	common sowthistle	AK, AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, HI, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY, Puerto Rico, Virgin Islands AB, BC, MB, NB, NF, NS, ON, PE, PQ, SK, YT	Forb/herb	Annual	Introduced	3b
Asteraceae Cichorioideae Lactuceae Stephanomeriinae	Stephanomeria cichoriacea	chicoryleaf wirelettuce	CA	Forb/herb Subshrub	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae Stephanomeriinae	Stephanomeria minor	lesser wirelettuce	AZ, CA, CO, ID, MT, ND, NM, NV, OR, TX, UT, WA, WY BC, SK	Forb/herb Subshrub	Perennial	Native	3b
Asteraceae Cichorioideae Lactuceae Crepidinae	Taraxacum officinale	common dandelion	AK, AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA,HI, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY, Puerto Rico	Forb/herb	Perennial	Native Introduced	3b
			AB, BC, LB, MB, NB, NF, NS, NT, NU, ON, PE, PQ,				

			SK, YT				
Asteraceae Cichorioideae Cardueae Centaureinae	Acroptilon repens	hardheads	AR, AZ, CA, CO, IA, ID, IL, IN, KS, KY, MI, MN, MO, MT, ND, NE, NM, NV, OH, OK, OR, SD, TX, UT, WA, WI, WY AB, BC, MB, ON, SK	Forb/herb	Perennial	Introduced	3с
Asteraceae Cichorioideae Cardueae Carduinae	Cirsium arvense	Canada thistle	AK, AL, AR, AZ, CA, CO, CT, DC, DE, IA, ID, IL, IN, KS, KY, MA, MD, ME, MI, MN, MO, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OR, PA, RI, SD, TN, UT, VA, VT, WA, WI, WV, WY AB, BC, MB, NB, NF, NS, NT, ON, PE, PQ, SK, YT	Forb/herb	Perennial	Introduced	3c
Asteraceae Cichorioideae Cardueae Carduinae	Cynara scolymus	globe artichoke	AZ, CA	Forb/herb	Perennial	Introduced	3c
Asteraceae Asteroideae Anthemideae Artemisiinae	Artemisia dracunculus	tarragon	AK, AZ, CA, CO, IA, ID, IL, KS, MA, MN, MO, MT, ND, NE, NM, NV, NY, OK, OR, SD, TX, UT, WA, WI, WY AB, BC, MB, SK, YT	Forb/herb	Perennial	Native	3d Economic
Asteraceae Asteroideae Anthemideae Chrysantheminae	Chrysanthemum cinerariifolium	pyrethrum	Cultivated	Forb/herb	Perennial	Cultivated	3d
Asteraceae Asteroideae	Eupatoriadelphus (Eupatorium)	spotted trumpetweed	CT,GA, IA, IL, IN, KY, MA, MD, ME, MI, MN, NC, NH,	Forb/herb	Perennial	Native	3d

Eupatorieae Eupatoriinae	maculatum		NJ, NY, OH, PA, RI, TN, VA, VT, WI, WY MB, NB, NS, ON, PE, PQ				
Asteraceae Asteroideae Helenieae Pectidinae	Tagetes erecta	Aztec marigold	AR, CA, CT, FL, KY, LA, MA, MD, MO, NC, NY, OH, OK, PA, SC, UT, VA, Puerto Rico, Virgin Islands	Forb/herb	Annual	Introduced	3d Ornamenta I
Asteraceae Asteroideae Heliantheae Coreopsidinae	<i>Dahlia</i> sp.	dahlia	Cultivated	Forb/herb Subshrub	Perennial	Cultivated	3d Ornamenta I
Asteraceae Asteroideae Heliantheae Helianthinae	Helianthus annuus	common sunflower	AK, AL, AR, AZ, CA, CO, CT, DC, DE, FL, GA, HI, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY, Puerto Rico AB, BC, MB, NB, NF, NS, NT, ON, PE, PQ, SK	Forb/herb	Annual	Native	3d Ornamenta I Economic
Asteraceae Asteroideae Vernonieae Vernoniinae	Stokesia laevis	Stokes' Aster	AL, FL, GA, LA, MS, NC, SC	Forb/herb	Perennial	Native	3d Ornamenta I
Asteraceae Gnaphalioideae Gnaphalieae Cassiniinae	Antennaria dioica	stoloniferous pussytoes	АК	Forb/herb	Perennial	Native	3d
Asteraceae Gnaphalioideae Gnaphalieae Gnaphaliinae	Gnaphalium audax		Not Present in USA or Canada				

Liliaceae	Allium cepa	garden onion	AR, CA, CT, IL, IN, KS, KY, LA, MA, ME, MI, MO, MS, MT, NC, NH, NY, OH, OR, PA, SC, TX, VT, WI	Forb/herb	Perennial	Introduced	5 Economic
Asclepiadaceae	Asclepias syriaca	common milkweed	AL, AR, CT, DC, DE, GA, IA, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NH, NE, NJ, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, VA, VT, WI, WV	Forb/herb	Perennial	Native	5
Chenopodiaceae	Beta vulgaris	common beet	AL, CA, CT, MA, ME, MI, MO, MT, NC, NH, NY, OR, PA, SC, TX, UT, VA, WV	Forb/herb	Annual Biennial	Introduced	5 Economic
Lamiaceae	Monarda fistulosa	wild bergamot	AL, AR, AZ, CO, CT, DC, DE, GA, IA, ID, IL, IN, KS, KY, LA, MA, MD, ME, MI, MN, MO, MS, MT, NC, ND, NE, NH, NJ, NM, NV, NY, OH, OK, OR, PA, RI, SC, SD, TN, TX, UT, VA, VT, WA, WI, WV, WY	Forb/herb Subshrub	Perennial	Native	5
Papaveraceae	Papaver nudicale	Iceland poppy	Cultivated AK YT	Forb/herb	Biennial Perennial	Cultivated	5 Ornamenta I
Apiaceae	Petroselinum crispum	parsley	AR, CA, CT, FL, GA, HI, IA, ID, KS, LA, MA, MD, MI, MS, MT, NC, NV, NY, OH, PA, RI, TX, SC, UT, WA, Puerto Rico	Forb/herb	Annual Biennial	Introduced	5 Economic
Fabaceae	Phaseolus vulgaris	kidney bean	CT, DC, FL, KY, MA, ME, MI, MO, MT, NC, NH, NY, OH, PA, RI, SC, UT, VA, WY Puerto Rico, Virgin Islands	Forb/herb	Annual	Introduced	5 Economic

Fabaceae	Pisum sativum	garden pea	CA, CT, FL, IL, KS, KY, LA, MA, ME, MI, MO, MS, NC, NH, NY, OK, OR, PA, SC, UT, VA, WA	Forb/herb Vine	Annual	Introduced	5 Economic
Fabaceae	Trifolium repens	white clover	Throughout the USA and Canada	Forb/herb	Perennial	Introduced	5 Economic
Fabaceae	Vicia faba	horsebean	CA, CT, DC, MA, MD, ME, MT, NY, OR, PA, VA, VT, WA	Forb/herb Vine	Annual	Introduced	5 Economic

<sup>1</sup>Test Categories: 1. Genetic types of target weed; 2. Species of the same (or closely related) genus; 3. Species in the same family as the target weed (3a. Plants in same subfamily; and 3d. Plants in same family); 4. Threatened and endangered species in the same family; 5. Species in other families.

Appendix 3. Host-specificity testing: experimental methodology, analysis and results

Hawkweeds are an important economic and environmental problem in both New Zealand and North America. Initial host-specificity tests for *C. urbana* were conducted by Centre for Agriculture and Biosciences International (CABI) in Switzerland and Landcare Research, New Zealand (summarized in Grosskopf et al., 2002). An application for approval to import and release *C. urbana* in New Zealand was submitted to the Environmental Risk Management Authority (ERMA) in November 2000 and release was approved in 2001. Unlike New Zealand, North America has numerous, native hawkweeds. Therefore a separate testing program was conducted at CABI to address these native hawkweed concerns. Test results from the New Zealand and North American projects are both presented here.

All host-specificity tests were conducted in the laboratory or common garden at CABI in Delémont, Switzerland (47°21'N, 7°22'E) and in the quarantine facility at Landcare Research Ltd., Lincoln, New Zealand.

#### Test plant list for New Zealand

A basic test plant list (see Table 3.1) was compiled (Landcare Research Ltd, Lincoln, New Zealand) following the procedures outlined by Wapshere (1974) and Harris and Zwölfer (1968). Plant species known as hosts of other *Cheilosia* species. (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. Most plants belonged to the family Asteraceae, and in particular the tribe Lactuceae. All hawkweeds adventive to New Zealand were included except *H. pollichiae* Sch. Bip., for which no seeds were available. Many of these test species have relevancy to the North American testing based on phylogenetic relationships among plant taxa used, and with some of the species tested having co-generic representation in North America.

Plant species <sup><i>a</i>,<i>b</i></sup>	Category <sup>c,d</sup>	No. replicates
Asteraceae		Tophones
Tribe: Lactuceae		
Pilosella officinarum Vaill. EUR <sup>e</sup>	Target weed	41
<i>P. officinarum</i> $NZ^a$	Target weed	5
P. officinarum NZ	Target weed	9
<i>P. aurantiaca</i> L.	Target weed	9
<i>P. caespitosa</i> Dumort. EUR	Target weed	7
P. caespitosa NZ	Target weed	7
P. caespitosa US	Target weed	6
<i>P. piloselloides</i> ssp. <i>praealta</i> Vill. ex Gnochat	Target weed	11
<i>P.</i> x <i>stoloniflora</i> (Waldst. & Kit.) F. W. Schultz & Sch. Bip.	Naturalized <sup>c</sup>	10
Hieracium argillaceum Jordan	Naturalized	8
<i>H. lepidulum</i> (Stenström) Omang	Target weed	9
H. murorum L.	Naturalized <sup>c</sup>	8
H. sabaudum L.	Naturalized	8
Cichorium intybus L.	Cultivated	9
Embergeria grandifolia (Kirk) Boulos	Native	8
Hypochoeris radicata L.	Naturalized	8
Kirkianella novae-zelandiae (Hook. f.) Allan <sup>a</sup>	Native	5
Lactuca sativa L.	Cultivated	6
Leontodon taraxacoides (Vill.) Mérat	Naturalized	8
Microseris scapigera (Sol. ex A. Cunn.) Sch. Bip.	Native	8
Picris hieracioides L.	Native	8
Sonchus kirkii Hamlin	Native	8 7
Sonchus kirki Hammi Sonchus oleraceus L.	Naturalized	7
	Naturalized	6
Taraxacum officinale Weber	Cultivated	0 7
<i>Tragopogon porrifolius</i> L. <u>Tribe: Anthemideae</u>	Cultivated	1
Artemisia dracunculus L.	Cultivated	10
	Cultivated	
Chrysanthemum cinerariifolium (Trev.) Vis. Tripleurospermum perforatum (Mérat) Laínz		7 6
	Cheilosia sp.	0
<u>Tribe: Astereae</u>	NI-4	C
<i>Olearia avicenniaefolia</i> (Raoul) Hook. f. Tribe: Heliantheae	Native	6
	Culture	C
Helianthus annuus L.	Cultivated	6
Tribe: Inuleae		E
Helichrysum bellidioides (Forster f.) Willd. <sup>a</sup>	Native	5
Helichrysum bracteatum (Vent.) Andrews	Cultivated	8
Gnaphalium audax D. Drury	Native	5
Raoulia hookeri Allan <sup>a</sup>	Native	5
<u>Tribe: Senecioneae</u>		7
Senecio monroi Hook. f. (= Brachyglottis monroi)	Native	6
Petasites paradoxus (Retz.) Baumg.	<i>Cheilosia</i> sp.	6
Senecio jacobaea L.	<i>Cheilosia</i> sp.	9
Tribe: Cardueae		-
Carduus acanthoides L.	<i>Cheilosia</i> sp.	6
Carduus nutans L.	<i>Cheilosia</i> sp.	6

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

### Table 3.1 (continued)

Plant species <sup><i>a,b</i></sup>	Category <sup>c,d</sup>	No. replicates
Carthamus tinctorius L.	Cultivated	6
Cirsium palustre (L.) Scop.	Cheilosia sp.	6
Cirsium vulgare (Savi) Ten.	Cheilosia sp.	6
Cynara scolymus L.	Cultivated	8
Apiaceae		
Aegopodium podagraria L.	Cheilosia sp.	6
Petroselinum crispum (Miller) A. W. Hill	Cultivated	6
Boraginaceae		
Cynoglossum officinale L.	<i>Cheilosia</i> sp.	5
Brassicaceae	_	
Brassica oleracea L.	Cultivated	6
Cannaceae		
Canna indica L.	Cultivated	7
Crassulaceae		
Sempervivum sp. L.	<i>Cheilosia</i> sp.	6
Caryophyllaceae	-	
Dianthus barbatus L.	Cultivated	6
Cyperaceae		
Carex testacea Boott	Native	6
Ericaceae		
Gaultheria crassa Allan <sup>a</sup>	Native	5
Fabaceae		
Trifolium repens L.	Cultivated	6
Iridaceae		
Gladiolus communis L.	Cultivated	6
Lamiaceae		-
Mentha sp. L.	Cultivated	6
Liliaceae		-
<i>Allium cepa</i> L.	Cultivated	7
Malvaceae		,
Althea rosea L.	Cultivated	6
Myrtaceae		Ũ
Leptospermum scoparium J. R. & G. Forst.	Native	6
Oleaceae		Ŭ
Olea europaea L.	Cultivated	6
Poaceae	Cultitudu	v
<i>Festuca novae-zelandiae</i> J. B. Armstr.	Native	6
Poa colensoi Hook. f.	Native	6
Agrostis tenuis Sibth.	Cultivated	6
Polygonaceae	Cultivated	0
Rumex acetosella L.		6
Primulaceae		0
Primula sp. L.	<i>Cheilosia</i> sp.	5
Proteaceae	Chenosia sp.	5
Knightia excelsa R. Br. <sup>a</sup>	Native	5
Ranunculaceae	manve	5
	Nativo	5 <sup>f</sup>
Clematis forsteri Gmel.	Native	<i>У</i>

#### Table 3.1 (continued)

Plant species <sup><i>a,b</i></sup>	Category <sup>c,d</sup>	No. replicates
Clematis paniculata Gmel.	Native	1 <sup>f</sup>
Ranunculus repens L.	<i>Cheilosia</i> sp.	6
Rhamnaceae		
Discaria toumatou Raoul	Native	2
D. toumatou <sup>a</sup>	Native	5
Rosaceae		
Filipendula ulmaria Maxim.	<i>Cheilosia</i> sp.	6
Rutaceae		
Citrus sp.	Culitvated	6
Scrophulariaceae		
Antirrhinum majus L.	Cultivated	9
Theaceae		
Camellia japonica L.	Cultivated	6
Solanaceae		
Lycopersicon esculentum Miller	Cultivated	6
Urticaceae		
Urtica dioica L.		6
Vitaceae		
Vitis vinifera L.	Cultivated	8

<sup>a</sup> Plant species tested in quarantine facilities of Landcare Research, New Zealand.

<sup>b</sup> 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.

<sup>c</sup> Hawkweed species not regarded as a noxious weed in New Zealand but attack by Cheilosia species is desired.

<sup>d</sup> "Cheilosia sp." refers to recorded host plants of other Cheilosia species.

<sup>e</sup> EUR, NZ, and USA refer to the origin of the seeds from which the plants were grown, i.e. Europe, New Zealand and USA.

<sup>f</sup>Fifteen instead of five neonate larvae were transferred per replicate.

#### Test plant list for North America

Ornamentals and crop plants within the Asteraceae and from other families had been tested prior to release of *C. urbana* and *C. psilophthalma* in New Zealand. For this reason, the North American test plant list focused on adding: (i) target weeds from the genus *Pilosella* to explore whether *C. urbana* can develop on these plants, (ii) *Hieracium* species in the subgenera *Hieracium* and *Chionoracium* which are native to North America, and (iii) native and economic plants in the family Asteraceae, especially the Lactuceae tribe and plants with biochemical similarities with the target weeds (Wilson and Birdsall, 2001) (Table 3.2). Additional plant species were tested due to their availability, although they were not part of the list. There are no threatened and endangered species or species at risk in the genus *Hieracium* in North America.

	Test plant species <sup>a</sup>	Origin <sup>b</sup>	Status
Asteraceae			
Tribe Lactuceae			
Genus: Pilosella	P. aurantiaca L. ID	Ι	Target weed
	P. aurantiaca L. MT	Ι	Target weed
	P. caespitosa Dumort. ID	Ι	Target weed
	P. floribunda Wimm. et Grab. MI	Ι	Target weed
	P. glomerata Froel. BC	Ι	Target weed
	P. glomerata EWA	Ι	Target weed
	P. officinarum Vaill.	Ι	Target weed
	P. piloselloides Vill. MT	Ι	Target weed
Genus: Hieracium			-
Subgenus: Hieracium	H. canadense Michx.	Ν	Native
5	H. umbellatum L.	Ν	Native
Subgenus: Chionoracium	H. albiflorum Hook.	Ν	Native
C	<i>H. argutum</i> Nutt.	Ν	Native
	H. bolanderi Gray	Ν	Native
	H. carneum Greene	Ν	Native
	H. fendleri Schultz-Bip.	N	Native
	H. gracile Hook.	N	Native
	H. gronovii L.	N	Native
	<i>H. longiberbe</i> 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 I	Native
	Crepis intermedia Gray	N	Native
	-	N	Native
	Krigia biflora (Walt) Blake Lygodesmia juncea (Pursh) D. Don	N	Native
		N	Native
	Microseris nutans (Hook.) Schultz-Bip.		
	Microseris troximoides (Gray) Greene	N N	Native
	Prenanthes sagittata (Gray) A. Nels.		Native
	Stephanomeria tenuifolia (Raf.) Hall	N	Native
	<i>Taraxacum laevigatum</i> (Willd.) DC <sup>c</sup>	I	Same tribe
	Taraxacum lyratum (Ledeb.) $DC^c$	N	Native
T 1 A (1 1 1	Tragopogon dubius Scop. <sup>c</sup>	I	Same tribe
Tribe: Anthemideae	Artemisia dracunculus L. <sup>c</sup>	N	Cultivated
Tribe: Arctoteae	<i>Gazania rigens</i> (L.) Gaertn. <sup><i>c</i></sup>	I	Ornamental
Tribe: Astereae	Aster laevis L. <sup>c</sup>	l	Ornamental
Tribe: Calenduleae	Calendula officinalis L. <sup>c</sup>	l	Ornamental
Tribe: Cardueae	Cirsium undulatum (Nutt.) Sprengel	N	Native
Tribe: Eupatorieae	Eupatorium maculatum L. <sup>c</sup>	N	Native
Tribe: Gnaphalieae	Antennaria dioica (L.) Gaertn. <sup>c</sup>	N	Native
Tribe: Helenieae	Tagetes erecta L. <sup>c</sup>	I	Ornamental
Tribe: Inuleae	Inula helenium L. <sup>c</sup>	Ι	
Tribe: Mutisieae	<i>Gerbera jamesonii</i> Bolus ex Hooker f. <sup>c</sup>	Ι	Ornamental
Tribe: Vernonieae	Stokesia laevis Greene <sup>c</sup>	Ν	Native

<b>Table 3.2.</b> Su	upplemental test	plant list used for screening Cheilosia urbana for North America	a.
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<sup>a</sup> States or provinces where the test plant seeds originate from, ID: Idaho, BC: British Columbia, EWA: East

Washington, MT: Montana; <sup>*b*</sup> origin of the test plants: I = introduced into North America, N = native to North America; <sup>*c*</sup> plants tested in addition to the actual test plant list.

#### General considerations on test design

In all tests, potted plant material was used to ensure that test conditions were as optimal as possible for this root-mining insect. Because the researchers were not able to get mating in captivity, gravid females were caught in the field when they oviposited on hawkweed plants. When kept in small vials they readily produced fertile eggs.

Because the females do not discriminate between suitable and unsuitable host plants under confinement, no-choice larval transfer tests were carried out to determine the fly's fundamental host-range (plants on which the larvae can develop under optimal conditions). It should be noted that these tests give extremely conservative test results and should be complemented by less restrictive tests to reflect the ecological host-range (plants which will be chosen by the female in the field and on which the larvae can develop).

During no-choice tests, only a restricted number of plant species supported the development to mature larva or adult. To determine whether these non-target species would be accepted for oviposition by *C. urbana* in the presence of one of the target weeds, they were subsequently exposed in single-choice oviposition tests. Although the target weeds were generally highly preferred, nearly all test plants received eggs under these conditions. Therefore, all subsequent tests were conducted under open-field conditions to simulate as much as possible natural oviposition behavior of females.

During the flight period of *C. urbana*, plants are still in the rosette stage and therefore rosettes were offered in tests. The target weeds (controls) and test plants matched in phenology and size as much as possible.

#### No-choice larval transfer tests

#### No-choice larval transfer tests for New Zealand

No-choice larval transfer tests were carried out at CABI in Delémont, Switzerland, and/or at the quarantine facility at Landcare Research Ltd., Lincoln, New Zealand, with test plant species not available at CABI.

**Methods.** All no-choice tests were carried out with neonate larvae obtained from eggs laid by gravid, field-collected *C. urbana* females. Field-collected, gravid *C. urbana* females were kept individually in vials provided with honey water and a *Bellis perennis* flower head as a pollen source. Eggs laid in the tubes were transferred into Petri-dishes lined with moist filter paper and checked three to four times per day for hatching larvae. Batches of five freshly hatched larvae were transferred onto each potted plant. Because the larvae feed on the roots in the soil, the larvae were transferred onto the stem of the plant as close as possible to the soil's surface. All pots were covered with gauze bags and kept in a polythene-covered garden tunnel for up to seven days to protect the larvae from extreme weather conditions, such as heavy rain fall. All plants were then embedded in a garden bed throughout the summer and watered if necessary. All garden beds in the CABI garden containing hawkweed 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*. The soil of all pots was sieved and carefully checked for *C. urbana* larvae

between mid-August and the end of September. Immature stages of the fly were transferred into small vials filled with humid soil to rear them to adulthood.

**Results.** Of the 72 plant species tested, mature larvae of *C. urbana* were only found on plants within the genera *Pilosella* and *Hieracium* (Subgenus *Hieracium*). None of the larvae survived on *H. murorum*. The survival rate of *C. urbana* larvae on other hawkweed species differed significantly ( $F_{11, 121} = 6.97$ , P < 0.001) and ranged from 0.6 larvae (12.5 percent survival) per replicate on *H. argillaceum* to 4.3 larvae (86.7 percent survival) on *P. caespitosa* of U.S. origin (Table 3.3). Fewer larvae survived on *P. × stoloniflora* and *H. argillaceum*, than on the target plants. *Cheilosia urbana* adults emerged from all hawkweed species on which larvae had been found. Survival from neonate larva to adult varied between hawkweed species ( $F_{11, 121} = 5.76$ , *P* < 0.001) and ranged from 2.5 percent on *H. argillaceum* to 80.0 percent on U.S. *P. caespitosa*. Fewer adults emerged from *H. argillaceum* and *P. × stoloniflora* (a hybrid between *P. aurantiaca* L. and *P. officinarum*), than most of the target weeds (Table 3.3).

Plant species <sup>a</sup>	No.	No. L1	Mature larvae		Weigh	nt of retrieved	Larvae devel	loping
	plants	transferred <sup>b</sup>	recovere	recovered Larvae		to adult		
			Mean $\pm$ SE	%°	п	Mean $\pm$ SE	Mean $\pm$ SE	%°
Genus Pilosella								
P. aurantiaca	9	45	$2.6\pm0.56$	51.1	23	$24.6\pm0.64$	$2.2\pm0.55$	44.4
P. caespitosa EUR	7	35	$2.9\pm0.55$	57.1	20	$19.5\pm1.22$	$2.3\pm0.42$	45.7
P. caespitosa NZ	7	35	$2.9\pm0.34$	57.1	20	$16.0\pm0.55$	$2.1\pm0.51$	42.9
P. caespitosa USA	6	30	$4.3\pm0.33$	86.7	26	$23.2\pm0.65$	$4.0\pm0.37$	80.0
P. officinarum EUR	41	205	$3.1\pm0.20$	62.0	127	$21.7\pm0.52$	$2.3\pm0.24$	46.8
P. officinarum NZ	9	45	$2.8\pm0.47$	55.6	25	$19.5\pm0.96$	$2.2\pm0.57$	44.4
P. piloselloides	11	55	$2.5\pm0.47$	50.9	28	$21.6\pm1.18$	$1.9\pm0.46$	38.2
P. ×stoloniflora	10	50	$1.4\pm0.45$	28.0	14	$16.5\pm0.77$	$0.6\pm0.31$	12.0
Genus Hieracium								
Subgenus Hieracium								
H. argillaceum	8	40	$0.6\pm0.50$	12.5	5	$15.1\pm0.70$	$0.1\pm0.13$	2.5
H. lepidulum	9	45	$2\pm0.41$	40.0	18	$20.0\pm1.25$	$1.2\pm0.32$	24.4
H. murorum	8	40	0	0	-	-	0	0
H. sabaudum	8	40	$1.9\pm0.52$	37.5	15	$20.3\pm0.92$	$1.5\pm0.46$	30.0

**Table 3.3.** Summary of no-choice larval transfer tests with *Cheilosia urbana* conducted during 1997-1999 for New Zealand. Only results for the genera *Pilosella* and *Hieracium* are shown, because no development occurred on any of the other 64 plant species tested.

<sup>a</sup> Origin of plants: EUR: Europe, NZ: New Zealand, USA: USA: United States of America.

<sup>b</sup> Five newly hatched *C. urbana* larvae were transferred onto each plant.

<sup>c</sup> Percentage: number of mature larvae retrieved or adults emerged, divided by the total number of neonate larvae transferred.

#### No-choice larval transfer tests for North America

No-choice larval transfer tests for North America were carried out at CABI in Delémont, Switzerland.

**Methods.** Tests were carried out between 2000 and 2008. The experimental set up was similar to the testing for New Zealand. In order to augment the number of larvae tested and render results even more reliable, seven larvae were transferred instead of five.

**Results.** Neonate larvae developed to adulthood on all *Pilosella* species tested, i.e. on all target weeds, ranging from 7.1 percent on the European population of *P. officinarum* to 52.4 percent on *P. aurantiaca* from Idaho (Table 3.4). Larval *C. urbana* also completely developed and emerged as adults on six out of 17 native North American *Hieracium* species. Except for *H. canadense,* survival rates were much lower than on the target weeds; ranging from 0.9 percent for *H. longipilum* to 6.1 percent for *H. scabrum*. Hence, the fundamental host range of *C. urbana* includes species in the genera *Pilosella* and *Hieracium* sub-genera *Hieracium* and *Chionoracium*. None of the plant species outside these two genera supported development and were therefore excluded as host plants and further testing.

#### Single-choice oviposition tests

To investigate whether *C. urbana* shows an oviposition preference when offered test plants and target weeds simultaneously, single-choice oviposition tests were conducted with all native North American *Hieracium* species available and four additional species that did not support larval development.

**Methods.** Field-collected *C. urbana* females were used in 368 single-choice oviposition tests carried out between 2000 and 2009. For each test, a female was transferred onto a clay pot (18 cm diameter) covered with a gauze bag and containing a plant of *P. caespitosa* ID control) and one plant of a test species. Females were left on plants for approximately 8 hours during the day, after which they were retrieved and re-used in another set of tests. The pots were kept on a window bench in the laboratory. Afterwards, the number of eggs laid on each plant was counted by careful visual inspection and removal of the eggs.

**Results.** In total, 175 of the *C. urbana* females laid eggs in the tests. Females laid eggs on all hawkweed species offered, but the oviposition ratio (test plant:target plant) varied considerably among species. In 106 of the replications no eggs were found on either plant species. *Pilosella aurantiaca, P. floribunda,* and *P. piloselloides* were preferred over *P. caespitosa* for oviposition. *Pilosella caespitosa* was nevertheless preferred over most native *Hieracium* species (oviposition ratios < 1) (Table 3.5). *Hieracium carneum* was the only native North American *Hieracium* species which had an oviposition ratio close to 1.0 indicating that neither the control nor the test plant was preferred. *Hieracium gracile* had the lowest oviposition ratio of the native hawkweeds, close to 0, followed by *Hieracium scouleri* with 0.05 and *H. umbellatum* with 0.06. No or few eggs were laid on four test species in other genera.

Plant species <sup>a</sup>	No. plants <sup>b</sup>	No. L1 transferred <sup>c</sup>	Mature larvae re	covered	Larvae devel to adult	
	1		Mean ± SE	%	Mean ± SE	%
ASTERACEAE						
Tribe: Lactuceae						
Genus: Pilosella						
P. aurantiaca ID	9	63	$3.9\pm0.3$	55.6	$3.7\pm0.3$	52.4
<i>P. aurantiaca</i> MT	8	56	$4.3\pm0.6$	60.7	$3.0\pm0.8$	42.9
P. caespitosa ID	95	665	$1.5 \pm 0.2$	21.7	$0.8\pm0.1$	12.0
P. floribunda USA	9	63	$1.8 \pm 0.3$	25.4	$1.2 \pm 0.3$	17.5
P. glomerata BC	18	126	$0.9\pm0.3$	12.7	$0.7\pm0.3$	10.3
P. glomerata EWA	9	63	$4.1\pm0.5$	58.7	$2.1 \pm 0.7$	30.2
P. officinarum EUR	6	42	$2.3\pm0.5$	33.3	$0.5\pm0.3$	7.1
P. officinarum USA	12	84	$1.9\pm0.4$	27.4	$1.2\pm0.3$	21.4
P. piloselloides USA	9	63	$3.1\pm0.5$	44.4	$2.1 \pm 0.4$	30.2
Genus: Hieracium						
Subgenus: Hieracium						
*H. canadense	12	84	$1.2 \pm 0.3$	16.7	$0.6\pm0.3$	8.3
H. lachenalii	2	14	0.0	-	-	-
H. murorum	12	84	0.0	-	-	-
*H. umbellatum	12	84	$0.3\pm0.4$	4.8	$0.1 \pm 0.1$	1.2
Subgenus: Chionoracium						
*H. albiflorum	3	21	0.0	-	-	-
*H. argutum	5	35	0.0	-	-	-
*H. bolanderi	5	35	0.0	-	-	-
*H. carneum	22	154	$0.1\pm0$	0.6	-	-
*H. fendleri	12	84	0.0			-
*H. gracile	1	7	0.0			-
*H. greenei	7	49	0.0			-
*H. gronovii	12	84	$0.3\pm0.2$	3.6	$0.3\pm0.2$	3.6
*H. horridum	5	35	0.0	-	-	-
*H. longiberbe	2	14	0.0	-	-	-
*H. longipilum	17	119	$0.2\pm0.1$	2.5	$0.1 \pm 0.1$	0.9

Table 3.4. Summary of no-choice larval transfer tests with *Cheilosia urbana* conducted during 2000–2008 for North America.

Plant species <sup>a</sup>	No. plants <sup>b</sup>	No. L1 transferred <sup>c</sup>	Mature larvae re	covered	Larvae deve to adu	
			Mean ± SE	%	Mean ± SE	_%
*H. parryi	6	42	0.0			-
*H. scabrum	7	49	$0.4\pm0.3$	6.1	$0.4\pm0.3$	6.1
*H. scouleri	26	182	0.0	-	-	-
*H. venosum	6	42	$0.3\pm0.2$	4.8	$0.2\pm0.2$	2.4
Agoseris grandiflora	8	56	0.0	-	-	-
Antennaria dioica	2	14	0.0	-	-	-
Catananche caerula	9	63	0.0	-	-	-
Crepis atribarba	1	7	0.0	-	-	-
Crepis intermedia	8	56	0.0	-	-	-
Hypochaeris radicata	12	84	0.0	-	-	-
Krigia biflora	10	70	0.0	-	-	-
Lactuca serriola	6	42	0.0	-	-	-
L. sativa	3	21	0.0	-	-	-
L. virosa	6	42	0.0	-	-	-
Lygodesmia juncea	5	21	0.0	-	-	-
Microseris nutans	3	21	0.0	-	-	-
M. troximoides	8	56	0.0	-	-	-
Prenanthes sagittata	9	63	0.0	-	-	-
Sonchus asper	6	42	0.0	-	-	-
S. arvensis	12	84	0.0	-	-	-
Stephanomeria cichoriacea	4	28	0.0	-	-	-
S. virgata	9	63	0.0	-	-	-
Taraxacum laevigatum	10	70	0.0	-	-	-
T. lyratum	2	14	0.0	-	-	-
Tragopogon dubius USA	7	49	0.0	-	-	-
Other Tribes			0.0		-	-
Gazania splendens	9	63	0.0	-	-	-
Aster chinensis	6	42	0.0	-	-	-
A. laevis	6	42	0.0	-	-	-
Calendula officinalis	6	42	0.0	-	-	-

Table 3.4. Continued: Summary of no-choice larval transfer tests with *Cheilosia urbana* conducted during 2000–2008 for North America

Plant species <sup>a</sup>	No. plants <sup>b</sup>	No. L1 transferred <sup>c</sup>	Mature larvae recovered		Larvae dev to adu	
	plants	unisterrea	Mean ± SE	%	Mean ± SE	%
Carthamus tinctorius	3	21	0.0			
Cirsium undulatum	11	77	0.0	-	-	-
Eupatorium maculatum	6	42	0.0	-	-	-
Antennaria dioica	8	56	0.0	-	-	-
Tagetes erecta	6	42	0.0	-	-	-
Dahlia sp.	3	21	0.0	-	-	-
Inula helenium	9	63	0.0	-	-	-
Gerbera jamesonii	6	42	0.0	-	-	-
Stokesia laevis	8	56	0.0	-	-	-
OTHER FAMILIES			0.0			
Asclepias syriaca	9	63	0.0	-	-	-
Beta vulgaris	6	42	0.0	-	-	-
Monarda fistulosa	9	63	0.0	-	-	-
Papaver nudicaule	9	63	0.0	-	-	-
Phaseolus vulgaris	6	42	0.0	-	-	-
Pisum sativum	6	42	0.0	-	-	-
Vicia faba	6	42	0.0	-	-	-

Table 3.4. Continued: Summary of no-choice larval transfer tests with *Cheilosia urbana* conducted during 2000–2008 for North America

<sup>a</sup> Origin of plants: BC: British Columbia; EWA East Washington; EUR: Europe; ID: Idaho; MT: Montana.

<sup>b</sup> Results refer to valid replicates only, i.e. plants that were still alive upon evaluation of the tests or pots in which larvae were found.

<sup>c</sup> Five newly hatched *C. urbana* larvae (L1) were transferred onto each plant.

\*Hawkweed species indigenous to North America.

Test plant <sup>a</sup>	No.	No	No. eggs		eggs	Oviposition ratio <sup>b</sup>
	repl.	Test	Control	Test	Control	(test/control)
Pilosella aurantiaca USA	10	143	71	66.8	33.2	2.01
P. floribunda USA	12	118	33	78.1	21.9	3.58
P. piloselloides USA	15	217	117	65.0	35.0	1.85
Subgenus Hieracium						
*H. canadense	18	85	212	28.6	71.4	0.40
*H. umbellatum	21	14	226	5.8	94.2	0.06
Subgenus Chionoracium						
<sup>*</sup> H. albiflorum	13	96	158	37.8	62.2	0.61
*H. argutum	10	26	153	14.5	85.5	0.17
*H. carneum	12	98	102	49.0	51.0	0.96
<sup>*</sup> H. fendleri	16	70	237	22.8	77.2	0.30
<sup>*</sup> H. gracile	22	1	403	0.2	99.8	0.00
*H. gronovii	22	53	251	17.4	82.6	0.21
*H. longipilum	27	32	402	7.4	92.6	0.08
*H. scabrum	15	102	321	24.1	75.9	0.32
*H. scouleri	13	11	210	5.0	95.0	0.05
*Krigia biflora	4	2	90	2.2	97.8	0.02
*Microseris sp.	10	32	175	15.5	84.5	0.18
*M. nutans	8	0	93	0	100.0	0.00
*Prenanthes sagittata	1	0	3	0	100.0	0.00

**Table 3.5.** Single-choice oviposition tests with *Cheilosia urbana* 2000–2004, using *Pilosella caespitosa* (ID) as the control plant.

<sup>*a*</sup>, \* plant species indigenous to North America.

<sup>b</sup>, Number of eggs laid on test plant divided by the number of eggs on control plant.

### **Open-field oviposition tests**

To further assess the susceptibility of native North American plant species to *C. urbana* under natural conditions, multiple-choice open-field tests were established in the CABI garden in Delémont, Switzerland between 2005 and 2008. All plants were potted in 13-centimeter (cm) clay pots with standard potting soil modified with vermiculite or sand for plant species associated with sandy soil. In all experiments the mean number of eggs or larvae per plant species is given, comprising all pots with living plants, including those that did not contain any immature stages of *C. urbana*.

### **Open-field test 2005**

**Methods.** Two plots (64 and 90 potted plants), consisting of eight native North American *Hieracium* species. and Eurasian *P. caespitosa* plants were established in two neighboring garden beds in the CABI garden from 28 April onwards. In 2005, the flight period of *C. urbana* started on April 22 and lasted until the second half of May. Each row of the two plots contained one pot of each hawkweed species. Between September and October, the soil of all pots was sieved and any *Cheilosia* larva found was retrieved and identified to species. Larvae were then transferred into small vials filled with humid soil to rear them to adulthood.

**Results.** Eight North American *Hieracium* species were tested in 2005. Larvae were only located on *H. gronovii*, *H. scouleri*, and *H. venosum*; averaging 0.1–0.3 larvae per plant (Table 3.6). No larvae were retrieved from pots containing *H. bolanderi*, *H. carneum*, *H. scabrum*, and *H. albiflorum*. However survival of *H. albiflorum* and *H. argutum* plants was poor due to environmental conditions. In contrast nearly all *P. caespitosa* control pots contained larvae (an average of 5.6 larvae was found per pot). The maximum number of larvae found in pots of native North American *Hieracium* species was 1, whereas up to 11 *C. urbana* larvae were retrieved from the control pots.

Test plant	No. of	No.	Plants with larvae <sup>a</sup>		Mean No.
	plants	valid	No.	%	larvae $(\pm SE)^a$
	exposed	replicates			
P. caespitosa ID	18	18	16	88.9	$5.6 \pm 0.9$
*H. albiflorum	28	2	0	0	0
*H. argutum	10	0	-	-	-
*H. bolanderi	18	12	0	0	0
*H. carneum	18	13	0	0	0
*H. gronovii	18	9	3	33.3	$0.3\pm0.2$
*H. scabrum	18	12	0	0	0
*H. scouleri	18	10	1	10.0	0.1
*H. venosum	8	4	1	25.0	0.3

**Table 3.6.** Results of open-field combined oviposition and larval development tests with *Cheilosia urbana* in 2005 (data of the two plots were pooled).

\*hawkweed species native to North America.

<sup>*a*</sup>results refer to the number of plants evaluated, i.e. dead plants were excluded from analysis.

### **Open-field test 2006**

In 2005, many native North American *Hieracium* species died before they could be checked for larval development, which resulted in numerous invalid replicates. From 2006 onwards, all plants were checked for eggs instead. Although the researchers were not able to distinguish the eggs of *C. urbana* and *C. psilophthalma*, mature larvae and pupae of both species can reliably be separated. Therefore, all eggs retrieved in open-field tests were transferred onto *P. aurantiaca*, a plant which is very suitable for the development of both *Cheilosia* species, and all larvae found in September/October were determined to species level.

**Methods.** On April 20, 2006, four plots were established in the CABI garden. To increase the density of gravid females, 73 field-collected *C. urbana* females were released in the garden between May 5 and May 18. To increase oviposition pressure, most of the indigenous *Pilosella* patches naturally established in the CABI garden were sprayed with herbicide and/or covered with sand or sawdust shortly after the emergence period of the flies.

<u>Plots 1 and 2</u> were established in two neighboring garden beds, and designed as a two-phase experiment. In the first phase all hawkweed plants (test and control plants) were exposed, representing a choice plus control situation. In the second phase, all *P. caespitosa* and *P*.

*aurantiaca* plants were removed, representing a choice minus control situation. All plants were checked for eggs on April 29, May 5–7 (plus control situation) and on May 23–25 (choice minus control situation).

Plot 1 (Fig. A) consisted of seven pots each of two target weeds, *P. caespitosa* and *P. aurantiaca*, and eight native North American hawkweed species (70 plants in total). Plot 2 consisted of nine pots each of *P. caespitosa*, six native North American *Hieracium* species and the naturalized *H. lachenalii* (72 plants in total). In both plots, pots with grass, such as *Lolium perenne*, were interspersed to provide shading for the hawkweed plants.

<u>Plots 3 and 4</u> consisted of six pots each of one target weed, *P. caespitosa*, the European species *H. murorum* and *H. lachenalii*, and three native hawkweed species (36 plants each). Plot 3 was located at a sunny spot in the CABI garden whereas plot 4 was located in the forest. They were checked for unhatched eggs and egg chorions (hatched eggs) on April 29/30 and May 31/June 1, 2006.

Plants in all plots were arranged in a randomized block design. The flight period of both hoverfly species started in the second half of April (April 22 for *C. urbana*) and lasted until the second half of May.

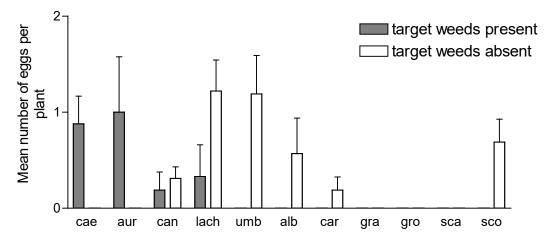


Figure A. Design of open-field test established with Cheilosia urbana in 2006, plot 1.

**Results.** <u>Plot 1 and 2</u>: A total of 80 eggs and egg chorions were retrieved from control and test plants. Twenty-nine *C. urbana* but no *C. psilophthalma* puparia were retrieved from the *P. aurantiaca* plants used to rear the larvae, indicating that the majority, if not all eggs collected from the exposed plants, were likely laid by *C. urbana* females.

In the presence of the target weeds, *P. caespitosa* and *P. aurantiaca*, only two species both of the subgenus *Hieracium*; *H. canadense* (NA), and *H. lachenalii* (Eur) were accepted for oviposition (Figure B, dark bars). Representatives in the North American subgenus *Chionoracium* remained free of infestation. In contrast, in the absence of *P. caespitosa* and *P. aurantiaca*, five native *Hieracium* species and *H. lachenalii* were accepted for oviposition, some to a similar degree as the target weeds (Figure B, white bars).

The mean number of *C. urbana* eggs retrieved from *P. caespitosa* and *P. aurantiaca*, ranged between 0.9 and 1.0 eggs per pot. Half of the *P. caespitosa* plants had eggs and 43 percent of the *P. aurantiaca* plants.



**Figure B.** Results of open-field oviposition test with *Cheilosia urbana* in 2006 (plots 1+2). Species labels indicate the first letters of the *Hieracium* and *Pilosella* species exposed: *P. caespitosa* (cae), *P. aurantiaca* (aur), *H. canadense* (can), *H. lachenalii* (lach), *H. umbellatum* (umb), *H. albiflorum* (alb), *H. carneum* (car), *H. gracile* (gra), *H. gronovii* (gro), *H. scabrum* (sca), and *H. scouleri* (sco). Dark grey bars indicate the number of eggs retrieved from test plants in the presence of the target weeds *P. caespitosa* and *P. aurantiaca* while white bars indicate the number of eggs retrieved in the absence of the target weeds. All pots with plants exposed were included in the analysis.

<u>Plot 3 and 4</u>: In plot 3, eggs were laid onto *P. caespitosa, H. lachenalii*, and *H. albiflorum* (Table 3.7). Most eggs were retrieved from *P. caespitosa* with 83.3 percent of the plants attacked, whereas half of the *H. lachenalii* plants were attacked, but only one out of six *H. albiflorum* plants. No eggs were retrieved from *H. canadense, H. murorum*, or *H. gracile*. No eggs were found on any of the plants exposed in the forest (plot 4, Table 3.8).

Test plant	No. of pots	Plants attacked		Mean no. eggs
	exposed	<u>No.</u>	%	per pot $\pm$ SE (range) <sup><i>a</i></sup>
P. caespitosa ID	6	5	83.3	$1.3 \pm 0.4 \ (0-3)$
*H. canadense	6	0	0	0
H. lachenalii	6	3	50.0	$0.5 \pm 0.2$ (0-1)
H. murorum	6	0	0	0
*H. albiflorum	6	1	16.7	$0.2 \pm 0.2$ (0-1)
*H. gracile	6	0	0	0

Table 3.7. Results of open-field oviposition test with Cheilosia urbana in in 2006 (plot 3).

\*hawkweed species indigenous to North America.

<sup>*a*</sup> all plants exposed were included in the analysis.

Test plant	No. of pots	Plar	nts attacked	Mean no. eggs per pot <sup><i>a</i></sup>
	exposed	No.	%	1 1
P. caespitosa ID	6	0	0	0
*H. canadense	6	0	0	0
H. lachenalii	6	0	0	0
H. murorum	6	0	0	0
*H. albiflorum	6	0	0	0
*H. gracile	6	0	0	0

Table 3.8. Results of open-field oviposition test with Cheilosia species in 2006 (plot 4, forest).

\*hawkweed species indigenous to North America, <sup>a</sup> all plants exposed were included in the analysis.

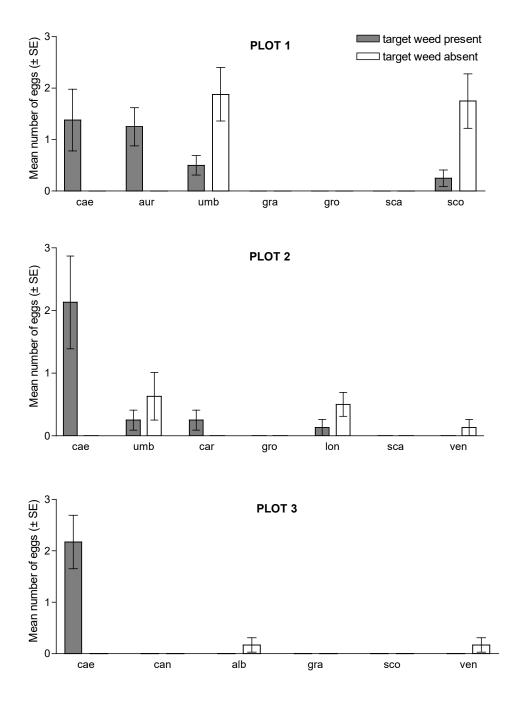
#### **Open-field test 2007**

**Methods.** On April 16 and 17, 2007, three plots were established in the garden at CABI. As in 2006, all three plots were set up as a two-phase experiment. In the first phase all *Hieracium* and *Pilosella* species (test and control plants), were exposed to naturally occurring gravid *Cheilosia* females, representing a choice plus control situation. Then, in the second phase, all *P. caespitosa* and *P. aurantiaca* plants were removed, representing a choice minus control situation. Between April 23 and 24, all plants in the choice plus control situation were checked for eggs, and between April 25 and May 2, all plants in the choice minus control situation were checked. Because some test plants were available in smaller numbers than others (due to high mortality during overwintering or difficulties in growing them), not all hawkweed species could be exposed in all plots.

Plot 1 consisted of eight pots each of two target weeds, *P. caespitosa* and *P. aurantiaca*, and five native North American hawkweed species, *H. scouleri*, *H. scabrum*, *H. gronovii*, *H. umbellatum*, and *H. gracile* (56 plants in total). Plot 2 consisted of eight pots each of *P. caespitosa* and six *Hieracium* species native to North America, *H. scabrum*, *H. gronovii*, *H. carneum*, *H. longipilum*, *H. venosum*, and *H. umbellatum* (56 plants in total). In both plots, pots with grass, *Lolium* species, were interspersed to provide shading for the hawkweed plants. Plot 3 consisted of six pots each of *P. caespitosa* and five *Hieracium* species native to North America, *H. canadense*, *H. albiflorum*, *H. venosum*, *H. gracile*, and *H. scouleri* (36 plants in total).

All plants were arranged in a randomized block design. The exact start of the flight period of *C*. *urbana* was not determined but it was assumed that it started earlier than in 2006 due to unusually warm spring weather. The first two *C*. *urbana* females were observed visiting *Pilosella* plants for oviposition on April 18.

As in 2006, *Cheilosia* eggs were not left on the different test plants to complete development but were retrieved and kept separately in Petri-dishes. Hatching larvae were transferred onto potted *P. aurantiaca* plants to allow completion of development and exact identification to species level, whether *C. urbana* or *C. psilophthalma*. In mid-September 2007 immature stages were retrieved from all *P. aurantiaca* plants and identified to species level.



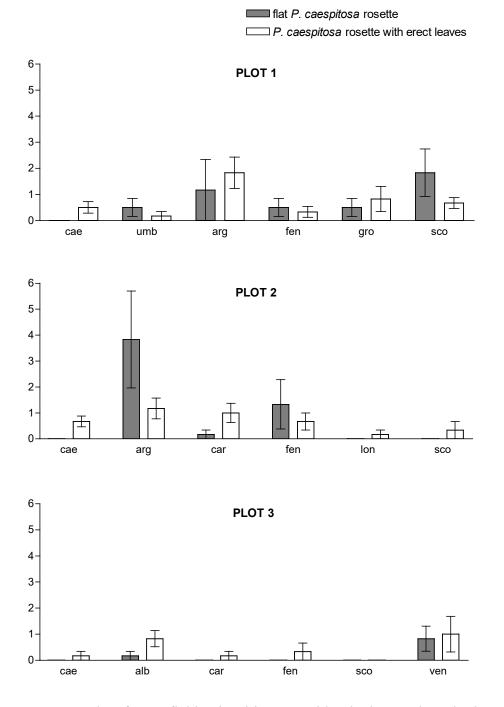
**Figure C.** Results of open-field oviposition test with *Cheilosia urbana* in the garden at CABI in 2007. The species labels indicate the first letters of the *Pilosella* and *Hieracium* species exposed: *P. caespitosa* (cae), *P. aurantiaca* (aur), *H. canadense* (can), *H. umbellatum* (umb), *H. albiflorum* (alb), *H. carneum* (car), *H. gracile* (gra), *H. gronovii* (gro), *H. longipilum* (lon), *H. scabrum* (sca), *H. scouleri* (sco), *H. venosum* (ven). Dark grey bars indicate the number of eggs retrieved from plants in the presence of the target weed *P. caespitosa* while white bars indicate the number of eggs retrieved in the absence of the target weed.

**Results.** In the presence of the target weed, *P. caespitosa*, four native North American hawkweed species, *H. umbellatum* (subgenus *Hieracium*), *H. carneum*, *H. longipilum*, and *H. scouleri* (subgenus *Chionoracium*) were accepted for oviposition (Figure C, dark bars). In the absence of *P. caespitosa*, five native North American *Hieracium* species were accepted for oviposition (Figure C, white bars). Apart from plot 1, more eggs were found on the target *P. caespitosa* than the test species. None of the test plants exposed in Plot 3 were accepted for oviposition in the presence of the control, whereas on average two eggs were laid on *P. caespitosa* plants.

#### **Open-field test 2008**

Methods. On April 23, 2008, three plots were established in the garden at CABI. All plots consisted of 36 potted plants arranged in a randomized block design; six pots of P. caespitosa and six pots of five different native North American Hieracium species. The native hawkweed species exposed in one or several plots were H. albiflorum, H. argutum, H. carneum, H. fendleri, H. gronovii, H. longipilum, H. scouleri, and H. venosum in the subgenus Chionoracium, and H. umbellatum in the subgenus Hieracium. The plants were either grown from seeds or shipped from the United States as rosettes. The tests were intended as two-phase experiments, as in 2006 and 2007 (see above). However, no eggs were found on any of the control plants exposed during the first check on May 6, whereas Cheilosia eggs were found on most of the other hawkweed species exposed. This was most probably because the P. caespitosa rosettes used were sown in spring 2008 and their leaves were close to the ground, which might have inhibited the normal egg-laying behavior of females; they usually walk down a leaf with an extended ovipositor and oviposit at the rosette base or into the leaf axil. Therefore, the experimental set-up was adjusted. Instead of exposing exclusively test plants in the second phase, the newly grown P. caespitosa rosettes were replaced with overwintered P. caespitosa plants with erect leaves. All plants were checked for eggs a second time on June 12, 2008.

**Results.** No eggs were found on any of the *P. caespitosa* rosettes with flat leaves exposed (Figure D). However, even on the *P. caespitosa* rosettes with erect leaves, exposed in the second phase of the experiments, only few eggs were found on the control plants. In 2008, no more than one egg per *P. caespitosa* pot was found whereas in previous years up to five eggs were found. All native North American *Hieracium* species were accepted for oviposition.



**Figure D.** Results of open-field oviposition test with *Cheilosia urbana* in the garden at CABI in 2008. The species labels indicate the first letters of the *Hieracium* and *Pilosella* species exposed: *P. caespitosa* (cae), *H. umbellatum* (umb), *H. albiflorum* (alb), *H. argutum* (arg), *H. carneum* (car), *H. fendleri* (fen), *H. gronovii* (gro), *H. longipilum* (lon), *H. scouleri* (sco), and *H. venosum* (ven). Dark grey bars indicate the number of eggs retrieved from plants in the presence of *P. caespitosa* rosettes with flat leaves, while white bars indicate the number of eggs retrieved in the presence of *P. caespitosa* rosettes with erect leaves.

### Impact on target and non-target plants

Because *C. urbana* larvae can develop on several native North American *Hieracium* species in nochoice larval transfer tests and some of those species were accepted for oviposition by gravid females in open-field tests, an impact experiment using different larval densities was carried out in 2008 at CABI Switzerland.

**Methods.** Different larval densities were transferred onto *P. caespitosa* representing the target weed and the native North American *H. gronovii. Hieracium gronovii* was chosen because *C. urbana* larvae can develop on this plant (3.9 percent survival was recorded in no-choice larval transfer tests). In addition, *H. gronovii* was accepted for oviposition in the open-field test in 2005.

All rosettes used in the impact experiment were grown from seeds in a standard potting soil in the greenhouse. To avoid unnaturally vigorous growth, grass was used as a competitor. Two tufts of *Festuca rubra* were added to each pot. At the beginning of the experiment the longest leaf of *P*. *caespitosa* was on average  $6.2 \pm 0.21$  cm (range: 3.3–9.1) and the longest leaf of *H. gronovii* was on average  $2.3 \pm 0.15$  cm (range: 0.8–4.3) long. There was no significant difference in the length of the longest leaf of *P. caespitosa* rosettes ( $F_{3,44} = 1.36$ , P = 0.268) between the pots assigned to the different larval densities, nor of *H. gronovii* rosettes ( $F_{3,28} = 0.273$ , P = 0.844) before the experiment was set up. Between May 9 and 15, 2008, four densities of C. urbana larvae (0, 3, 6, or 12 larvae) were transferred onto 12 P. caespitosa plants and eight H. gronovii plants per density treatment (48 and 32 plants in total, respectively). All pots were kept in a greenhouse to protect the freshly transferred larvae from heavy rain. On May 23, all plants were covered with gauze bags and embedded in sawdust in a garden bed. The grass in the pots was cut three times, in April, May, and July to prevent it from out-competing the hawkweeds. All hawkweed plants were measured between September 19 and 26, 2008, and plant phenostage (rosette/bolting) and plant state (alive/dead) recorded. The biomass of the plants was harvested and dried for 24 hours at 80°C and its weight (g) recorded. Between October 6 and 8, the soil from all pots was sieved and searched for C. urbana puparia to determine their survival rate at the different densities.

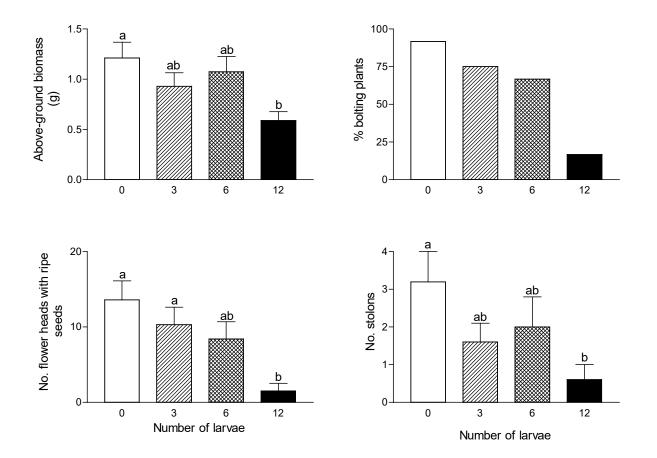
The proportion of dead rosettes (in the case of *H. gronovii*) and the proportion of bolting plants (in the case of *P. caespitosa*) were analyzed using binary linear regression with larval density and the length of the longest leaf as covariates. The number of flower heads with ripe seeds, the number of stolons of *P. caespitosa*, and above-ground biomass of both species were compared using one-way ANOVAs with larval density as a factor. To obtain homogeneity of variances, the number of flower heads and stolons were ( $\ln + 0.5$ )-transformed. Where larval densities were found to have a significant effect, means were compared using Tukey's HSD test.

**Results.** Plants of *P. caespitosa* with longer leaves at the beginning of the experiment bolted more readily than plants with shorter leaves (Wald = 7.82, P = 0.005). Ninety-two percent of the control plants formed flower heads whereas only 17 percent of the plants exposed to 12 *C. urbana* larvae bolted (Wald = 10.65, P = 0.014). Herbivory by *C. urbana* also significantly reduced the number of flower heads with ripe seeds ( $F_{3, 44} = 6.040$ , P = 0.002), the number of stolons ( $F_{3, 44} = 3.431$ , P = 0.025), and above-ground biomass ( $F_{3, 44} = 3.880$ , P = 0.015) (see Figure E). The biomass of plants onto which 12 larvae were transferred was reduced by 51 percent compared to plants without larvae, the number of flower heads with ripe seeds with ripe seed was reduced by 89 percent and the number of stolons by

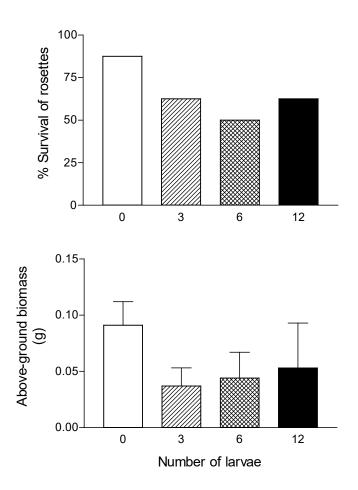
81 percent.

None of the *H. gronovii* plants bolted during the experiment. Plant survival was highest in the control plant group (with zero larvae) and lowest in the group exposed to six larvae (see Figure F). However, larval density had no significant effect on the mortality of *H. gronovii* rosettes (Wald = 2.37, P = 0.50). Plants with longer leaves at the beginning of the experiment tended to survive better than plants with shorter leaves (Wald = 3.81, P = 0.051). Larval density did not significantly affect the above-ground biomass of plants harvested after the experiment ( $F_{3, 27} = 2.079$ , P = 0.127).

Only four *C. urbana* puparia were found on *P. caespitosa* plants and none in the *H. gronovii* pots. The number of immature *C. urbana* recovered from no-choice larval transfer tests and from the pots used in the impact experiment was very low in 2008. The reason for this is unclear.



**Figure E.** Effect of four larval densities of *Cheilosia urbana* on different plant parameters of *Pilosella caespitosa*. Bars are untransformed means + SE of 12 plants except for the percentage of bolting plants (Tukey's HSD Test, homogenous groups are represented by the same letters, P < 0.05).



**Figure F.** Effect of four larval densities of *Cheilosia urbana* on survival of *Hieracium gronovii* rosettes (Wald = 2.37, P = 0.50) and above-ground biomass ( $F_{3, 27} = 2.079$ , P = 0.127).

# Appendix 4. Release Strategy for *Cheilosia urbana*.

## A. Protocol for releasing the agent

#### Geographical or host source

All flies used for host-range tests were field-collected in the Swiss Jura and the southern Black Forest in south-western Germany. These two regions are less than 100 kilometers apart and genetic exchange is therefore likely. Because mating could not be obtained under caged conditions, mated females that landed on hawkweed rosettes for oviposition were caught in the field and kept in small vials in which they laid eggs.

#### Method to ensure proper identification

Because *C. urbana* and *C. psilophthalma* are sympatric species that develop on plants in the genus *Pilosella*, there is a risk of confusing the identity of the two species. However, mature larvae, puparia and adults can be easily distinguished using a dissecting microscope. Voucher specimens of *C. urbana* and *C. psilophthalma* will be kept for comparative purposes and also sent to North America for deposit in national collections in the United States and Canada. *Cheilosia mutabilis* adults emerge several months later than *C. urbana*; therefore, there is not a concern for misidentification.

#### Protocol to ensure the absence of natural enemies

*Cheilosia urbana* will be reared at CABI on potted hawkweed plants covered with gauze bags to reduce contamination by other organisms. *Cheilosia urbana* will be shipped to North America in the pupal stage, and will be received in containment, where they will be kept caged and inspected for identity and the emergence of parasitoids or other unwanted organisms before field release. Any contaminants will be removed and destroyed through autoclaving. Voucher specimens of *Cheilosia* species and any accompanying parasitic insects or other organisms also will be preserved and kept for identification purposes in the respective containment facilities. Preserved vouchers of the contaminating organisms also will be lodged in the national collections of the receiving countries. Puparia and adults of *C. urbana* will be sampled and inspected for the presence of pathogens. Upon field release of *C. urbana* in New Zealand, all five shipments of *C. urbana* and/or *C. psilophthalma* sent by CABI between 2002 and 2006 had been screened by an insect pathologist, but in none of them were any insect pathogenic micro-organisms found.

### Specific location of rearing facility

Quarantine handling and rearing to adulthood for U.S. releases will be conducted at the arthropod containment facilities at the Montana State University, Bozeman.

#### Intended sites for initial release

Initial releases are proposed for sites in Lincoln and Flathead Counties, MT. Releasing at multiple release sites essentially represents a bet-hedging strategy and, to some degree, may mitigate failure of *C. urbana* to establish. Releases will be made at different elevational or latitudinal locations, and also on different host *Pilosella* species to determine the optimum conditions for establishment and efficacy of *C. urbana*.

#### Number of hoverflies to be released

*Cheilosia urbana* will be released as adults or in the form of infested potted plants (see below). Actual numbers released per site will depend upon the availability of flies, though target release sizes will be at least 30 adults per site or 15 potted plants.

### Timing of releases

*Cheilosia urbana* is a spring-emerging species. The first occurrence of gravid females at CABI depended strongly on the weather conditions and ranged from early April to early May in different years. Similarly, researchers in the United States will plan for spring releases, coordinated with optimum host phenology (pre-bolt stage), weather conditions, and the presence of pollen or nectar sources.

### Release methods

To date it is not possible to obtain mating of *C. urbana* in captivity. The use of field cages of different sizes and different netting, screened rearing cages, or gauze-covered pots were not successful in allowing flies to mate and lay viable eggs. Therefore gravid females will be field-collected in Europe and placed in cages. Viable eggs obtained will be transferred to potted plants at CABI and the resulting puparia and larvae will be shipped to containment facilities in North America. The resulting F1 males and females will be identified to species and then released at field sites. An alternative method would be to obtain viable *C. urbana* eggs from CABI and to inoculate hawkweed plants grown free of pests from seeds within rearing facilities at Montana State University. These infested plants will be transplanted early spring the following year, thus allowing the adults to emerge naturally. This could avoid potential problems handling the flies and keeping them for extended periods in the lab after emergence, especially if release sites are distant from the quarantine facility and the emergence period is extended. To better concentrate fly populations, releases will be made onto relatively isolated patches of the target host and proximity to a forest margin. Cages will be set-up if needed to contain or recover adults.

### **B.** Monitoring plans

Monitoring of the site(s) will be carried out by personnel at Montana State University in the United States. Once sufficient releases are made, the help of local cooperators also will be enlisted in spring during the oviposition period of the fly. In the 1–3 years following field releases, plants in the field will be closely monitored for establishment by inspecting 50–100 randomly chosen plants in field plots visually for eggs of *C. urbana*. In addition, when weather conditions are favorable, hawkweed

plants can be checked for ovipositing females during the oviposition period of the fly. Because *C. urbana* is fairly mobile, long-term monitoring, including surrounding hawkweed patches, may be necessary to determine establishment.

Common Name	Determination	Impact/Effects
San Diego ambrosia	May affect, not likely to adversely affect (MANLAA)	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect San Diego ambrosia or its critical habitat.
Encinitas baccharis	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Encinitas baccharis.
Sonoma sunshine	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Sonoma sunshine.
Decurrent false aster	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect decurrent false aster.
Florida brickell-bush	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Florida brickell-bush or its designated critical habitat.
Cape Sable thoroughwort	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Cape Sable thoroughwort or its designated critical habitat.
Florida golden aster	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its

**Appendix 5.** May affect determinations for listed plants in the contiguous United States.

		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect Florida golden aster.
Fountain thistle	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
		survival and reproduction because it occurs in the same sub-family as
		invasive hawkweeds. However, in host specificity testing, C. urbana
		developed only on <i>Hieracium</i> and <i>Pilosella</i> species. The surrogate
		<i>Cirsium undulatum</i> was tested and did not support development of <i>C</i> .
		<i>urbana</i> . Therefore, environmental release of <i>C. urbana</i> may affect, but
		is not likely to adversely affect Fountain thistle.
Chorro Creek bog	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
thistle		survival and reproduction because it occurs in the same sub-family as
		invasive hawkweeds. However, in host specificity testing, <i>C. urbana</i>
		developed only on <i>Hieracium</i> and <i>Pilosella</i> species. The surrogate
		<i>Cirsium undulatum</i> was tested and did not support development of <i>C</i> .
		<i>urbana</i> . Therefore, environmental release of <i>C. urbana</i> may affect, but
Suisun thistle	MANLAA	is not likely to adversely affect Chorro Creek bog thistle.
Suisun thistle	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
		survival and reproduction because it occurs in the same sub-family as
		invasive hawkweeds. However, in host specificity testing, <i>C. urbana</i>
		developed only on <i>Hieracium</i> and <i>Pilosella</i> species. The surrogate
		<i>Cirsium undulatum</i> was tested and did not support development of <i>C</i> .
		<i>urbana</i> . Therefore, environmental release of <i>C. urbana</i> may affect, but
La Graciosa thistle	MANLAA	is not likely to adversely affect Suisun thistle or its critical habitat.
La Graciosa inistie	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
		survival and reproduction because it occurs in the same sub-family as
		invasive hawkweeds. However, in host specificity testing, <i>C. urbana</i>
		developed only on <i>Hieracium</i> and <i>Pilosella</i> species. The surrogate
		<i>Cirsium undulatum</i> was tested and did not support development of <i>C. urbana</i> . Therefore, environmental release of <i>C. urbana</i> may affect, but
		is not likely to adversely affect La Graciosa thistle or its critical habitat.
Pitcher's thistle	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
r itelier s tilistie	MANLAA	survival and reproduction because it occurs in the same sub-family as
		invasive hawkweeds. However, in host specificity testing, <i>C. urbana</i>
		developed only on <i>Hieracium</i> and <i>Pilosella</i> species. The surrogate
		<i>Cirsium undulatum</i> was tested and did not support development of <i>C</i> .
		<i>urbana</i> . Therefore, environmental release of <i>C. urbana</i> may affect, but
		is not likely to adversely affect Pitcher's thistle.
Sacramento	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
Mountains thistle		survival and reproduction because it occurs in the same sub-family as
mountains unsue		invasive hawkweeds. However, in host specificity testing, <i>C. urbana</i>
		developed only on <i>Hieracium</i> and <i>Pilosella</i> species. The surrogate
		<i>Cirsium undulatum</i> was tested and did not support development of <i>C</i> .
		<i>urbana</i> . Therefore, environmental release of <i>C. urbana</i> may affect, but
		is not likely to adversely affect Sacramento Mountains thistle.
Otay tarplant	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		menuced the most closery related plants to invasive nawkweeds, only

		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Otay tarplant or its designed original babitat.
	MANLAA	designated critical habitat.
Gaviota tarplant	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Gaviota tarplant or its designated critical habitat.
Smooth coneflower	MANLAA	ě
		<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect smooth coneflower.
Ash Meadows	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
sunray		survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Ash Meadows sunray or its designated critical habitat.
Willamette daisy	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Willamette daisy or its designated critical habitat.
<b>T</b>		designated efficient naoran.
Parish's daisy	MANLAA	Cheilosia urbanacould potentially attack this plant, affecting itssurvival and reproduction because it belongs in the same family ashawkweeds (Asteraceae). However, in host specificity tests thatincluded the most closely related plants to invasive hawkweeds, onlyplants in the genera Hieracium and Pilosella supported development ofC. urbana. Therefore, APHIS has determined that release of C. urbanamay affect, but is not likely to adversely affect Parish's daisy or itsdesignated critical habitat.
Parish's daisy Zuni fleabane	MANLAA	<ul> <li>Cheilosia urbana could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i>. Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Parish's daisy or its designated critical habitat.</li> <li><i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i>. Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect parish's daisy or its designated critical habitat.</li> </ul>
		<ul> <li>Cheilosia urbana could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i>. Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Parish's daisy or its designated critical habitat.</li> <li><i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of a survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of</li> </ul>

		hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect San Mateo woolly sunflower.
Ash Meadows gumplant	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Ash Meadows gumplant or its designated critical habitat.
Virginia sneezeweed	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Virginia sneezeweed.
Pecos sunflower	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Pecos sunflower or its designated critical habitat.
Schweinitz's sunflower	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Schweinitz's sunflower.
Whorled sunflower	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect whorled sunflower or its designated critical habitat.
Santa Cruz tarplant	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Santa Cruz tarplant or its designated critical habitat.

Labrasida daira	MANILAA	Chailagin unhang aguld not ontigilly -tt1- this -1t - fftime 't
Lakeside daisy	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect lakeside daisy.
Texas prairie dawn-	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
flower		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera Hieracium and Pilosella supported development of
		C. urbana. Therefore, APHIS has determined that release of C. urbana
		may affect, but is not likely to adversely affect Texas prairie dawn-
		flower.
Burke's goldfields	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera Hieracium and Pilosella supported development of
		C. urbana. Therefore, APHIS has determined that release of C. urbana
		may affect, but is not likely to adversely affect Burke's goldfields.
Contra Costa	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
goldfields		survival and reproduction because it belongs in the same family as
-		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera Hieracium and Pilosella supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect Contra Costa goldfields
		or its designated critical habitat.
Beach layia	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera Hieracium and Pilosella supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect beach layia.
San Francisco	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
lessingia		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect San Francisco lessingia.
Heller's blazingstar	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
iner o oneingour		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect Heller's blazingstar.
Some blogingston	MANILAA	
Scrub blazingstar	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its

<b></b>		
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C</i> where <i>C</i> where <i></i>
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
Santa Crazz	MANLAA	may affect, but is not likely to adversely affect scrub blazingstar.
Santa Cruz	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
malacothrix		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
<b>T 1 1 1 1</b>	MANT A A	may affect, but is not likely to adversely affect Santa Cruz malacothrix.
Island malacothrix	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
Mohr's Barbara	MANLAA	may affect, but is not likely to adversely affect island malacothrix.
	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
buttons		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
C I i la	NANT AA	may affect, but is not likely to adversely affect Mohr's Barbara buttons.
San Joaquin wooly-	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
threads		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C</i> where <i>C</i> where <i></i>
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect San Joaquin wooly-
San Francisco Peaks	MANLAA	threads.
	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
ragwort		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect San Francisco Peaks
		ragwort or its designated critical habitat.
White-rayed	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
pentachaeta		survival and reproduction because it belongs in the same family as
pentaenaeta		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect white-rayed pentachaeta.
Lyon's pentachaeta	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
Lyon's pentaenaeta		survival and reproduction because it belongs in the same family as
L		survival and reproduction occause it belongs in the same family as

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		hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect Lyon's pentachaeta.
Ruth's golden aster	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
Ruth 5 golden üster		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect Ruth's golden aster.
Hartweg's golden	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
sunburst		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect Hartweg's golden
		sunburst.
San Joaquin adobe	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
sunburst		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera Hieracium and Pilosella supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect San Joaquin adobe
		sunburst.
Layne's butterweed	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera Hieracium and Pilosella supported development of
		C. urbana. Therefore, APHIS has determined that release of C. urbana
		may affect, but is not likely to adversely affect Layne's butterweed.
Houghton's	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
goldenrod		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
<u> </u>		may affect, but is not likely to adversely affect Houghton's goldenrod.
Short's goldenrod	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C</i> , where <i>C</i> are a plus has determined that release of <i>C</i> where
		<i>C. urbana.</i> Therefore, APHIS has determined that release of <i>C. urbana</i>
Dhua Didaa	MANILAA	may affect, but is not likely to adversely affect Short's goldenrod.
Blue Ridge	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
goldenrod		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that

Malheur wire-lettuce	MANLAA	included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Blue Ridge goldenrod. <i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same tribe as invasive hawkweeds. However, in host specificity tests, no plants tested
		exhibited symptoms or supported development of <i>C. urbana</i> except for <i>Hieracium</i> and <i>Pilosella</i> species. Two surrogate species, <i>Stephanomeria minor</i> (= <i>S. tenuifolia</i> ) and <i>S. cichoriacea</i> , were tested and no development occurred. Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect Malheur wire-lettuce or its designated critical habitat.
California	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
taraxacum		survival and reproduction because it belongs in the same tribe as
		invasive hawkweeds. However, in host specificity tests, no plants tested
		supported development of <i>C. urbana</i> except for <i>Hieracium</i> and <i>Pilosella</i>
		species. Three surrogate <i>Taraxacum</i> species ( <i>laevigatum</i> , officinale, and
		<i>lyratum</i> ) were tested and no development occurred. Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to
		adversely affect California taraxacum or its designated critical habitat.
Ashy dogweed	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its
Tishy dogweed		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		C. urbana. Therefore, APHIS has determined that release of C. urbana
		may affect, but is not likely to adversely affect ashy dogweed.
Last Chance	MANLAA	Cheilosia urbana could potentially attack this plant, affecting its
townsendia		survival and reproduction because it belongs in the same family as
		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
Big-leaved	MANLAA	may affect, but is not likely to adversely affect Last Chance townsendia.Cheilosia urbana could potentially attack this plant, affecting its
crownbeard		survival and reproduction because it belongs in the same family as
ero miocuru		hawkweeds (Asteraceae). However, in host specificity tests that
		included the most closely related plants to invasive hawkweeds, only
		plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of
		<i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i>
		may affect, but is not likely to adversely affect big-leaved crownbeard.

Desert yellowhead	MANLAA	<i>Cheilosia urbana</i> could potentially attack this plant, affecting its survival and reproduction because it belongs in the same family as hawkweeds (Asteraceae). However, in host specificity tests that included the most closely related plants to invasive hawkweeds, only plants in the genera <i>Hieracium</i> and <i>Pilosella</i> supported development of <i>C. urbana</i> . Therefore, APHIS has determined that release of <i>C. urbana</i> may affect, but is not likely to adversely affect desert yellowhead.
Golden paintbrush	May affect beneficially	Mouseear hawkweeds are encroaching on the habitat of golden paintbrush in Thurston County, Washington (FWS, 2000). Therefore, release of <i>C. urbana</i> may affect beneficially the golden paintbrush.

(FWS) U.S. Fish and Wildlife Service. 2000. Recovery Plan for the Golden Paintbrush (*Castilleja levisecta*). U.S. Fish and Wildlife Service, Portland, Oregon. 51 pp. Available: <u>https://www.fws.gov/oregonfwo/documents/RecoveryPlans/Golden\_Paintbrush\_RP.pdf</u> Accessed: 21 February, 2017.

## **Appendix 6.** Response to comment on draft environmental assessment.

A commenter asked how the release of *Chelosia urbana* is expected to affect the native hawkweeds of North America?

**Response:** Approximately 23 native species of hawkweeds (i.e., *Hieracium*) are found in North America. Of these, 17 species were tested (at some level) during the determination of the potential host range of *Cheilosia urbana*. The hoverfly developed to mature larvae on two native species in the subgenus *Hieracium*, (*H. umbellatum* and *H. canadense* (some authors have placed this species in the subgenus *Chionoracium*)). Survival rates to mature larva were 4.8 percent and 16.7 percent, respectively, and development to adulthood was 1.2 and 8.3 percent, respectively. In contrast, only five out of 15 native North American *Hieracium* species in the subgenus *Chionoracium* supported development to mature larvae with development rates ranging from 0.6 to 6.1 percent. Adults developed on four species: *H. gronovii, H. longipilum, H. scabrum*, and *H. venosum*.

When given the choice between a native North American *Hieracium* species and *Pilosella caespitosa* in single-choice oviposition tests, *C. urbana* females laid less than 10 percent of their eggs on *H. umbellatum*, *H. gracile*, *H. longipilum* and *H. scouleri*. Because females preferred *P. aurantiaca*, *P. floribunda*, and *P. piloselloides* over *P. caespitosa*, the preference would likely have been even stronger when offering these species. Open-field oviposition tests with *C. urbana* carried out between 2006 and 2008 showed that *C. urbana* mostly prefers *P. caespitosa* over native North American *Hieracium* spp. However, this hoverfly also laid eggs onto ten of the 12 native hawkweed species tested; although oviposition was highly variable between years and test plots and sometimes occurred in absence of the target hawkweed.

To better interpret test results, the data of open-field oviposition tests obtained between 2006 and 2008 were pooled, and the overall acceptance and suitability of target and test plant species for *C. urbana* (i.e., the average number of eggs laid onto the different test plants exposed in open-field tests multiplied by the average survival rate to adulthood (from neonate larva to adult fly)) was calculated (Figure 1). The suitability value was below two for native North American *Hieracium* species in the subgenus *Hieracium* (range: 0.7–1.7) and between zero and 0.7 for species in the subgenus *Chionoracium*, while the target weeds *P. caespitosa* and *P. aurantiaca* had an overall acceptance and suitability of almost 11 and 47, respectively. The low overall suitability of native *Hieracium* species is confirmed by results of the open-field test established in 2005, where subsequent larval development was also monitored. While nearly 90 percent of control plants (*P. caespitosa*) were attacked and had a maximum of 11 larvae, only 10–33 percent of exposed native *Hieracium* species were attacked and a maximum of only one *C. urbana* larva was found.

Paynter et al. (2015) concluded that quantitative laboratory testing data can help predict risk of nontarget attack in the field. In New Zealand, researchers found the probability of host use was positively correlated with relative performance for both no-choice survival tests and no-choice oviposition tests. These performance ratings were a ratio (of either larval survival, or oviposition) of non-target plant species divided by that of the target host plant. Moreover, a combined risk score for no-choice tests (multiplying the relative performance scores for no-choice survival and oviposition tests) resulted in a distinct threshold (scores between 0.21 and 0.33) for probability of host use (including minor spillover attack) that rose from zero to a near certainty. Choice oviposition test data exhibited a similar pattern to no-choice data. When converting results of no-choice survival tests and no-choice oviposition tests for *C. urbana*, the performance ratings generally fell below the threshold scores determined by Paynter et al. (2015) depending upon the target host selected (e.g., *P. caespitosa* verses *P. aurantiaca*) (range of 0.01 to 0.30).

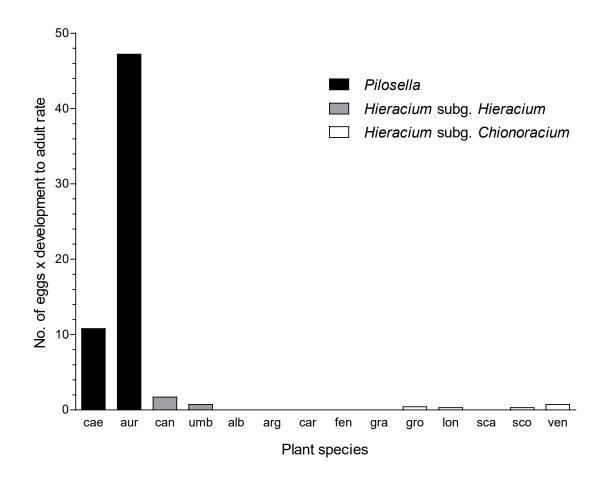
In an impact experiment using *P. caespitosa* and the native North American *H. gronovii*, larval feeding did not significantly affect growth of the native. This result is most probably due to high initial mortality of *C. urbana* larvae transferred onto the non-target plant. The survival rate of *C. urbana* larvae transferred onto *H. gronovii* was 3.6 percent in no-choice larval transfer tests carried out between 2000 and 2008, whereas an average of 21.7 percent of transferred larvae survived on *P. caespitosa*. We conclude that it is very unlikely that *C. urbana* will have a measurable impact on native *Hieracium* species considering their low suitability for *C. urbana* larval survival.

The fundamental and ecological host ranges of *C. urbana* are restricted to species in the genera *Pilosella* and *Hieracium*. Native Asteraceae outside the genera *Pilosella* and *Hieracium* are therefore not considered to be at significant risk of non-target attack. Tests showed that *C. urbana* might attack native North American *Hieracium* species in the subgenera *Hieracium* and *Chionoracium* in the field. However, single-choice and open-field oviposition tests indicate that the fly generally has a strong preference for the European invasive *Pilosella* species and that impact on native species, should they be attacked, is expected to be minimal because of low larval survival.

From the results of host-specificity tests conducted in Switzerland and New Zealand, it can be concluded that *C. urbana* will perform best on alien invasive hawkweeds in the genus *Pilosella*. The probability of attack of native North American hawkweed species in the subgenera *Hieracium* and *Chionoracium* is likely in cases where they grow intermixed with invasive hawkweeds, but the magnitude of impact is expected to be minor and possibly short-lived, which is supported by the low suitability for development of these species.

### **Reference:**

Paynter, Q., S. V. Fowler, A. H. Gourlay, P. G. Peterson, L. A. Smith, and C. J. Winks. 2015. Relative performance on test and target plants in laboratory tests predicts the risk of non-target attack in the field for arthropod weed biocontrol agents. Biological Control 80: 133–142. **Figure 1.** Overall acceptance and suitability of target and test plant species for *Cheilosia urbana*, calculated as the average number of eggs laid in open-field tests carried out between 2006 and 2008, multiplied by the average survival rate from neonate larva to adulthood in no-choice larval transfer tests. The letters indicate the first letters of the *Hieracium* and *Pilosella* species exposed: *P. caespitosa* (cae) and *P. aurantiaca* (aur) in the genus *Pilosella*, *H. canadense* (can) and *H. umbellatum* (umb) in the subgenus *Hieracium*, and the remaining *Hieracium* species in the subgenus *Chionoracium*, i.e. *H. albiflorum* (alb), *H. argutum* (arg), *H. carneum* (car), *H. fendleri* (fen), *H. gracile* (gra), *H. gronovii* (gro), *H. longipilum* (lon), *Hieracium scabrum* (sca), *H. scouleri* (sco), and *H. venosum* (ven).



#### **Decision and Finding of No Significant Impact**

for

#### Field Release of the Hoverfly *Cheilosia urbana* (Diptera: Syrphidae) for Biological Control of Invasive *Pilosella* Species Hawkweeds (Asteraceae) in the contiguous United States. July 2019

The U.S. Department of Agriculture (USDA), Animal and Plant Health Inspection Service (APHIS) is proposing to issue permits for environmental release of the insect *Cheilosia urbana* (Diptera: Syrphidae). This agent would be used for the biological control of invasive hawkweeds, *Pilosella* species (Asteraceae), in the contiguous United States. Before permits are issued for release of *C. urbana*, APHIS must analyze the potential impacts of its release into the contiguous United States in accordance with USDA, APHIS National Environmental Policy Act implementing regulations (7 Code of Federal Regulations Part 372). APHIS has prepared an environmental assessment (EA) that analyzes the potential environmental consequences of this action. The EA is available from:

U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine Pests, Pathogens, and Biocontrol Permits 4700 River Road, Unit 133 Riverdale, MD 20737 http://www.aphis.usda.gov/plant\_health/ea/index.shtml

The EA analyzed the following two alternatives in response to a request for permits authorizing environmental release of C, urbana: (1) no action, and (2) issue permits for the release of C. *urbana* for biological control of invasive hawkweeds (preferred alternative). A third alternative, to issue permits with special provisions or requirements concerning release procedures or mitigating measures, was considered. However, this alternative was dismissed because no issues were raised that indicated that special provisions or requirements were necessary. The No Action alternative, as described in the EA, would likely result in the continued use at the current level of chemical, mechanical, cultural, and biological controls for the management of invasive hawkweeds. These control methods described are not alternatives for decisions to be made by APHIS, but are presently being used to control invasive hawkweeds in the United States and may continue regardless of permit issuance for field release of C. urbana. Notice of this EA was made available in the Federal Register on May 28, 2019 for a 30-day public comment period. APHIS received 8 comments on the EA by the close of the comment period. Six comments were in favor of the release of the biological control agents. One comment was a general comment against APHIS but raised no substantive issues. One comment asked a question regarding impacts on native hawkweeds. This comment is addressed in appendix 6 of the EA.

I have decided to authorize APHIS to issue permits for the environmental release of *C. urbana*. The reasons for my decision are:

- *Chelosia urbana* is sufficiently host specific and poses little, if any, threat to the biological resources, including non-target plant species, of the contiguous United States.
- *Chelosia urbana* is not likely to adversely affect federally listed threatened and endangered species or their critical habitats in the contiguous United States.
- *Chelosia urbana* poses no threat to human health.
- No negative cumulative impacts are expected from release of Chelosia urbana.
- There are no disproportionate adverse effects to minorities, low-income populations, or children in accordance with Executive Order 12898 "Federal Actions to Address Environmental Justice in Minority Populations and Low-income Populations" and Executive Order 13045, "Protection of Children from Environmental Health Risks and Safety Risks."
- While there is not total assurance that the release of *C. urbana* into the environment will be reversible, there is no evidence that this organism will cause any adverse environmental effects.

I have determined that there would be no significant impact to the human environment from the implementation of the preferred alternative and, therefore, no Environmental Impact Statement needs to be prepared.

Steven Crook, Director Permitting and Coordination Compliance U.S. Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine

8/19

Date