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Annual Report 2016

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January 2017

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CABI Ref: VM10031
Issued January 2017

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Sponsored by:
Ministry of Forests, Lands and Natural Resource Operations, British Columbia
Alberta Invasive Species Council
Montana Noxious Weed Trust Fund through Montana State University

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Summary

1. Oxeye daisy, *Leucanthemum vulgare*, is a rhizomatous perennial that is native to Europe and that has become an aggressive invader in North America, particularly in pastures and meadows. While mowing and chemical control can be effective methods to control local infestations of oxeye daisy, there is a lack of methods suitable for the sustainable management of this invasive plant across invaded landscapes. Therefore, a project to investigate prospects for classical biological control of oxeye daisy was initiated in 2008.

2. In January 2016, we received comments on the test plant list that had been submitted to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) and the Canadian Biocontrol Review Committee (CBRC) in 2013. Based on the comments by the reviewers and after discussion with our North American partners we decided to add a few more native North American species to the list.

3. In 2016, additional no-choice larval development tests were conducted with the root-mining tortricid moth *Dichrorampha aeratana* using ten test plant species and varieties. Plants were dissected in autumn 2016. One test species was attacked: a few larvae were found in *Matricaria discoidea* plants dissected in August; but none were found during dissections in September.

4. In addition, a multiple-choice field cage test was set up with *D. aeratana* using four plant species that had been attacked under no-choice conditions or showed ambiguous results in previous tests. Plants were dissected in autumn. Larvae were found in only 25% of the oxeye daisies exposed and one larva was found in one plant of the ornamental *Ismelia carinata*.

5. In 2016, we started working with a new biological control candidate, the root-galling tephritid fly *Oxyna nebulosa*. A total of 382 galls were collected from three sites in the Czech Republic and no-choice tests were set up with 17 plant species and varieties. In autumn, plants were inspected for galls; galls were found on 44% of the control plants, but not on any of the test plants exposed.

6. A multiple-choice field cage test was set up with *O. nebulosa*, exposing three Shasta daisy varieties and oxeye daisy as control plants. All plants were dissected in autumn, but no galls or larvae were found on any of the plants exposed.

7. No studies were conducted with the flower-head attacking tephritid fly *Tephritis neesii* in 2016. However, its larvae and pupae were found in flower heads of Shasta daisy growing in the CABI garden. This indicates that Shasta daisies form part of the ecological host range of *T. neesii* and we decided to stop working with this species and eliminate it from the list of potential agents.

8. In summary, work in 2016 advanced well and the data collected are encouraging. In 2017, we will continue and possibly complete no-choice larval development tests with *D. aeratana.* Depending on results, we will prepare a petition for field release. In addition, we will collect more galls of *O. nebulosa* and continue with investigations on the biology and host-range of this potential biological control agent.

1. Introduction

Oxeye daisy (*Leucanthemum vulgare* Lam.; syn. *Chrysanthemum leucanthemum* L.) is a perennial herb of the Asteraceae family with showy flower heads. Originating from Europe, oxeye daisy has been introduced to many other parts of the world, including North America, South America, New Zealand, Australia, Hawaii, China and Pakistan (Holm *et al.*, 1979) as a contaminant of seed, as an ornamental or as a medicinal plant. In the north-eastern USA and the Canadian province of Quebec *L. vulgare* was reported to have naturalized by the 18th century (Fernald, 1903; Lavoie *et al*., 2012). It was introduced into the north-western USA as a contaminant of forage and grass seeds in the late 19th century (Forcella, 1985). Today, oxeye daisy occurs throughout most of temperate North America. Common oxeye daisies in Europe are represented by two morphologically very similar species: the diploid *L. vulgare* and the tetraploid *L. ircutianum.* Both species have been introduced to North America but field surveys have revealed that *L. vulgare* is much more common (Fernald, 1903; Mulligan, 1958, 1968; Stutz *et al*., 2014, 2016a).

In North America, oxeye daisy has become a particularly aggressive invader in pastures and meadows. Cattle generally avoid oxeye daisy and therefore any pasture infested with dense stands of the plant will produce less forage for grazing. Under high stocking rates livestock may physically damage oxeye daisy plants by trampling, but the subsequent overgrazing of desirable vegetation and soil disturbance will worsen the infestation (Olsen *et al.*, 1997). Persistent mowing and chemical applications can be effective methods to control local infestations of oxeye daisy. Application of fertilizer in pastures or meadows stimulates the growth of forage species and can also be an effective method to reduce oxeye daisy density (Cole, 1998). However, there is a lack of methods suitable for the sustainable management of this invasive plant across invaded landscapes. Classical biological control, i.e. the intentional introduction of host-specific natural enemies from the area of origin of an invasive plant into its exotic range, could be a valid option. A field survey revealed that *L. vulgare* in North America has largely escaped its leaf and root herbivores, and flower head herbivores are completely absent in the introduced range. Therefore, many empty feeding niches are potentially available for biological control agents in North America (Stutz *et al*., 2016a). In 2008, a project was initiated to investigate prospects for the biological control of oxeye daisy in North America. Initially, the project was financed by the Ministry of Forests, Lands and Natural Resource Operations of British Columbia. In 2010, the Montana Noxious Weed Trust Fund, through Montana State University and the US Department of Agriculture (USDA) Forest Service, joined in to form a North American consortium for the biological control of oxeye daisy. In 2012 and 2013, additional funding was provided by the Canadian Agricultural Adaptation Program with support from the Wyoming Biological Control Steering Committee, the Alberta Association of Agriculture Fieldmen, Canadian Pacific, Enbridge Pipelines Inc. and the Peace Region Forage Seed Association, and in 2015 and 2016 by the Alberta Invasive Species Council.

Based on literature surveys, eight species were prioritized as potential biological control agents because records suggested they have restricted host plant ranges: the root-mining moths *Dichrorampha aeratana* and *D*. *baixerasana*, the shoot-mining moth *D*. *consortana*, the root-feeding weevils *Cyphocleonus trisulcatus* and *Apion stolidum,* the root-galling tephritid fly *Oxyna nebulosa*, the flower-head attacking fly *Tephritis neesii* and the flower-head attacking weevil *Microplontus campestris*. *Microplontus campestris, C. trisulcatus* and *A. stolidum* were subsequently dropped from the list of potential agents owing to a lack of impact on seed output or host specificity (Schaffner *et al*. 2011, Stutz *et al*., 2012, 2014, 2015, 2016b). In 2016, work has concentrated on the two root-feeding herbivores *D. aeratana* and *O. nebulosa.*

2. Work Programme for Period under Report

The following work programme was proposed for 2016:

***Dichrorampha aeratana* (Lep., Tortricidae)**

- Continue and if possible complete no-choice larval development tests;
- Set up multiple-choice cage test with *Achillea ptarmica*, *Ismelia carinata* and *Matricaria chamomilla*;
- Start preparing a petition for field release.

***Oxyna nebulosa* (Dipt., Tephritidae)**

- Collect galls in Germany and the Czech Republic;
- Establish a rearing colony and study biology;
- Conduct host-range testing with critical test plant species.

3. Test Plant List

In January 2016, we received comments on the test plant list that had been submitted in 2013 to the Technical Advisory Group for Biological Control Agents of Weeds (TAG) and the Canadian Biocontrol Review Committee (CBRC) by Alec McClay (Stutz et al., 2014). Based on the comments by the reviewers and after discussion with our North American partners we decided to add a few more native North American species to the list which are either in the same tribe as oxeye daisy (*Artemisia tridentata*, *A. scopulorum*, and *A. arctica*), or representatives of more distantly related Asteraceae tribes (*Echinacea* sp., *Erigeron* sp. and *Symphyotrichum* sp.). Some of these species were included in host-range tests with *Dichrorampha aeratana* in 2016; the remaining ones will be tested in 2017.

4. *Dichrorampha aeratana* Pierce & Metcalfe (Lep., Tortricidae)

During a literature survey conducted at the beginning of this project, we found that 15 *Dichrorampha* species were reported to develop on *Leucanthemum* species. Three of these species, i.e. the root-mining *D. aeratana* and *D. baixerasana* and the shoot-mining *D. consortana,* are considered to be monophagous on oxeye daisy. In 2008, we found a site with a large population of *D. aeratana* in southern Switzerland, and we therefore decided to initially focus our laboratory and field studies on this species. The larvae of *D. aeratana* feed and overwinter inside the roots of oxeye daisy (Plate 1). Around March–April they leave the roots and pupate in the soil. Adult *D. aeratana* fly in May and June.

From 2011 onwards, we conducted no-choice larval development tests and found larvae in several varieties of the ornamental Shasta daisy (*Leucanthemum* × *superbum*) and in a few other test plant species. Shasta daisies as well as single *Leucanthemella serotina* and *Matricaria occidentalis* plants were also attacked under multiple-choice cage conditions. Shasta daisies were also attacked under open-field conditions, but at much lower levels than oxeye daisy. In an impact experiment, *D. aeratana* reduced both below-ground biomass and the number of flowers of potted *L. vulgare* plants by 62% but, had no measurable impact on the Shasta daisy variety *Leucanthemum* × *superbum* Amelia.

Plate 1. *Dichrorampha* larva in root of oxeye daisy (left) and adult *D. aeratana* (right).

4.1. Rearing and field collections

Unlike previous years, overwintering survival of *D. aeratana* in 2016 was very low: from 28 April to 6 May only 13 females and 34 males emerged from the more than 80 potted oxeye daisy plants that had been infested with 5–10 larvae each in 2015. The reason for the extremely low overwintering survival is unclear, but it might be connected with the exceptionally high temperatures in late autumn and winter 2015/16. These high temperatures might have triggered pupation in late autumn instead of early spring as usual. The pupae might then have suffered high mortality, since they are probably not adapted to long periods of cold temperatures.

To increase the number of adults and larvae available for host-range tests, a field trip to southern Switzerland (Plate 2) was conducted on 16 May 2016 and 21 females and 20 males were collected. To further increase our rearing colony an additional field trip to southern Switzerland was conducted on 26 September and 317 rosettes were collected. All rosettes were dissected and a total of 167 larvae were found. All live larvae (n = 139) were transferred on potted oxeye daisies and, together with the rearing plants set up in spring and autumn, our rearing colony now comprises 86 potted plants infested with 5–10 larvae each.

Plate 2. Meadow in southern Switzerland where adults and larvae of *Dichrorampha aeratana* were collected (left) and set-up for a multiple-choice cage test with *D. aeratana* (right).

4.2. No-choice larval development tests

METHODS In May 2016, we set up no-choice larval development tests with 1–10 replicates of ten test plant species and varieties (Table 1). Plants from four *L. vulgare* populations (from the Czech Republic, Alberta, British Columbia, and Colorado) and one *L. ircutianum* population (from Austria) were used as controls. Five freshly hatched larvae were transferred with a thin paintbrush onto the petioles of each of the potted test and control plants. The pots were kept for one day in the laboratory and then most were transferred to field cages in the CABI garden. Plant species that had been heavily attacked by slugs in the garden in previous years (*Arctanthemum arcticum* and *Daucus carota*) and plants that were still relatively small when infested with larvae (*Symphyotrichum laeve* and *Echinacea purpurea*) as well as five oxeye daisy plants were transferred to an unheated greenhouse instead. In August and September 2016 all plants were dissected for larvae.

RESULTS On average 59% of the larvae that had been transferred in spring were found alive in *L. vulgare,* but only 28% of the transferred larvae were recovered alive from *L. ircutianum* (Table 1). Larval survival was similar for all tested *L. vulgare* populations. Larval survival was higher in oxeye daisies that were kept outside than in those kept in the greenhouse, and only two larvae were found in one of the oxeye daisy plants kept in the greenhouse. A single larva was also found alive in each of three *Matricaria discoidea* plants, a species in which no larvae had been found during previous testing in 2011. The larvae were still very small and were all found in plants dissected in August (n = 5), while none were found during plant dissections in September (n = 5). *Matricaria discoidea* started to senesce in August but the roots were still intact when the plants were dissected in September. No larvae were found in any of the other test plant species.

Table 1. Results of no-choice larval development tests for *Dichrorampha aeratana* from 2011 to 2016

Test plant species	2011–2015			2016		
	No. replicates	% plants infested[a]	% larvae found/plant (mean ± SE)	No. replicates	% plants infested	% larvae found/plant (mean ± SE)
Tribe Anthemideae						
Subtribe Leucantheminae						
Leucanthemum vulgare	171	93.6	49.7 ± 2.1	20	75.0	59.0 ± 9.5
Leucanthemum ircutianum	127	86.7	48.0 ± 3.0	5	80.0	28.0 ± 10.2
Leucanthemum × superbum Alaska	7	42.9	10.0 ± 4.0			
Leucanthemum × superbum Amelia	6	100.0	43.3 ± 8.0			
Leucanthemum × superbum Crazy Daisy	4	100.0	25.0 ± 5.0			
Leucanthemum × superbum Marconi Double	5	20.0	4.0 ± 4.0			
Leucanthemum × superbum Silver Princess	3	66.7	13.2 ± 6.7			
Leucanthemum × superbum Snow Lady	5	20.0	5.0 ± 4.4			
Leucanthemum × maximum	3	100.0	26.7 ± 6.7			
Subtribe Anthemidinae						
Anthemis arvensis	15	0.0				
Anthemis cotula	13	54.3	7.7 ± 5.1			
Tanacetum camphoratum[b]	7	0.0				
Tanacetum cinerariifolium	7	0.0				
Tanacetum huronense[b]	6	0.0		1	0.0	
Tanacetum vulgare	7	0.0				
Tripleurospermum inodorum	7	0.0				
Subtribe Matricariinae						
Matricaria chamomilla	23	8.7	1.7 ± 1.2			
Matricaria discoidea[b]	7	0.0		10	30.0	6.0 ± 3.1
Matricaria occidentalis[b]	18	38.9	2.2 ± 1.5			
Achillea alpina[b]	14	0.0				
Achillea borealis[b]	11	0.0				
Achillea ptarmica	22	4.5	0.9 ± 0.9			
Subtribe Santolininae						
Chamaemelum nobile	7	0.0				
Santolina chamaecyparissus	7	0.0				
Subtribe Glebionidinae						
Glebionis coronaria	7	0.0				
Glebionis segetum	7	0.0				
Ismelia carinata	7	14.3	2.9 ± 2.9	6	0.0	
Argyranthemum frutescens	8	0.0				
Subtribe Artemisiinae						
Artemisia biennis[b]	7	0.0				
Artemisia campestris[b]	7	0.0				
Artemisia californica[b]	7	0.0				
Artemisia cana[b]	7	0.0				
Artemisia dracunculus[b]	7	0.0				

Test plant species	2011–2015			2016		
	No. replicates	% plants infested[a]	% larvae found/plant (mean ± SE)	No. replicates	% plants infested	% larvae found/plant (mean ± SE)
Artemisia filifolia[b]	10	0.0				
Artemisia frigida[b]	7	0.0				
Artemisia ludoviciana[b]	7	0.0				
Artemisia scopulorum[b]	1	0.0				
Artemisia spinescens[b]				1	0.0	
Artemisia tridentata[b]	7	0.0				
Artemisia vulgaris[b]	7	0.0				
Arctanthemum arcticum (ornamental)	7	0.0				
Arctanthemum arcticum[b]	4	0.0		2	0.0	
“Chrysanthemum × grandiflorum” Garden Mums	9	0.0				
“Chrysanthemum× grandiflorum” Morden Canary				7	0.0	
“Chrysanthemum × grandiflorum” Morden Delight				7	0.0	
Leucanthemella serotina	14	21.4	4.3 ± 2.3			
Subtribe Cotulinae						
Cotula coronopifolia	6	0.0				
From other tribes						
Anaphalis margaritacea[b]	7	0.0				
Arnica chamissonis[b]	9	0.0				
Carthamus tinctorius	8	0.0				
Cichorium intybus	7	0.0				
Cirsium flodmanii[b]	6	0.0				
Coreopsis tinctoria[b]	7	0.0				
Cynara scolymus	7	0.0				
Daucus carota	5			8	0.0	
Echinacea purpurea				6	0.0	
Lobelia cardinalis[b]	7					
Eutrochium maculatum[b]	8	0.0				
Helenium autumnale[b]	7	0.0				
Helianthus annuus[b]	9	0.0				
Lactuca sativa	10	0.0				
Petroselinum crispum	7	0.0				
Senecio eremophilus[b]	7	0.0				
Solidago nemoralis[b]	9	0.0				
Symphyotrichum laeve[b]				6	0.0	
Tagetes lucida	7	0.0				

[a] Includes plants in which larvae or feeding traces (in the case of dead plants) were found.
[b] Plant species native to North America.

4.3. Multiple-choice tests in field cages

We set up a multiple-choice cage test with four species that had either supported development under no-choice conditions (*Matricaria chamomilla*) or had ambiguous results in previous tests (*Achillea ptarmica*, *Ismelia carinata* and *M. discoidea*).

METHODS We established four field cages (2 m × 2 m × 1.6 m) with potted plants of *A. ptarmica, I. carinata, M. chamomilla* and *M. discoidea* as well as *L. vulgare* as control plants. Three plants of each species were randomly distributed within each field cage (Plate 2). Owing to the limited number of plants available, *A. ptarmica* was only present in three of the four cages. From 5 to 18 May a total of six egg-laying females (2–3 females from our rearing colony and 3–4 females collected in the field in southern Switzerland) were released into each field cage. All plants were dissected in August or September and the number of larvae was counted.

RESULTS AND DISCUSSION Unfortunately, larvae were found in plants from only two of the four field cages and in total only three of the twelve control plants were attacked. In one of the cages 5.0 ± 2.6 larvae were found in *L. vulgare* and one larva was found in one of the three *I. carinata* plants. In addition, one larva was found in *L. vulgare* in another field cage. No larvae were found in any of the *M. chamomilla*, *M. discoidea* or *A. ptarmica* plants exposed. The low attack rates on the control plants are in contrast to those found in similar tests conducted in previous years where 85–100% of the control plants were attacked (Stutz *et al*., 2013, 2014) and might have been due to the release of field-collected females. *Dichrorampha aeratana* females are very short-lived and lay a large proportion of their eggs within a few days of emerging. Therefore, the field-collected females might already have been too old to lay a large number of eggs. As in previous years, all females had been checked for egg-laying by individually placing them in plastic cylinders containing oxeye daisy leaves and only females that laid eggs were released into the cages. However, it was later observed that no larvae emerged from a relatively large number of the eggs laid in the plastic cylinders, indicating that some of the females did not lay fertile eggs. This was only very occasionally observed in previous years.

4.4. Conclusions and outlook

Since 2011 we have conducted no-choice larval development tests with 64 test plant species and varieties for the moth *D. aeratana* (Table 1). Most of the species did not support larval development. A few larvae were found in six test plant species outside the genus *Leucanthemum* (*Anthemis cotula*, *Ismelia carinata*, *Leucanthemella serotina*, *Matricaria chamomilla*, *M. discoidea* and *M. occidentalis*) as well as in all tested Shasta daisy varieties. In addition, a single larva was found on one additional test plant species (*Achillea ptarmica*) but since a total of 22 plants were tested and only one larva was found on one of them it is very likely that it resulted from contamination with other *Dichrorampha* species. The annuals *M. discoidea* and *M. occidentalis* are the only species native to North America that have been attacked. At CABI, plants of these species usually senesce in August, which is long before larval development is completed. We therefore believe that it is unlikely that *D. aeratana* can successfully develop on *M. discoidea* or the closely related *M. occidentalis.* This was confirmed for *M. occidentalis* by development tests conducted in 2013/2014 and 2014/15, when a total of 11 *M. occidentalis* plants that had been infested with larvae in spring were overwintered in CABI's garden and no adults emerged from any of the plants. The fact that larvae found in *M. discoidea* in August 2016 were still very small and no larvae were found in plants dissected in

September supports our assumption that it is unlikely that *D. aeratana* can complete its development on *M. discoidea.*

Under multiple-choice cage conditions we found one larva each in single plants of *I. carinata, Leucanthemella serotina* and *M. occidentalis*, and fewer larvae in Shasta daisies than in oxeye daisies. In open-field tests we found no larvae in test plant species outside the genus *Leucanthemum* and only a few larvae in Shasta daisies. In addition, we found that development from egg to adult was much less frequent in all tested Shasta daisy varieties than in oxeye daisy. An impact experiment that had been set up with *L. vulgare* and one of the Shasta daisy varieties in 2013 revealed that *D. aeratana* has a negative impact on the biomass and number of flowers of oxeye daisies but no impact on the Shasta daisy variety exposed. We conclude that although *D. aeratana* may attack and complete development on Shasta daisies under field conditions it is unlikely to impact their ornamental value.

Host-range testing with *D. aeratana* is almost complete and the data collected in the last six years look encouraging. Only a few species that were added to the test plant list after the review by TAG and CBRC in 2016 as well as the native *Hulteniella integrifolia* still need to be tested under no-choice conditions. Although in past years several attempts were made to grow *H. integrifolia* from seeds and field-collected plants were shipped to CABI in 2016, none of the plants survived long enough to be included in the host-range tests. We are currently looking into the possibility of conducting no-choice tests for this species in the quarantine facility at Agriculture and Agri-Food Canada (AAFC) in Lethbridge. In addition, we will repeat the multiple-choice cage test with *Ismelia carinata*, *Matricaria chamomilla* and *M. discoidea.* Depending on the results, we will prepare a petition for field release in collaboration with our North American partners.

5. *Oxyna nebulosa* WIEDEMANN (Dipt., Tephritidae)

Oxyna nebulosa (syn. *Tephritis proboscidea*) is a root-galling tephritid fly (Plate 3) that is widely distributed across Europe. It is almost exclusively reported from *Leucanthemum* species although Lemée (1902, as cited in Houard, 1909), mentions *Tanacetum corymbosum* as an additional host plant. However, this has not been confirmed by other sources and it is therefore possible that *O. nebulosa* is monophagous on *Leucanthemum* species. Adults are reported to fly from late June to August (Baugnée, 2006). The species is relatively rare in Europe, but populations with relatively high attack rates were found during field surveys conducted in the Czech Republic in 2013. In addition, a few galls were found on oxeye daisy sites in and around Regensburg (Germany) in 2012.

5.1. Field collections, emergence and studies on the biology

From 14 to 15 April 2016, we visited the two sites in and around Regensburg where we had found a few galls of *O. nebulosa* in 2012 and collected about 200 plants. All plants were dissected in the laboratory, but only one gall containing one larva was found.

From 6 to 10 June, we visited several field sites in the northern parts of the Czech Republic where we had found galls of *O. nebulosa* in 2013. We collected a total of 382 galls from three sites. A few galls were dissected and one or several pupae were found inside (Plate 3). All galls were placed into plastic containers partly filled with moist vermiculite. The boxes were placed in a wooden shelter at ambient temperatures and regularly checked for adult emergence. From 17 June to 22 July, a total of 118 females and 101 males of *O. nebulosa* (Table 2) and a large number of parasitoids emerged. Emerging *O. nebulosa* were placed into plastic cylinders containing a small potted oxeye daisy rosette and small Petri dishes containing sugar, yeast and milk powder. The rosettes were regularly dissected for eggs and replaced by new ones. Females started to lay eggs about four days after emergence. Since the eggs were found only in the leaves and leaf axils (Plate 3) and not in the roots we later changed the set-up to test for egg-laying, using cut rosettes inserted in moist florist sponge instead of potted rosettes. Only females that laid eggs were used for subsequent host-range tests.

Table 2. Number of *Oxyna nebulosa* galls collected from three sites in the Czech Republic in 2016 and number of adults that emerged from these galls.

Site	No. galls collected	No. females emerged	No. males emerged	Total no. adults emerged	No. adults per gall
CZ20A	313	85	84	169	0.5
CZ21A	34	12	20	32	0.9
CZ23A	35	13	5	18	0.5
Total	382	110	109	219	0.6

Plate 3. Above: Galls of the tephritid fly *Oxyna nebulosa* on a root of oxeye daisy (left). The arrows show the pupae within the gall (right). Below: Adult of *O. nebulosa* on a shoot of oxeye daisy (left) and eggs of *O. nebulosa* in a leaf of oxeye daisy (right).

5.2. No-choice test

METHODS Between 27 June and 28 July we set up no-choice oviposition and development tests with *O. nebulosa*. One or two females and up to two males were placed onto individually potted, gauze-covered test and control (*L. vulgare* and *L. ircutianum*) plants. Small Petri dishes containing sugar, milk powder and yeast were placed in the pots as food sources. After 4–10 days the surviving flies were retrieved from the plants and the plants were moved to a gauze-covered field cage where they were embedded in sawdust. The retrieved females were individually placed for 2–3 days into plastic cylinders together with a rosette of oxeye daisy. Egg-laying females were then re-used for host-range testing. In total, we were able to expose 52 plants to *O. nebulosa*: 18 were controls and the remainder were plants of 17 test species and varieties (1–4 replicates per test plant species; Table 3). In September, all plants were visually checked for galls, and plants on which no galls were found were dissected. All attacked plants will be individually covered with gauze bags and regularly checked for adult emergence in spring 2017.

RESULTS In September, galls were found on 44% of the control plants (*L. vulgare* and *L. ircutianum*) but no galls or larvae were found on any of the test plant species exposed (Table 3). An average of 1.1 ± 0.4 galls per plant were found on the control plants.

Table 3. Results of no-choice oviposition and development tests with *Oxyna nebulosa* in 2016.

Test plant species	No. replicates	% plants attacked	No. galls per plant (mean ± SE)
Tribe Anthemideae			
Subtribe Leucantheminae			
Leucanthemum vulgare	15	46.7	1.2 ± 0.7
Leucanthemum ircutianum	3	33.3	0.7 ± 0.4
Leucanthemum × superbum Alaska	2	0.0	
Leucanthemum × superbum Crazy Daisy	2	0.0	
Leucanthemum × maximum	2	0.0	
Subtribe Anthemidinae			
Tanacetum camphoratum[a]	3	0.0	
Tanacetum corymbosum	2	0.0	
Tanacetum huronense[a]	3	0.0	
Tanacetum vulgare	1	0.0	
Subtribe Matricariinae			
Achillea alpina[a]	4	0.0	
Achillea borealis[a]	3	0.0	
Achillea ptarmica	1	0.0	
Subtribe Santolininae			
Chamaemelum nobile	2	0.0	
Subtribe Artemisiinae			
Artemisia campestris[a]	1	0.0	
Artemisia cana[a]	1	0.0	
Artemisia frigida[a]	1	0.0	
Arctanthemum arcticum (ornamental)	2	0.0	
"Chrysanthemum × grandiflorum" Garden Mums	1	0.0	
Leucanthemella serotina	3	0.0	

[a] Plant species native to North America.

5.3. Multiple-choice cage test

Since all potential biological control agents for oxeye daisy tested so far also attack the closely related Shasta daisy under no-choice conditions, we expected similar results for *O. nebulosa* and therefore also set up a multiple-choice cage test with *O. nebulosa* in which we exposed three Shasta daisy varieties.

METHODS One field cage containing two potted plants of each of the Shasta daisy varieties 'Alaska', 'Crazy Daisy' and 'Maximum' and six potted oxeye daisies (four *L. vulgare* and two *L. vulgare*) was set up in July. On 15 July, seven egg-laying females and four males were released into the cage. All plants were dissected in September.

RESULTS No larvae or galls were found on any of the control or test plants.

5.4. Conclusions and outlook

The fact that no galls were found on any of the test plants exposed under no-choice conditions indicates that *O. nebulosa* has a narrow host range. However, only 44% of the control plants developed galls and clearly, more replicates are necessary to reach meaningful conclusions. It is unclear whether the low number of galls found on oxeye daisies was the result of a low number of eggs laid by females and/or due to low larval survival. Unfortunately, some weeks after the exposure to *O. nebulosa* the plants were attacked by aphids, which resulted in partial defoliation. Since our observations in the laboratory indicated that females oviposit in leaves and leaf axils, defoliation may have meant some larvae did not reach the roots and died.

It is also unclear why no galls were found on any of the plants exposed in the field cage. Potentially, the flies might have been eaten by predators (e.g. spiders) soon after they were released into the field cage but before they could lay eggs. Equally, they might have died prematurely from lack of food, but several of the plants exposed in the cage had open flower heads and the flies therefore had access to pollen. Like the plants from the no-choice tests, the plants used in the multiple-choice test suffered from attack by aphids with the potential consequence that larvae died due to defoliation.

Early June proved to be the perfect time for the collection of *O. nebulosa* galls in the Czech Republic and the first adults emerged one week after the galls had been collected. Because the parasitism rate was relatively high the number of adults that emerged from the large number of collected galls was lower than expected. In 2017, we plan to find more field sites in the Czech Republic and to collect more galls. In addition, we plan to conduct more detailed studies on the biology of *O. nebulosa* in order to improve the methods for host-range tests. Furthermore, we plan to conduct no-choice tests with a small number of test plant species.

6. *Tephritis neesii* MEIGEN (Dipt., Tephritidae)

Another potential biological control candidate for oxeye daisy is the flower-head attacking fly *Tephritis neesii*. The larvae feed in the receptacle and on the developing seeds, thereby reducing seed output (Robinson, 2008). *Tephritis neesii* pupates in the flower heads and adults emerge in summer. It has one generation per year, and overwintering occurs in the adult stage. *Tephritis neesii* is very common in central and western Europe but seems to be rare in southern Europe.

No studies were conducted with *T. neesii* in 2016 but its larvae and pupae were found in flower heads of Shasta daisy growing in the CABI garden. Since this indicates that *T. neesii* would attack Shasta daisies growing in gardens in North America and because root herbivores are likely to be more effective than seed herbivores in controlling invasive oxeye daisies we decided to stop working with this species and eliminate it from the list of potential agents.

7. Work Programme Proposed for 2017

Based on the results of our work in 2016, we propose the following work programme for the coming season.

***Dichrorampha aeratana* (Lep., Tortricidae)**

- Continue and if possible complete no-choice larval development tests;
- Repeat multiple-choice cage test with *Ismelia carinata*, *Matricaria chamomilla* and *M. discoidea*;
- Provided a sufficient number of moths is available, set up open-field test with *I. carinata*, *M. chamomilla* and *M. discoidea*;
- Prepare petition for field release.

***Oxyna nebulosa* (Dipt., Tephritidae)**

- Collect galls in the Czech Republic;
- Establish a rearing colony and study biology;
- Improve methods for host-range tests;
- Conduct no-choice and multiple-choice tests with critical test plant species.

8. Acknowledgements

We thank Prof. Dr Christoph Oberprieler (University of Regensburg, Germany) and Ondřej Kauzál (Charles University in Prague) for their help with collecting *Oxyna nebulosa* in Regensburg and in the Czech Republic, respectively. We would also like to thank Florence Willemin, Lise Berberat and Christian Leschenne (all CABI) for plant propagation and Anouchka Perret-Gentil (CABI) for additional technical assistance.

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