



Annual Report 2015

Prospects for the biological control of oxeye daisy, *Leucanthemum vulgare*

S. Stutz, S. Ribeiro, H.L. Hinz and U. Schaffner

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Table of contents

Summary.....	1
1. Introduction	2
2. Work Programme for Period under Report.....	3
3. <i>Dichrorampha aeratana</i> PIERCE & METCALFE (Lep., Tortricidae).....	3
3.1. Susceptibility of various Shasta daisy varieties to <i>Dichrorampha aeratana</i>	4
3.2. No-choice larval development tests.....	5
3.3. Conclusions and outlook	8
4. <i>Cyphocleonus trisulcatus</i> HERBST (Col., Curculionidae)	8
4.1. No-choice oviposition and development tests	9
4.2. Multiple-choice field cage test	12
4.3. Multiple-choice open-field test.....	12
4.4. Impact experiment.....	14
4.5. Conclusions and outlook	15
5. <i>Tephritis neesii</i> MEIGEN (Dipt., Tephritidae).....	16
5.1. Overwintering experiment.....	16
5.2. Conclusions and outlook	17
6. <i>Oxyna nebulosa</i> WIEDEMANN (Dipt., Tephritidae).....	17
7. Work Programme Proposed for 2016	18
8. Acknowledgements	18
9. References.....	18

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 2015, additional no-choice larval development tests were conducted with the root-mining tortricid moth *Dichrorampha aeratana* using 11 test plant species. Plants were dissected in autumn 2015. Only *Ismelia carinata* and *Matricaria chamomilla* were infested with one larva each.
3. In spring 2015, adult emergence was recorded from a field cage test that had been set up in spring 2014 with nine Shasta daisy varieties to test if they differ in their suitability for larval development of *D. aeratana*. Adult emergence was very low on all of the tested Shasta daisy varieties and on average ten times lower than on oxeye daisy.
4. In 2015, we continued with no-choice oviposition and development tests with the root-feeding weevil *Cyphocleonus trisulcatus* using 34 test plant species and varieties. Larvae emerged from one of the test plants. A multiple-choice field cage test was set up with three plant species that had been attacked under no-choice conditions in previous tests. Plants were dissected six weeks later and a few larvae were found on *Matricaria chamomilla* and *M. occidentalis*. In an open-field test that was set up with Shasta daisies and oxeye daisies, a similar number of adults emerged from oxeye daisies as from Shasta daisies.
5. The impact experiment that was set up in 2014 revealed that *C. trisulcatus* reduced the number of flower heads and above ground biomass of potted oxeye daisies by 44% and below ground biomass by 68%.
6. In winter 2014/2015, different set-ups have been tested to improve the overwintering survival of the flower-head attacking fly *Tephritis neesii*. Overwintering survival was generally very low and lowest when flies were kept in field cages at ambient temperatures and highest when kept in cylinders in an incubator at constant temperatures.
7. In summary, work in 2015 advanced well and the data collected are encouraging. In 2016, 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. After discussions with our North American collaborators we decided to stop working on *C. trisulcatus* due to the high levels of attack on ornamental Shasta daisies under open-field conditions. Instead, we plan to start working with a new biological control candidate, the root-galling tephritid fly *Oxyna nebulosa*.

1. Introduction

Oxeye daisy (*Leucanthemum vulgare* Lam.; synonym *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 surveys revealed that *L. vulgare* is much more common (Fernald, 1903; Mulligan, 1958, 1968; Morton, 1981, Stutz *et al.*, 2014).

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 alternative. In 2008, a project was therefore 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 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 by the Alberta Invasive Species Council.

Based on literature surveys eight species were prioritized as potential biological control agents based on records of their restricted host range: 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 *Oxya nebulosa*, the flower-head attacking fly *Tephritis neesii* and the flower-head attacking weevil *Microplontus campestris*. *Microplontus campestris* and *A. stolidum* were subsequently dropped from the list of potential agents due to a lack of impact on seed output or host specificity, respectively (Schaffner *et al.* 2011, Stutz *et al.*, 2012, 2014, 2015). In 2015, work has concentrated on the two root feeding herbivores *D. aeratana* and *C. trisulcatus*.

2. Work Programme for Period under Report

The following work programme was proposed for 2015:

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

- Continue and if possible complete no-choice larval development tests;
- Record adult emergence from various Shasta daisy varieties and *Matricaria occidentalis*;
- Start preparing a petition for field release.

***Cyphocleonus trisulcatus* (Col., Curculionidae)**

- Continue no-choice oviposition and development tests;
- Conduct open-field test with Shasta daisies;
- Conduct multiple-choice cage test with *Glebionis coronaria*, *Matricaria chamomilla* and *M. occidentalis*;
- Continue investigations on the impact of *C. trisulcatus*.

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

- Test several set-ups to increase overwintering survival.

3. ***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 established no-choice larval development tests and found larvae on several varieties of the ornamental Shasta daisy (*Leucanthemum x superbum*) as well as on 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. An impact experiment revealed that *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 x superbum* Amelia.



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

3.1. Susceptibility of various Shasta daisy varieties to *Dichrorampha aeratana*

In 2014, a cage test with nine Shasta daisy varieties had been set up to investigate whether they differ in their suitability for larval development of *D. aeratana* (see section 3.5 in Stutz *et al.*, 2015).

METHODS In spring 2014, five field cages were set up with two plants each of nine Shasta daisy varieties as well as *L. vulgare* and *L. ircutianum*. Six females were released into each of the cages and the number of eggs found on each plant was recorded two weeks later. Plants with fewer than two eggs ($n = 38$) were rearranged in two new field cages and another four females were released. Two weeks later the number of eggs found on each plant was recorded again. Eggs were found on all except six Shasta daisy plants. In spring 2015, all plants were individually covered with gauze bags, and adult as well as parasitoid emergence was recorded from 21 April to 29 May to compare them with the number of eggs that had been found in 2014.

RESULTS An average of 2 adults and 1 parasitoid emerged from oxeye daisies and 0.2 adults and 0.04 parasitoids from Shasta daisies (Fig. 1). On average, 19% of the eggs laid on oxeye daisies and 4% of the eggs laid on Shasta daisies developed to adults. On average, adults or parasitoids emerged from 75% of the oxeye daisies and from 21% of the Shasta daisies. Adult emergence was very low on all of the tested Shasta daisy varieties.

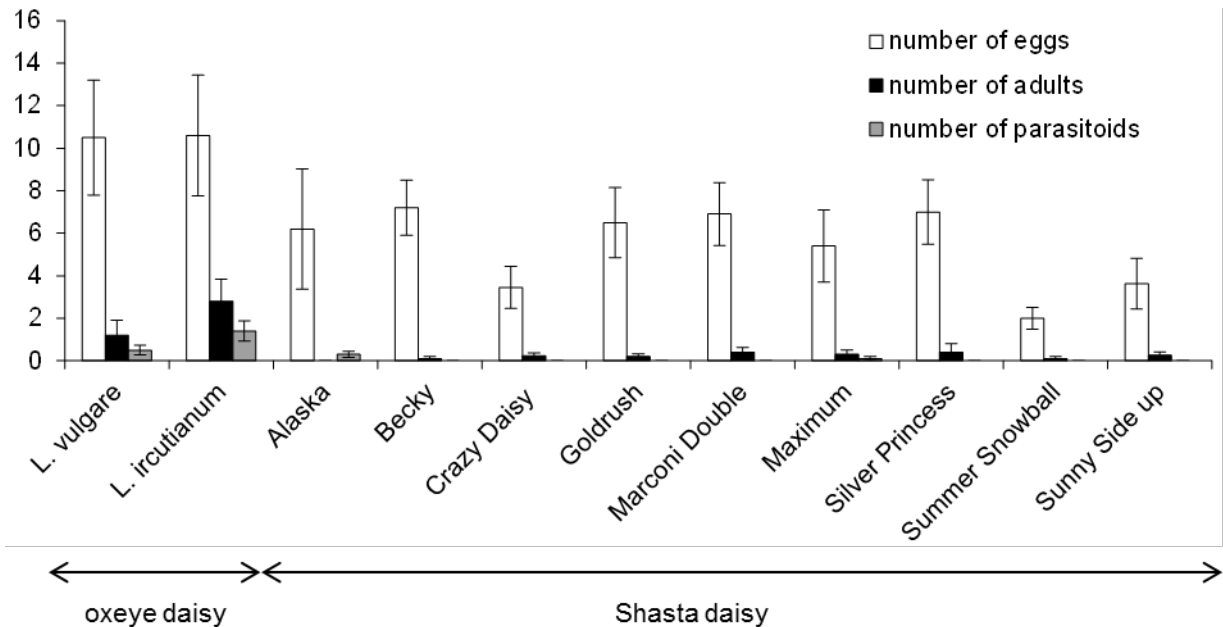


Fig. 1. Mean (\pm SE) number of eggs found on and number of adults and parasitoid emerged from oxeye daisy (*Leucanthemum vulgare* and *L. ircutianum*) and nine Shasta daisy varieties exposed to females of *Dichrorampha aeratana* in a field cage.

3.2. No-choice larval development tests

METHODS In May 2015, we set up no-choice larval development tests with 1–11 replicates of 11 test plant species (Table 1). Plants from four *L. vulgare* populations (from British Columbia, Montana and Washington) and one *L. ircutianum* population (from British Columbia) from the invaded range 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 transferred to field cages in the CABI garden. In August and September 2015 all plants were dissected for larvae.

RESULTS Similar to previous years, about half of the larvae that had been transferred in spring were found alive in the control plants (*L. vulgare* and *L. ircutianum*) (Table 1). Larval survival was similar for all tested *L. vulgare* and *L. ircutianum* populations. One larva was found alive in *Matricaria chamomilla*, a species in which one larva had been found in 2014. No larvae were found in any of the other test plant species. Interestingly, no larvae were found in *Achillea ptarmica*, a species in which one larva had been found in 2013.

Table 1. Results of no-choice larval development tests for *Dichrorampha aeratana* from 2011 to 2015

Test plant species	2011 – 2014			2015		
	No. replicates	% plants infested ^a	% larvae found/plant (mean ± SE)	No. replicates	% plants infested	% larvae found/plant (mean ± SE)
Tribe Anthemideae						
Subtribe Leucanthemeinae						
<i>Leucanthemum vulgare</i>	156	92.9	50.0 ± 3.2	15	100.0	46.7 ± 6.7
<i>Leucanthemum irtutianum</i>	124	86.3	48.1 ± 3.0	3	100.0	46.7 ± 17.6
<i>Leucanthemum</i> × <i>superbum</i> Alaska	7	42.9	10.0 ± 4.0			
<i>Leucanthemum</i> × <i>superbum</i> Amelia	6	100.0	43.3 ± 8.0			
<i>Leucanthemum</i> × <i>superbum</i> Crazy Daisy	4	100.0	25.0 ± 5.0			
<i>Leucanthemum</i> × <i>superbum</i> Marconi Double	5	20.0	4.0 ± 4.0			
<i>Leucanthemum</i> × <i>superbum</i> Silver Princess	3	66.7	13.2 ± 6.7			
<i>Leucanthemum</i> × <i>superbum</i> Snow Lady	5	20.0	5.0 ± 4.4			
<i>Leucanthemum</i> × <i>maximum</i>	3	100.0	26.7 ± 6.7			
Subtribe Anthemidinae						
<i>Anthemis arvensis</i>	15	0.0				
<i>Anthemis cotula</i>	13	54.3	7.7 ± 5.1			
<i>Tanacetum camphoratum</i> ^b	7	0.0				
<i>Tanacetum cinerariifolium</i>	7	0.0				
<i>Tanacetum huronense</i> ^b	6	0.0				
<i>Tanacetum vulgare</i>	7	0.0				
<i>Tripleurospermum inodorum</i>	7	0.0				
Subtribe Matricariinae						
<i>Matricaria chamomilla</i>	12	8.3	1.7 ± 1.7	11	9.1	1.8 ± 1.8
<i>Matricaria discoidea</i> ^p	7	0.0				
<i>Matricaria occidentalis</i> ^b	18	38.9	2.2 ± 1.5			
<i>Achillea alpina</i> ^b	14	0.0				
<i>Achillea borealis</i> ^b	11	0.0				
<i>Achillea ptarmica</i>	16	6.3	0.1 ± 0.1	6	0.0	
Subtribe Santolininae						
<i>Chamaemelum nobile</i>	7	0.0				
<i>Santolina chamaecyparissus</i>	7	0.0				
Subtribe Glebionidinae						
<i>Glebionis coronaria</i>	7	0.0				
<i>Glebionis segetum</i>	7	0.0				
<i>Ismelia carinata</i>	6	0.0		1	100.0	20.0
<i>Argyranthemum frutescens</i>	8	0.0				

Test plant species	2011 – 2014			2015		
	No. replicates	% plants infested ^a	% larvae found/plant (mean ± SE)	No. replicates	% plants infested	% larvae found/plant (mean ± SE)
Subtribe Artemisiinae						
<i>Artemisia biennis</i> ^b	7	0.0				
<i>Artemisia campestris</i> ^b	6	0.0		1	0.0	
<i>Artemisia californica</i> ^b				7	0.0	
<i>Artemisia cana</i> ^b	7	0.0				
<i>Artemisia dracunculus</i> ^b	7	0.0				
<i>Artemisia filifolia</i> ^b	2	0.0		8	0.0	
<i>Artemisia frigida</i> ^b	7	0.0				
<i>Artemisia ludoviciana</i> ^b	7	0.0				
<i>Artemisia scopulorum</i> ^b	1	0.0				
<i>Artemisia tridentata</i> ^b	7	0.0				
<i>Artemisia vulgaris</i> ^b	7	0.0				
<i>Arctanthemum arcticum</i> (ornamental)	7	0.0				
<i>Arctanthemum arcticum</i> ^b				4	0.0	
" <i>Chrysanthemum x grandiflorum</i> " gMums				9	0.0	
<i>Leucanthemella serotina</i>	14	21.4	4.3 ± 2.3			
Subtribe Cotulinae						
<i>Cotula coronopifolia</i>	6	0.0				
Other tribes						
<i>Anaphalis margaritacea</i> ^b	7	0.0				
<i>Arnica chamissonis</i> ^b	9	0.0				
<i>Carthamus tinctorius</i>	8	0.0				
<i>Cichorium intybus</i>	7	0.0				
<i>Cirsium flodmanii</i> ^b	6	0.0				
<i>Coreopsis tinctoria</i> ^b	7	0.0				
<i>Cynara scolymus</i>	4	0.0		3	0.0	
<i>Daucus carota</i>	2			3	0.0	
<i>Lobelia cardinalis</i> ^b				7	0.0	
<i>Eutrochium maculatum</i> ^b	8	0.0				
<i>Helenium autumnale</i> ^b	7	0.0				
<i>Helianthus annuus</i> ^b	9	0.0				
<i>Lactuca sativa</i>	6	0.0		4	0.0	
<i>Petroselinum crispum</i>	7	0.0				
<i>Senecio eremophilus</i> ^b	7	0.0				
<i>Solidago nemoralis</i> ^b	9	0.0				
<i>Tagetes lucida</i>	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.

3.3. Conclusions and outlook

Since 2011 we have established no-choice larval development tests with 58 test plant species and varieties for the moth *D. aeratana*. Most of the species did not support larval development. A few larvae were found in four test plant species outside the genus *Leucanthemum* as well as in all tested Shasta daisy varieties. In addition, a single larva was found in two additional test plant species but it is unclear if they resulted from contamination with other *Dichrorampha* species. The annual species *Matricaria occidentalis* is the only species native to North America that has been attacked. At CABI, plants of this 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. occidentalis*. This was confirmed by development tests conducted in 2013/2014 and 2014/2015, when a total of 11 *M. occidentalis* that had been infested with larvae in spring were overwintered in CABI's garden and no adults emerged from any of the plants. In open-field tests we found no larvae on test plant species outside the genus *Leucanthemum* and only a few larvae on Shasta daisies. In addition, we found that development from egg to adult is much lower on all tested Shasta daisy varieties compared to oxeye daisies. 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.

In 2016, we will continue with and complete no-choice larval development tests, assuming that all remaining test plants are available. In addition, we will set up a multiple-choice cage test with three plant species not exposed so far. Depending on the results, we will prepare a petition for field release in collaboration with our North American partners.

4. *Cyphocleonus trisulcatus* HERBST (Col., Curculionidae)

The root-feeding weevil *Cyphocleonus trisulcatus* (Plate 2) was identified as a promising potential biological control agent during our literature survey. Eggs are laid from May onwards and larvae feed internally and externally on roots of oxeye daisy. They pupate in the soil and adults start to emerge in August. *Cyphocleonus trisulcatus* appears to be quite rare in Europe, and most site records for this species date from more than 50 years ago (Hassler and Rheinheimer, 2010). During field trips to southern France in 2012 and 2013 we found a total of nine sites with several plants infested by *C. trisulcatus*, which enabled us to establish a rearing colony. A total of 446 weevils were kept for overwintering in cylinders at ambient temperatures or in an incubator set at 2°C, and more than 80% of the adults hibernated successfully until spring 2015.



Plate 2. Adult *Cyphocleonus trisulcatus* (left); egg and first instar larva of *C. trisulcatus* on roots of *Leucanthemum vulgare* (right).

4.1. No-choice oviposition and development tests

METHODS Before being used in host-range tests, all females were individually tested for egg laying by placing them for 2–3 days in small cups together with two leaves of oxeye daisy inserted in a moist florist sponge. Between 15 May and 17 July two egg-laying females were then placed onto individually potted, gauze-covered test and control (*L. vulgare* and *L. ircutianum*) plants. After 4–6 days the weevils were retrieved from the plant. To ensure that they were able to mate and feed on *L. vulgare* in between tests, we placed them individually for at least two days into small cups together with a male and leaves of oxeye daisies. Egg-laying females were then re-used for host-range testing. In total, we were able to expose 193 plants to *C. trisulcatus*: 54 were controls and the remainder were plants of 34 test species and varieties (1–8 replicates per test plant species; Table 2). All test plants and a few of the control plants were dissected 3–7 weeks after exposure to females, but as soon as larvae were found in one replicate of a test plant species, dissection was stopped and the remaining replicates were kept for adult emergence. All retained plants were individually covered with gauze bags and regularly checked for adult emergence. In early October all the plants were dissected and the soil around the roots was searched for pupae and additional adults.

RESULTS Adults started to emerge about 2.5 months after the plants were exposed to egg-laying females. On average a similar number of adults emerged from *L. vulgare* compared with *L. ircutianum* (Table 2). On one plant of *Achillea ptarmica* seven larvae were found, but no larvae or adults were found on the other four replicates of this test plant species or on any of the other test plant species.

Table 2. Results of no-choice oviposition and development tests with *Cyphocleonus trisulcatus*.

Test plant species	2014			2015		
	No. replicates	% plants attacked ^b	No. of adults emerged (mean ± SE)	No. replicates	% plants attacked ^b	No. of adults emerged (mean ± SE)
Tribe Anthemideae						
Subtribe Leucanthemeinae						
<i>Leucanthemum vulgare</i>	35	91	4.7 ± 0.6	43	89	6.7 ± 0.7
<i>Leucanthemum ircutianum</i>	8	80	3.8 ± 1.7	11	100	6.0 ± 1.4
<i>Leucanthemum</i> × <i>superbum</i> Alaska	7	70	2.0 ± 0.8			
<i>Leucanthemum</i> × <i>superbum</i> Becky	5	80	4.2 ± 2.3			
<i>Leucanthemum</i> × <i>superbum</i> Crazy Daisy	7	100	3.7 ± 0.4			
<i>Leucanthemum</i> × <i>superbum</i> Marconi Double	5	80	4.2 ± 1.7			
<i>Leucanthemum</i> × <i>superbum</i> Silver Princess	5	100	3.0 ± 0.9			
<i>Leucanthemum</i> × <i>superbum</i> Sunny side up	1	100	1.0 ± 0.0			
<i>Leucanthemum</i> × <i>maximum</i>	7	90	5.0 ± 1.9			
Subtribe Anthemidinae						
<i>Anthemis arvensis</i>	2	0		4	0	
<i>Anthemis cotula</i>	6	0				
<i>Tanacetum huronense</i> ^a	6	0		1	0	
<i>Tanacetum vulgare</i>	5	0		2	0	
<i>Tripleurospermum inodorum</i>	6	0				
Subtribe Matricariinae						
<i>Matricaria chamomilla</i>	4	100	1.7 ± 0.3			
<i>Matricaria occidentalis</i> ^a	4	80	0.7 ± 0.3			
<i>Achillea alpina</i> ^a	5	0		2	0	
<i>Achillea borealis</i> ^a	4	0		3	0	
<i>Achillea ptarmica</i>	3	0		5	20	0.0
Subtribe Santolininae						
<i>Santolina chamaecyparissus</i>				7	0	
<i>Chamaemelum nobile</i>	1	0		4	0	
Subtribe Glebionidinae						
<i>Argyranthemum frutescens</i>	5	0				
<i>Glebionis coronaria</i>	5	40	0.5 ± 0.4			
<i>Ismelia carinata</i>	3	0		2	0	

Test plant species	2014			2015		
	No. replicates	% plants attacked ^b	No. of adults emerged (mean ± SE)	No. replicates	% plants attacked ^b	No. of adults emerged (mean ± SE)
Subtribe Artemisiinae						
<i>Artemisia californica</i> ^a				7	0	
<i>Artemisia campestris</i> ^a	5	0		2	0	
<i>Artemisia cana</i> ^a	5	0		1	0	
<i>Artemisia filifolia</i> ^a				3	0	
<i>Artemisia frigida</i> ^a	5	0		3	0	
<i>Artemisia ludoviciana</i> ^a	5	0				
<i>Arctanthemum arcticum</i> ^a	5	0		8	0	
" <i>Chrysanthemum x glandiflorum</i> " gMums				7	0	
" <i>Chrysanthemum x glandiflorum</i> " Canary Num				1	0	
" <i>Chrysanthemum x glandiflorum</i> " Morden Delight				3	0	
<i>Leucanthemella serotina</i>	6	0		1	0	
Subtribe Cotulinae						
<i>Cotula coronopifolia</i>	2	0				
Other tribes						
<i>Solidago nemoralis</i> ^a	3	0		4	0	
<i>Anaphalis margaritacea</i> ^a				6	0	
<i>Helenium autumnale</i> ^a				3	0	
<i>Coreopsis tinctoria</i> ^a				5	0	
<i>Helianthus annuus</i> ^a				5	0	
<i>Eutrochium maculatum</i> ^a				2	0	
<i>Arnica chamissonis</i> ^a				7	0	
<i>Senecio eremophilus</i> ^a				4	0	
<i>Lactuca sativa</i>				7	0	
<i>Cichorium intybus</i>				5	0	
<i>Cirsium flodmanii</i> ^a	1	0		1	0	
<i>Carthamus tinctorius</i>				7	0	
<i>Cynara scolymus</i>				7	0	
<i>Daucus carota</i>				7	0	
<i>Lobelia cardinalis</i> ^a				4	0	

^a Plant species native to North America.

^b Larvae found upon dissection or adults emerged.

4.2. Multiple-choice field cage test

In 2014, three test species outside of the genus *Leucanthemum* had been attacked under no-choice conditions, the native North American *Matricaria occidentalis* and the commercially grown *M. chamomilla* and *Glebionis coronaria*. In 2015, we established a multiple-choice cage test to see whether these plants would also be attacked in the presence of oxeye daisy.

METHODS In May 2015, a total of four field cages with three plants each of *G. coronaria*, *M. chamomilla*, *M. occidentalis* and *L. vulgare* were established. On 28 May, eleven egg-laying females were released in two of the cages and this was repeated on 15 June for the remaining two cages. Ten days after the release of the females, all plants were removed from the cages and checked for females. Once all the females were retrieved, the plants were moved to a garden bed and embedded in sawdust. Four to six weeks later, all plants were dissected for larvae.

RESULTS Larvae were found on all of the control plants. In the two cages set up on 28 May a few larvae were found on three of the *M. chamomilla* and two of the *M. occidentalis* plants but no larvae were found on test plants set up on 15 June. No larvae were found on any of the *G. coronaria* plants exposed (Fig. 2).

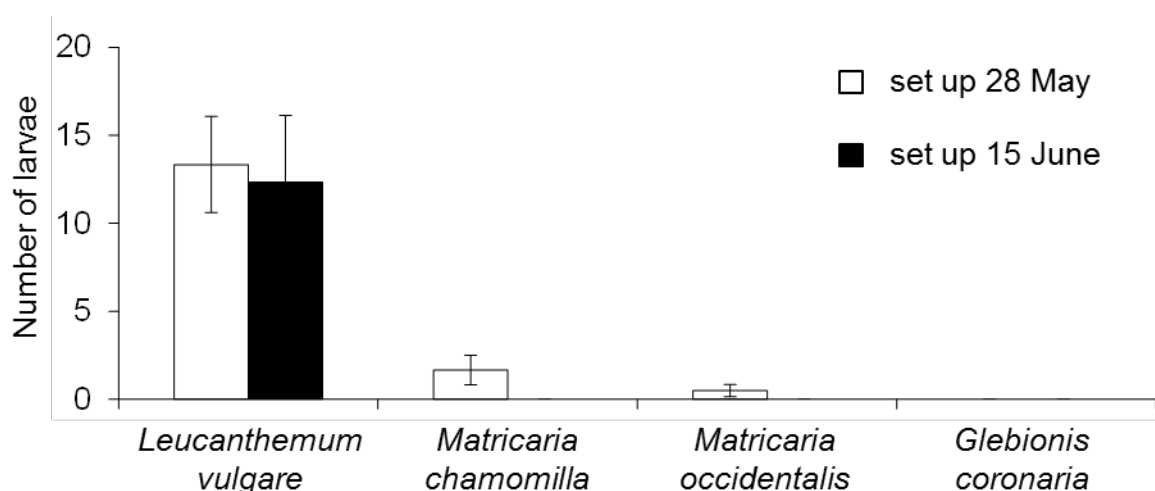


Fig. 2. Mean (\pm SE) number of larvae found on *Leucanthemum vulgare* and three test plant species exposed to females of *Cyphocleonus trisulcatus* in multiple-choice field cages. Half of the plants were exposed from 28 May to 9 June and the other half from 15 June to 25 June.

4.3. Multiple-choice open-field test

Since Shasta daisy varieties supported adult development of *C. trisulcatus* when exposed under no-choice conditions and plants were also attacked under multiple-choice cage conditions (Stutz *et al.*, 2015), we wanted to check whether the plants would be accepted for oviposition under open-field conditions.

METHODS Twelve potted plants each of four Shasta daisy varieties (Alaska, Becky, Crazy Daisy and *L. x maximum*) and 48 plants of *L. vulgare* (36 in the rosette stage and 12 with shoots) were arranged in eight lines radiating out at distances of 2.5, 5 and 10 m (Fig. 3, Plate 3). Between 16 and 18 June a total of 50 females of *C. trisulcatus* were released at the central point. One month later, all plants were removed, checked for females and individually covered with gauze bags. Until the

end of September all plants were regularly checked for adult emergence. In early October, all plants were dissected and the soil around the roots searched for pupae and additional adults.

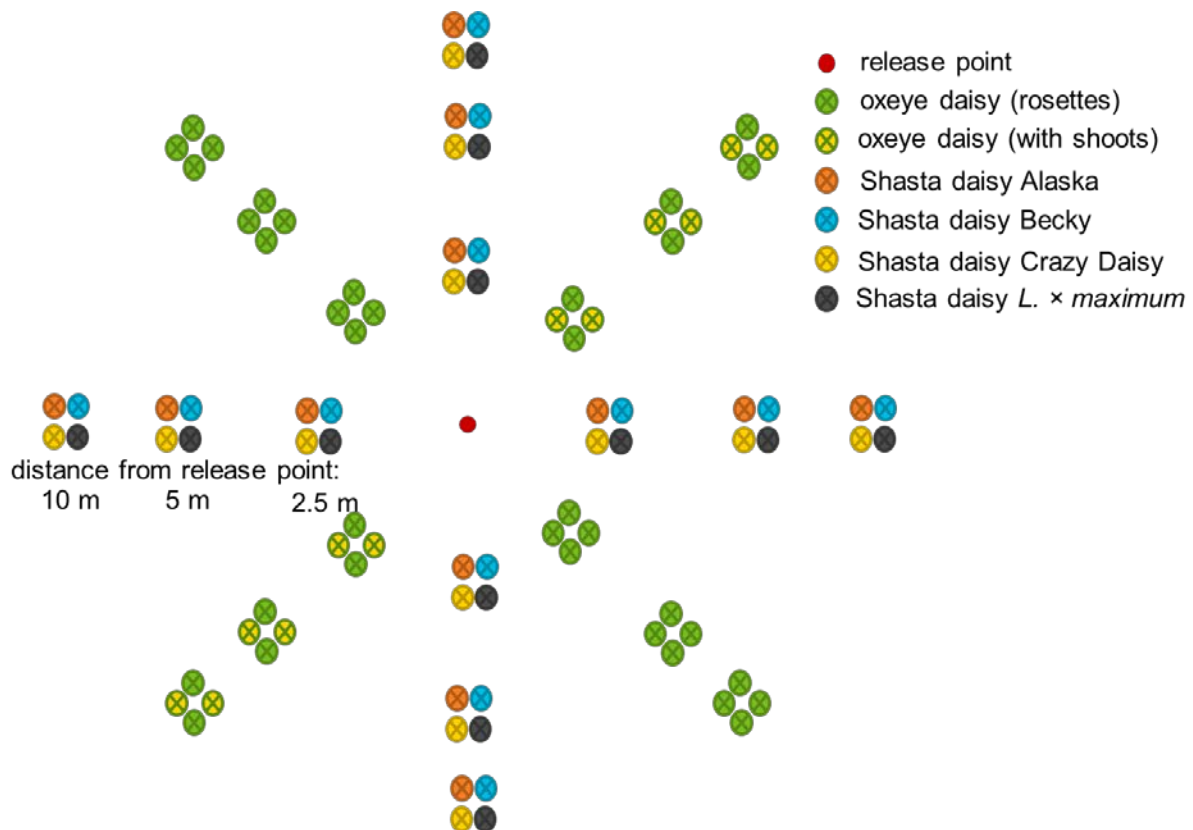


Fig. 3. Set-up of open-field test with *Cyphocleonus trisulcatus*. All Shasta daisies except the variety ‘Becky’ were in the rosette stage.

RESULTS Within a few days after the plants had been retrieved from the open-field test adults started to emerge from the Shasta daisy variety ‘Becky’. Since development from eggs to adults takes at least two months, it is likely that these plants had already been infested with *C. trisulcatus* before they were used in the open-field test. Since the Shasta daisy variety ‘Becky’ had been overwintered in a field cage, the most likely explanation is that this cage had been contaminated with *C. trisulcatus*. Except for the oxeye daisies with shoots, which had been sown in 2014 and were kept in another field cage, all other test and control plants had been sown in spring 2015 and were kept in a greenhouse or another field cage until the open-field test was set up. The Shasta daisy variety ‘Becky’ was therefore excluded from the data reported here. The proportions of plants attacked by *C. trisulcatus* was high for oxeye daisy rosettes as well as for Shasta daisies (adults emerged from 89% and 88% of the plants, respectively), but only one (i.e. 8%) of the oxeye daisy plants with shoots was attacked. All Shasta daisy varieties were attacked at an equally high rate, and a similar number of adults emerged. A similar number of adults emerged from oxeye daisy rosettes and Shasta daisies set up at 2.5 m and 10 m from the release point ($P > 0.1$) and only a slightly lower number of adults emerged from Shasta daisies compared to oxeye daisy rosettes set up at 5 m ($z = 2.3$, $P = 0.02$; Fig. 4). Ninety-two percent of the oxeye daisy rosettes and 100% of the Shasta daisies set up at a distance of 2.5 m from the release point were attacked by *C. trisulcatus* and the percentage of plants attacked at 10 m from the release point was still high (83% for oxeye daisy rosettes and 58% for Shasta daisy).



Plate 3. Oxeye daisy patch consisting of two rosettes and two plants in the flowering stage (left) and Shasta daisy patch consisting of plants of four different varieties (right) exposed to *Cyphocleonus trisulcatus* in the open-field test.

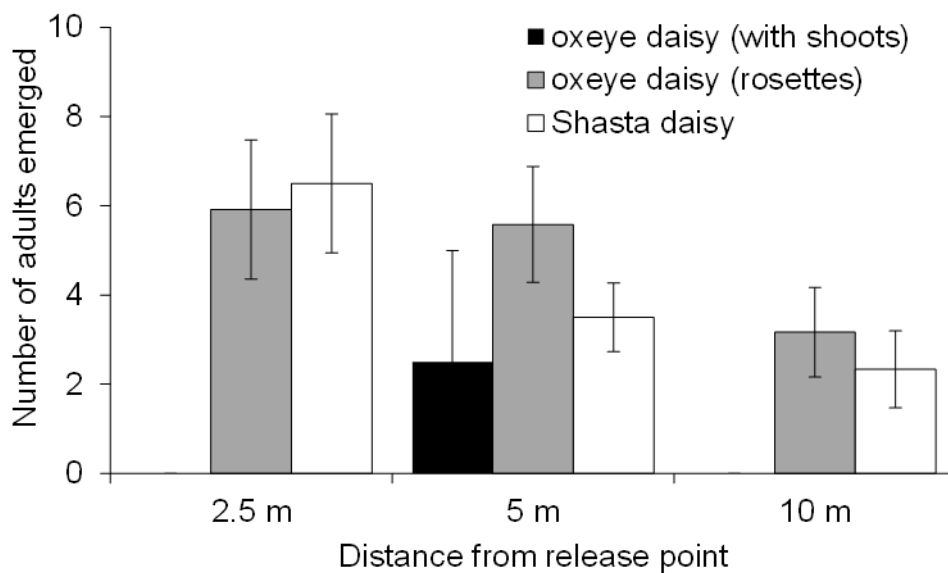


Fig. 4. Mean (\pm SE) number of adults that emerged from oxeye daisy and Shasta daisy exposed to females of *Cyphocleonus trisulcatus* in an open-field test.

4.4. Impact experiment

METHODS In order to assess the impact of larval feeding by *C. trisulcatus* an impact experiment with potted plants had been set up in summer 2014 (see section 4.3 in Stutz *et al.*, 2015). Half of the plants had been exposed to two females each for six days and an average of 7.2 ± 0.8 weevils had emerged from these plants. All plants were kept for overwintering to investigate if the reduction in size observed in autumn 2014 would lead to a reduction in the biomass and number of flower heads in the following summer. In July 2015, all 30 plants were harvested and the numbers

of stems and flower heads as well as the dried above- and belowground biomass determined.

RESULTS Plants infested with *C. trisulcatus* produced on average 44% fewer shoots and flower heads than non-infested control plants ($z = 6.2$, $P < 0.001$ for both; Fig. 5). Aboveground biomass was reduced by 44% and belowground biomass by 68% ($t = 4.1$, $P < 0.001$ and $t = 5.8$, $P < 0.001$, respectively; Fig. 5).

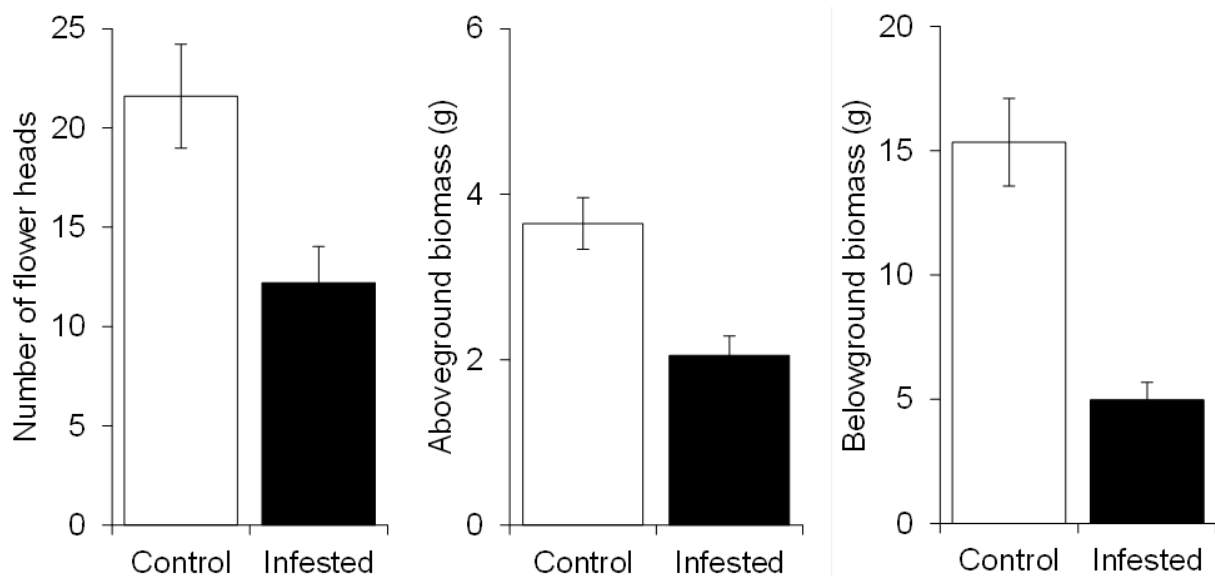


Fig. 5. Mean (\pm SE) number of flower heads (left), aboveground biomass (centre) and belowground biomass (right) of infested and uninfested (control) plants from the impact experiment with *Cyphocleonus trisulcatus*.

4.5. Conclusions and outlook

The no-choice oviposition and development tests with *C. trisulcatus* confirmed that the fundamental host range of this root-feeding weevil is restricted to the genus *Leucanthemum* and a few other closely related species. Under multiple-choice cage conditions we found a few larvae on *Matricaria chamomilla* and *M. occidentalis* but not on *Glebionis coronaria*. Interestingly, *M. chamomilla* and *M. occidentalis* were only attacked when the plants were exposed from 28 May to 9 June but not when they were exposed two weeks later. All three test plant species are annual plants and it is therefore likely that young plants are preferred over older plants. The phenology of *G. coronaria* plants used in the multiple-choice cage tests was already relatively advanced and tests would need to be repeated with younger plants in order to confirm that *C. trisulcatus* does not attack *G. coronaria* plants when they are in the rosette stage. Under open-field conditions, a similar number of adults emerged from Shasta daisies as from oxeye daisies, independent of the distance from a central release point. Since in North America Shasta daisies are widely grown as ornamentals we decided - after discussion with our North American partners - to stop working with *C. trisulcatus*.

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

Another potential biological control candidate for oxeye daisy is the flower-head attacking fly *Tephritis neesii* (Plate 4). 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. Usually only seed heads collected from plants flowering in May or June were infested; seed heads collected later in summer from plants that had regrown after mowing were not infested.



Plate 4. Adult *Tephritis neesii* mating on an oxeye daisy flower head (left); oxeye daisy flower head attacked by *T. neesii* larvae (right).

5.1. Overwintering experiment

In winter 2014/2015, we tested different set-ups to improve the overwintering survival of adults.

METHODS In October 2014, four rearing cages (50 cm × 30 cm) each containing 80 females and 90 males of *T. neesii* had been set up. The cages were partially filled with a 30-cm thick layer of either leaf litter (two cages) or crumpled paper towels (two cages). The leaf litter had been oven-dried in order to exclude any predators. In December, one cage of each set-up was moved to an underground shelter while the other two cages were kept in a wooden shelter at ambient temperatures. In addition, we set up five plastic cylinders containing ten males and ten females each. In December, the cylinders were made airtight to prevent desiccation and moved to an incubator which was set at 2°C. The flies in the cages and cylinders were provided with water and sugar. In April 2015 all surviving flies were counted.

RESULTS Overwintering survival was generally very low. Survival was lowest when flies were kept in a wooden shelter at ambient temperature and only marginally higher when they were kept in an underground shelter (Table 3). Overwintering survival was highest when the flies were kept in cylinders in an incubator set at 2°C, but overwintering survival was generally very low and lower than in the previous year when flies had been kept in cylinders in the wooden and underground shelters (survival of 36% and 29%, respectively).

Table 3. Overwintering survival of *Tephritis neesii* using different set-ups.

Set-up	Set up October 2014		Survival April 2015		
	No. females	No. males	No. females	No. males	% survival
Underground shelter with leaf litter	80	90	9	6	8.8
Underground shelter paper towel	80	90	5	3	4.7
Wooden shelter with leaf litter	80	90	1	1	1.2
Wooden shelter with paper towel	80	90	2	2	2.4
Cylinders with paper towel	48	50	11	7	18.4

5.2. Conclusions and outlook

Overwintering survival of *T. neesii* was generally very low for all of the set-ups tested in winter 2014/2015. Survival was also lower than in winter 2013/2014 when the flies had been kept in cylinders in wooden and underground shelters (survival of 36% and 29%, respectively). Our results indicate that plastic cylinders are better suited to hibernate *T. neesii* than rearing cages.

Due to the difficulties in hibernating *T. neesii* in numbers high enough to conduct host-specificity tests and because root herbivores are likely to be more effective in controlling invasive oxeye daisies than seed herbivores we decided to suspend work with *T. neesii*.

6. *Oxya nebulosa* WIEDEMANN (Dipt., Tephritidae)

Oxya nebulosa is a root-galling tephritid fly that is widely distributed across Europe. It is reported to be monophagous on *Leucanthemum* species (Plate 5). Adults are reported to fly from late June to August (Bagnée, 2006). The species is relatively rare in Europe, but during field surveys conducted in the area of Regensburg (Germany) and in the Czech Republic in 2012 and 2013 populations with relatively high attack rates had been found. In 2016, we plan to re-visit these sites to collect galls in order to establish a rearing colony and to begin host-range testing.



Plate 5. Gall of the tephritid fly *Oxya nebulosa* on a root of oxeye daisy

7. Work Programme Proposed for 2016

Based on our work in 2015, we propose the following work programme for the coming season.

***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.

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9. References

- Bagnée, J.Y. (2006) Contribution à la connaissance des Tephritidae de Belgique (Diptera: Brachycera). *Notes fauniques de Gembloux* 59, 63-113.
- Cole, D.E. (1998) Effect of competition on growth of ox-eye daisy (*Chrysanthemum leucanthemum* L.) in pastures and hay land. University of Alberta, Canada, Master's thesis, 156 pp.
- Fernald, M.L. (1903) *Chrysanthemum leucanthemum* and the American white weed. *Rhodora* 5, 177–181.
- Forcella, F. (1985) Final distribution is related to rate of spread in alien weeds. *Weed Research* 25, 181–191.
- Hassler, M. and Rheinheimer, J. (2010) *Die Rüsselkäfer Baden-Württembergs*. Regionalkultur Verlag, Heidelberg, Germany.
- Holm, L., Pancho, J.V., Herberger, J.P. and Plucknett, D.L. (1979) *A Geographical Atlas of World Weeds*. John Wiley & Sons, New York.
- Lavoie, C., Saint-Louis, A., Guay, G., Groeneveld, E. and Villeneuve, P. (2012) Naturalization of exotic plant species in north-eastern North America: trends and detection capacity. *Diversity and Distributions* 18, 180–190.
- Morton, J.K. (1981) Chromosome numbers of Compositae from Canada and the USA. *Botanical Journal of the Linnean Society* 82, 357–368.
- Mulligan, G.A. (1958) Chromosome races in the *Chrysanthemum leucanthemum* complex. *Rhodora* 60, 122–125.

- Mulligan, G.A. (1968) Diploid and tetraploid chromosome races of *Chrysanthemum leucanthemum* L. s.l. *Naturaliste Canadien* 95, 793–795.
- Olsen, B.E., Wallander, R.T. and Fay, P.K. (1997) Intensive cattle grazing of oxeye daisy (*Chrysanthemum leucanthemum*). *Weed Technology* 11, 176–181.
- Robinson, J. (2008) The evolution of flower size and flowering behaviour in plants: the role of pollination and pre-dispersal seed predation. University of Southampton, UK, Master's thesis, 224 pp.
- Schaffner, U., Zaquini, L. and Häfliger, P. (2011) Prospects for the biological control of oxeye daisy, *Leucanthemum vulgare*. Annual Report 2010. Unpublished report, CABI, Delémont, Switzerland.
- Stutz, S., Tateno, A., Hinz, H.L. and Schaffner, U. (2012) Prospects for the biological control of oxeye daisy, *Leucanthemum vulgare*. Annual Report 2011. Unpublished report, CABI, Delémont, Switzerland.
- Stutz, S., Sauvain, L., Inskip, J., Oliveira, E., Palmer, E., Hinz, H.L. and Schaffner, U. (2014) Prospects for the biological control of oxeye daisy, *Leucanthemum vulgare*. Annual Report 2013. Unpublished report, CABI, Delémont, Switzerland.
- Stutz, S., Sjolie, D., Elsby, M., Hinz, H.L. and Schaffner, U. (2015) Prospects for the biological control of oxeye daisy, *Leucanthemum vulgare*. Annual Report 2014. Unpublished report, CABI, Delémont, Switzerland.

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Ken Bloem	Brian Marschman
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Tim Collier	Mike Pitcairn
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Marc Eylar	Blake Schaan
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