Annual Report 2011

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CABI
Rue des Grillons 1, CH-2800 Delémont, Switzerland
Tel: ++ 41 32 421 4870
Fax: ++ 41 32 421 4871
Email: Europe-CH@cabi.org

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Summary

1. Work in 2011 focused on maintaining a colony of the root-feeding beetle *Eumolpus asclepiadeus* and monitoring adult emergence from larval transfer tests carried out in previous years.

2. In contrast to results recorded in 2010 from a larval transfer test carried out in 2009, in which most beetles emerged after one year of development, only very few beetles emerged in 2011 from larval transfers made in 2010.

In addition to one adult of *E. asclepiadeus* ex Ukraine that emerged from the native North American (NA) *Apocynum cannabinum*, 16 one-year-old larvae were found upon dissection of *Asclepias incarnata* NA, plus a total of three larvae were recovered from *A. syriaca* NA and *A. tuberosa* NA. No larval development was found on any of the other 15 plant species tested in 2010–11. Survival of one-year-old larvae of *E. asclepiadeus* ex France was as high on *A. incarnata* NA as on *V. rossicum*.

In summary, a few native NA non-target species in the genus *Asclepias* support complete larval development of *E. asclepiadeus*. In the presence of *Vincetoxicum,* oviposition and larval development occasionally occurred on *A. tuberosa* NA and *A. incarnata* NA. Very little or no adult feeding was recorded on potted non-target plants under choice conditions. Adult *E. asclepiadeus* will feed and reproduce when provided with excised leaves of some non-target species. Although little adult feeding occurred on intact non-target leaves (potted plants) in choice or no-choice tests, *E. asclepiadeus* naïve females were able to produce fertile eggs on *A. tuberosa* NA and to a lesser extent on *A. incarnata* NA. Very occasional larval development to the adult stage was also recorded on the native NA *Apocynum cannabinum* and *Cephalanthus occidentalis*.

3. Alicia Leroux started her Master's degree on the seed-feeding fly *Euphranta connexa* in 2011. Alicia is enrolled at the University of Manitoba, Winnipeg, Canada, where she is supervised by Prof. Neil Holliday, and will conduct her practical work at CABI in Switzerland under the supervision of André Gassmann and Hariet Hinz.

Development time to adult emergence of *E. connexa* decreased linearly as temperature increased from 15°C to 25°C. There was no emergence at 10°C, 30°C and 35°C. The dissection of one- to 20-day-old females showed that the first cycle of ovariole maturation takes about ten days. To study the impact of different *Vincetoxicum* species on the fecundity of *E. connexa,* an experiment was set up in 2011. The fecundity of females emerging from larvae reared on different *Vincetoxicum* species will be assessed in 2012.

4. In 2012, work will focus on monitoring the emergence of *Eumolpus asclepiadeus* and on maintaining a colony of the beetle population from Ukraine. Studies on the biology of *Euphranta connexa* will be continued and methods will be developed for testing this candidate agent.

1. Introduction

Swallow-worts are herbaceous perennials in the family Apocynaceae (subfamily Asclepiadoideae). Two European species of swallow-worts, *Vincetoxicum nigrum* (black swallow-wort) (synonym: *Cynanchum louiseae*) and *V. rossicum* (pale swallow-wort) (synonym: *C. rossicum*) are now naturalized in north-eastern North America, and considered invaders of natural areas and abandoned pastures (Lawlor, 2000). The earliest record of *V. nigrum* in the USA is from Massachusetts in 1854 and *V. rossicum* was recorded in New York state in 1897 (Sheeley and Raynal, 1996). *Vincetoxicum nigrum* is native to the Mediterranean regions of France, Italy and Spain whereas *V. rossicum* is native to south-east Ukraine and Russia.

In the USA, *V. nigrum* is distributed from Maine through to Kansas and also in California (Tewksbury *et al*., 2002). This vine is commonly found in open habitats and along forest margins (DiTommaso *et al*., 2005). *Vincetoxicum rossicum* has a growth pattern and habitat preference similar to *V. nigrum* but also invades forest understories (DiTommaso *et al*., 2005). It has a discontinuous distribution that extends from the Great Lakes through to New England and the Mid-Atlantic states (Tewksbury *et al*., 2002).

There are no reliable reports of herbivores attacking *V. nigrum* and *V. rossicum* in Europe. Herbivore surveys conducted during 2006 in Switzerland, France, Germany and Ukraine located the common herbivores documented by Tewksbury *et al*. (2002) on the closely related species *V. hirundinaria* and also documented the first occurrence of *Hypena opulenta* on *V. rossicum* and *V. scandens* (Weed and Gassmann, 2007). However, no herbivores have yet been collected in Europe from *V. nigrum.*

Five potential insect biological control agents were selected for more in-depth study, i.e. the leaf-feeding noctuids *Abrostola asclepiadis* and *H. opulenta*, the leaf-feeding chrysomelid *Chrysolina aurichalcea* ssp. *asclepiadis*, the root-feeding chrysomelid *Eumolpus asclepiadeus*, and the seed-feeding tephritid *Euphranta connexa.* Sforza (2011) uses the name *Chrysochus asclepiadeus* for *Eumolpus asclepiadeus*.

Host-range studies at the University of Rhode Island (URI), USA, for *Chrysolina aurichalcea* ssp. *asclepiadis* have shown that this species is unsuitable for biological control of *Vincetoxicum* due to potential risk of attack on native North American (NA) *Asclepias* and native NA Asteraceae (Weed and Casagrande, 2011). In contrast, research indicates that both *H. opulenta* and *Abrostola asclepiadis* pose little risk to native NA plants and are promising agents against *Vincetoxicum* spp. A petition has been submitted for the release of *H. opulenta* in North America.

Alicia Leroux from the University of Manitoba, Winnipeg, Canada, started a Master’s degree on the biology and host range of *Euphranta connexa* at CABI in Switzerland in collaboration with the University of Manitoba. This study is being supervised by Prof. Neil J. Holliday (University of Manitoba) and Drs. André Gassmann and Hariet L. Hinz (CABI).

Because of limited funding, the main objectives for CABI in 2011 were restricted to the recording of adult emergence of *Eumolpus asclepiadeus* from the 2009 and 2010 host-specificity tests and to studies on *Euphranta connexa*. This report presents work carried out in 2011 at CABI.

2. *Eumolpus asclepiadeus* (Coleoptera: Chrysomelidae)

Eumolpus asclepiadeus ranges throughout central and eastern Europe and into Asia and is considered rare and protected in some countries (e.g. Germany). This species is reported to be a specialist herbivore of *V. hirundinaria* (Dobler *et al*., 1998). In 2006, we collected adults on *V. hirundinaria* in Kiev, Ukraine, in mid-June, and on *V. hirundinaria* in southern Switzerland in early September. The species was only found in open habitats receiving full sun. We determined that in outdoor conditions at CABI, most *E. asclepiadeus* individuals require one to two years to complete their life cycle while very occasionally adults will emerge in the third year.

Between 2007 and 2009 at URI, no-choice larval transfer tests were conducted for *E. asclepiadeus* ex Ukraine larvae on *V. hirundinaria*, *V. rossicum*, *V. nigrum* and 26 test plant species, 15 native to NA (Gassmann *et al*., 2010). Live beetle larvae were found on five native NA *Asclepias* species (i.e. *A. fascicularis*, *A. incarnata*, *A*. *speciosa*, *A. tuberosa* and *A. viridiflora*) and the native NA *Funastrum angustifolium* four months after larval transfer (Gassmann *et al*., 2010). All larvae recovered during dissections were transferred onto new plants of the same species so that they could finish development. From plants infested in 2007 and 2008 at URI, adults emerged in 2009 from the native NA *A. fascicularis, A. incarnata, A. speciosa* and *A. tuberosa*. Larval transfer tests carried out at CABI in 2008–10 also indicated that *A. incarnata* NA and *A. tuberosa* NA are suitable host plants for larval development of *E. asclepiadeus* (Gassmann *et al*., 2010).

In 2008–09, a few larvae were observed developing on *A. incarnata* NA in a single-choice oviposition test with *V. hirundinaria* and *V. nigrum* (Gassmann *et al*., 2010). In 2009, a few larvae were collected from *A. tuberosa* NA in a multiple-choice field cage test four weeks after set-up (Gassmann *et al*., 2010).

In 2010–11, no-choice and choice testing were continued to determine whether *E. asclepiadeus* can complete development to the adult stage and reproduce on non-target plants.

2.1. Adult emergence and plant dissections

2.1.1. Adult emergence from larval transfers made in 2009

METHODS. In 2009, 2300 larvae (20 per plant) were transferred onto eight plant species, five of which are non-target taxa. All plants were placed into raised beds and adult emergence monitored in 2010 and 2011. All dead plants were dissected in July–August 2011.

RESULTS AND DISCUSSION. In contrast to our previous observations, a relatively high number of adults emerged from the target *Vincetoxicum* spp. one year after the larval transfers (Table 1). Beetle emergence occurred from 26 June until 8 July 2010. A few additional adults emerged from *V. nigrum* (three adults) and *V. rossicum* (five adults) in 2011. The rate of larval development was similar for the three target weeds but the ratio of number of larvae or pupae (larvae–pupae) to number of adults recovered in 2010 suggests that larval development was faster on *V. nigrum* than on *V. rossicum* and *V. hirundinaria*. On *Vincetoxicum* spp., 50–75% of the adults emerged from dead plants and numerous live larvae were found upon dissection of dead plants. One adult emerged from *Cephalanthus occidentalis* NA in 2011. No larval development was recorded on the other four test plant species.

2.1.2. Adult emergence from larval transfers made in 2010

Because of the discrepancy in time for larval development to the adult stage between the larvae transferred in 2006–07 and 2009 (Gassmann *et al*., 2011), we decided to rear and test the two-year and one-year 'strains' of *E. asclepiadeus* (ex *V. hirundinaria*, Ukraine) separately. We have also tested separately a one-year 'strain' of *E. asclepiadeus* (ex *V. hirundinaria,* France) which was kindly provided by Dr René Sforza, USDA-ARS-EBCL (US Department of Agriculture – Agricultural Research Service – European Biological Control Laboratory), Montpellier, France.

METHODS. Adults that emerged from larval transfers made in 2008 (LT 2008) and 2009 (LT 2009) and adults collected in the field in the French Jura in 2010 were reared as in previous years. Batches of ten or more mating pairs were placed into separate rearing containers to collect eggs and were fed for a couple of days with cut shoots of their respective larval hosts (i.e. *V. hirundinaria*, *V. nigrum* or *V. rossicum*), but then, because of plant shortage, with cut shoots of *V. hirundinaria* collected in the area around Delémont. Rearing containers consisted of transparent cylinders (16 cm high, 11 cm diameter) or larger ventilated plastic boxes containing shoots of *Vincetoxicum* spp*.* inserted into moist blocks of florist foam enclosed in plastic foil. A moistened square of florist foam with parallel lines engraved into it served as an oviposition substrate. Eggs were collected twice a week and plant shoots were changed when necessary. Egg collection was interrupted once all plants available for host-range testing had been used. Remaining adults were killed.

In 2010, a total of 8600 larvae were transferred onto three *Vincetoxicum* spp. and 21 test plant species, 13 of which are native to NA. All plants were placed into raised beds and adult emergence monitored in 2011. Dead plants were dissected in autumn 2010 and late summer 2011. All other plants are being kept in the centre's garden to monitor adult emergence in 2012.

RESULTS AND DISCUSSION. Results for the two-year and one-year 'strains' of *E. asclepiadeus* (ex *V. hirundinaria*, Ukraine) are presented separately (Tables 2 and 3) although no genetic divergence was recorded between these two 'strains' (Gassmann *et al.*, 2011). In contrast, the mean genetic divergence between specimens from France and Ukraine ranged between 2.9% and 3.1%, so they should be treated at least at the level of well-differentiated subspecies with potentially different biological properties.

Table 1. Larval transfers of *Eumolpus asclepiadeus* in 2009 and beetle recovery in 2010–11.

Species[a]	2009 No. of replicates (plants)	2009 Total no. of larvae transferred	2010 No. of adults / larvae–pupae recovered (% survival)	2011 No. of adults / larvae–pupae recovered (% total survival)
Family Apocynaceae Tribe Asclepiadeae Subtribe Tylophorinae				
Vincetoxicum hirundinaria	25	500	53 / 40 (18.6%) *22 plants dissected*	0 / 0 (18.6%)
Vincetoxicum nigrum	25	500	78 / 11 (17.8%) *20 plants dissected*	3 / 0 (18.4%)
Vincetoxicum rossicum	25	500	45 / 31 (15.2%) *22 plants dissected*	5 / 0 (16.2%)
Subtribe Asclepiadinae				
Asclepias currassavica[a]	8	160	0 *all plants dissected*	-
Asclepias fasicularis[a]	8	160	0 *all plants dissected*	-
Asclepias fruticosa	8	160	0 *all plants dissected*	-
Periploca graeca	8	160	0	0 / 0
Family Rubiaceae				
Cephalanthus occidentalis[a]	8	160	0	1 / 0 (0.63%)

[a] Plant species native to North America.

In contrast to results recorded in 2010 from a larval transfer test carried out in 2009, in which most beetles emerged after one year of development, only very few beetles emerged in 2011 from larval transfers made in 2010 (Tables 2–4). No adults of the two-year ‘strain’ of *E. asclepiadeus* ex Ukraine emerged from *Vincetoxicum* spp., but dissection of dead plants indicated a very high survival rate for one-year-old larvae on *V. hirundinaria* and *V. nigrum* (Table 2); surprisingly, only one larvae was recorded on *V. rossicum*. In contrast, larval survival on *V. rossicum* was higher than on *V. hirundinaria* for the one-year ‘strain’ of the beetle ex Ukraine and three adults emerged from *Vincetoxicum* spp. (Table 3). In addition to one adult of the two-year ‘strain’ ex Ukraine that emerged from *Apocynum cannabinum* NA, 16 one-year-old larvae of the same ‘strain’ were found upon dissection of *Asclepias incarnata* NA (Table 2). Finally, three larvae of the one-year ‘strain’ ex Ukraine were recovered upon dissection of *A. syriaca* NA and *A. tuberosa* NA (Table 3). No larval development was found on any of the other 14 plant species tested.

Only one adult of *E. asclepiadeus* ex France emerged from each of *V. hirundinaria* and *V. nigrum* (Table 4). Recovery of one-year-old larvae on *V. nigrum* differed from that observed for *E. asclepiadeus* ex Ukraine. Survival of one-year-old larvae of *E. asclepiadeus* ex France was as high on *A. incarnata* NA as on *V. rossicum*.

In summary, adult emergence from larval transfers made in 2010 was very low in 2011 for the three strains of *E. asclepiadeus* tested. Much higher adult emergence is expected in 2012 as indicated by a high but variable survival rate of one-year-old larvae on *Vincetoxicum* spp. between the three strains tested. Occasional larval development was recorded on *Apocynum cannabinum* NA and *Asclepias syriaca* NA. *Asclepias incarnata* NA and *A. tuberosa* NA were confirmed as suitable host plants for larval development of *E. asclepiadeus.* Final results will be available in 2012 when beetle emergence will be recorded and all plants will be dissected.

Table 2. Larval transfer tests carried out in 2010 for *Eumolpus asclepiadeus* (ex Ukraine, two-year 'strain'; 2008 generation) and beetle recovery in 2011.

Plant species[a]	2010		2011			
	No. of replicates (plants)	Total no. of larvae transferred	No. of adults emerged	No. of dead plants dissected in 2011	No. of larvae found at dissection of dead plants (% survival / dead plants dissected)	Total no. of adults + larvae–pupae recovered (total % survival in Sept. 2011)
Family Apocynaceae						
Tribe Asclepiadeae						
Subtribe Tylophorinae						
Vincetoxicum hirundinaria	8	240	0	4	50 (41.7)	50 (20.8)
Vincetoxicum nigrum	8	240	0	4	64 (53.3)	64 (26.7)
Vincetoxicum rossicum	8	240	0	2	1 (1.7)	1 (0.4)
Subtribe Asclepiadinae						
Asclepias curassavica	5	150	0	5	0	0
Asclepias fascicularis[a]	5	150	0	3	0	0
Asclepias fruticosa	5	150	0	5	0	0
Asclepias incarnata[a]	5	150	0	2	16 (26.7)	16 (10.7)
Asclepias speciosa[a]	5	150	0	1	0	0
Asclepias syriaca[a]	5	150	0	4	0	0
Asclepias tuberosa[a]	5	150	0	4	0	0
Subtribe Cynanchinae						
Cynanchum laeve[a]	5	150	0	-	-	0
Subtribe Metastelmatinae						
Funastrum angustifolium[a]	5	150	0	2	0	0
Tribe Ceropegieae						
Ceropegia woodii	5	150	0	5[c]	0	0
Tribe Malouetieae						
Pachypodium lamerei	5	150	0	5[c]	0	0
Tribe Apocyneae						
Apocynum cannabinum[a]	5	150	1[b]	-	-	1 (0.7)
Tribe Vinceae						

Plant species[a]	2010		2011			
	No. of replicates (plants)	Total no. of larvae transferred	No. of adults emerged	No. of dead plants dissected in 2011	No. of larvae found at dissection of dead plants (% survival / dead plants dissected)	Total no. of adults + larvae–pupae recovered (total % survival in Sept. 2011)
Amsonia tabernaemontana[a]	5	150	0	-	-	0
Tribe Carisseae						
Carissa macrocarpa	5	150	0	-	-	0
Family Gentianaceae						
Centaurium erythraea	5	150	0	2	0	0
Family Rubiaceae						
Cephalanthus occidentalis[a]	5	150	0	-	-	0

[a] Plant species native to North America.
[b] Found dead in emergence cage.
[c] All plants were alive at dissection.

Table 3. Larval transfer tests carried out in 2010 for *Eumolpus asclepiadeus* (ex Ukraine, one-year 'strain'; 2009 generation) and beetle recovery in 2011.

Plant species[a]	2010		2011			
	No. of replicates (plants)	Total no. of larvae transferred	No. of adults emerged	No. of dead plants dissected in 2010/11	No. of larvae found at dissection of dead plants (% survival / dead plants dissected)	Total no. of adults + larvae – pupae recovered (total % survival in Sept. 2011)
Family Apocynaceae						
Tribe Asclepiadeae						
Subtribe Tylophorinae						
Vincetoxicum hirundinaria	10	300	2	4	25 (20.8)	27 (9.0)
Vincetoxicum nigrum	10	300	1	3	51 (56.7)	52 (17.3)
Vincetoxicum rossicum	11	330	0	6	51 (28.3)	51 (15.5)
Subtribe Asclepiadinae						
Asclepias curassavica	5	150	0	2	0	0
Asclepias fascicularis[a]	8	240	0	6	0	0
Asclepias fruticosa	5	150	0	5	0	0
Asclepias incarnata[a]	5	150	0	-	-	0
Asclepias speciosa[a]	5	150	0	-	-	0
Asclepias syriaca[a]	7	210	0	4	2 (1.7)	2 (1.0)
Asclepias tuberosa[a]	7	210	0	6	1 (0.6)	1 (0.5)
Asclepias viridiflora[a]	5	150	0	1	0	0
Subtribe Cynanchinae						
Cynanchum acutum	6	180	0	2	0	0
Cynanchum laeve[a]	5	150	0	-	-	0
Subtribe Gonolobinae						
Matelea gonocarpos[a] (=*suberosa*)	5	150	0	-	-	0
Subtribe Metastelmatinae						
Funastrum angustifolium[a]	5	150	0	1	0	0
Tribe Ceropegieae						
Ceropegia woodii	5	150	0	5[b]	0	0
Stapelia gigantea	5	150	0	5[b]	0	0

Plant species[a]	2010		2011			
	No. of replicates (plants)	Total no. of larvae transferred	No. of adults emerged	No. of dead plants dissected in 2010/11	No. of larvae found at dissection of dead plants (% survival / dead plants dissected)	Total no. of adults + larvae – pupae recovered (total % survival in Sept. 2011)
Tribe Malouetieae						
Pachypodium lamerei	5	150	0	5[b]	0	0
Tribe Apocyneae						
Apocynum cannabinum[a]	5	150	0	-	-	0
Tribe Vinceae						
Amsonia tabernaemontana[a]	5	150	0	1	0	0
Tribe Carisseae						
Carissa macrocarpa	5	150	0	-	-	0
Family Gentianaceae						
Centaurium erythraea	5	150	0	-	-	0
Family Rubiaceae						
Cephalanthus occidentalis[a]	5	150	0	-	-	0

[a] Plant species native to North America.
[b] All plants alive at dissection.

Table 4. Larval transfer tests carried out in 2010 for *Eumolpus asclepiadeus* (ex France) and beetle recovery in 2011.

Plant species[a]	2010		2011			
	No. of replicates (plants)	Total no. of larvae transferred	No. of adults emerged	No. of dead plants dissected in 2011	No. of larvae found at dissection of dead plants (% survival / dead plants dissected)	Total no. of adults + larvae – pupae recovered (total % survival in Sept. 2011)
Family Apocynaceae Tribe Asclepiadeae Subtribe Tylophorinae						
Vincetoxicum hirundinaria	5	150	1	4	41 (34.2)	42 (28.0)
Vincetoxicum nigrum	5	150	1	3	8 (8.9)	9 (6.0)
Vincetoxicum rossicum	5	150	0	3	12 (13.3)	12 (8.0)
Subtribe Asclepiadinae						
Asclepias curassavica	5	150	0	5	0	0
Asclepias fruticosa	3	80	0	3	0	0
Asclepias incarnata[a]	5	150	0	3	12 (13.3)	12 (8.0)
Asclepias tuberosa[a]	5	150	0	2	0	0.0
Subtribe Cynanchinae						
Cynanchum laeve[a]	5	150	0	-	-	0
Family Gentianaceae						
Centaurium erythraea	5	150	0	2	0	0

[a] Plant species native to North America.

Table 5. Single-choice adult feeding, adult survival and reproduction tests for *Eumolpus asclepiadeus* (ex France) on *Asclepias* spp. and *Vincetoxicum* spp. in 2010 and beetle recovery in 2011.

	A. tuberosa – A. incarnata (5 replicates)		*A. fruticosa – A. incarnata* (5 replicates)		*A. curassavica – A. fruticosa* (5 replicates)		*V. hirundinaria – V. hirundinaria* (5 replicates – control)	
	A. tuberosa	*A. incarnata*	*A. fruticosa*	*A. incarnata*	*A. curassavica*	*A. fruticosa*	*V. hirundinaria*	*V. hirundinaria*
Adult feeding[a]	(+)	+	-	(-)	-	-	++	++
Mean max. female longevity (days ± SD)	23.2 ± 7.1		14.4 ± 4.9		10.8 ± 4.0		**>** 49.8 ± 16.1	
Beetle recovery in 2011	0	0	0	0	0	0	0	0

[a] – no feeding; (-) some nibbling on one occasion; (+) some nibbling in all replicates; + some feeding on 1–2 leaves in all replicates; ++ regular feeding.

2.2. Single-choice adult feeding, adult survival and reproduction tests in 2010–11

METHODS. Single-choice tests were conducted for *E. asclepiadeus* beetles collected in early July 2010 from the Jura Region in France by René Sforza, USDA-ARS-EBCL. Tests were established with *A. tuberosa* NA, *A. incarnata* NA, *A. fruticosa* and *A. curassavica,* and *V. hirundinaria* as a control.

Two potted test (*Asclepias*) plants were placed together into a large 30-litre pot and surrounded by soil; the control was set up in the same way with two *V. hirundinaria* plants. At the same time, a no-food control was established in ventilated plastic cylinders half-filled with soil and stored under the same conditions, protected from direct sunshine. One mating pair of *E. asclepiadeus* was used in each replicate. The following combinations were set up on 5 July 2010:

- *A. tuberosa* NA – *A. incarnata* NA (five replicates)
- *A. fruticosa* – *A. incarnata* NA (five replicates)
- *A. curassavica* – *A. fruticosa* (five replicates)
- *V. hirundinaria* – *V. hirundinaria* (five replicates; control)
- No food (five replicates; control)

The large pots were covered with a gauze bag and one mating pair of *E*. *asclepiadeus* was released into each bag. Beetle survival and feeding were recorded every week. All pots were overwintered in an unheated greenhouse and dissected in early May 2011 to record numbers of mature larvae and pupae.

RESULTS AND DISCUSSION. *Asclepias incarnata* NA was the preferred host for adult feeding in the *A. tuberosa* NA – *A. incarnata* NA test, perhaps because of the much bigger size of the *A. incarnata* NA plants compared to *A. tuberosa* NA (Table 5). Slight feeding was recorded on *A. incarnata* NA on one occasion only in the *A. fruticosa* – *A. incarnata* NA tests and the females survived for a shorter time too. Regular feeding was recorded in the *V. hirundinaria* – *V. hirundinaria* tests, and both males and females lived much longer; in three out of five replicates, both males and females were still alive after nearly two months when the test was stopped. In the *A. tuberosa* NA – *A. incarnata* NA test, the males were found dead within one week in three out of five replicates, and replaced. No adult survived longer than eight days in the no-food control. In summary, some nibbling occurred on *A*. *tuberosa* NA while for some reason more significant but inconsistent feeding was recorded on *A. incarnata* NA. No feeding was recorded on *A. fruticosa* and *A. curassavica*. Female longevity was shorter on the non-target hosts than on *V. hirundinaria*.

Most *A. fruticosa* and *A. curassavica* plants did not survive the winter, but all *V. hirundinaria* were alive in spring 2011. Quite surprisingly, no larvae or pupae were recorded on any of the plants used in this test. This is perhaps due to the overwintering conditions and a possible excess of watering.

2.3. No-choice adult feeding and reproduction tests with naïve adults in 2010–11

METHODS. Preliminary adult feeding and reproduction tests with naïve adults have been conducted with *A. tuberosa* NA, *A. incarnata* NA, *V. nigrum* and *V. rossicum*. Naïve adults (= adults which have not fed on any plants) of *E. asclepiadeus* ex Ukraine and emerging from the 2008 (LT 2008) and 2009 (LT 2009) larval transfer tests were used separately. All tests were set up on 25 June – 2 July 2010.

Pots were covered individually with a gauze bag and one mating pair of *E. asclepiadeus* was released onto each pot. Mesh gauze was placed on the soil surface of each potted plant (to prevent eggs from being deposited deep into the soil) and covered with a layer of 2–3 cm of finely sieved soil to facilitate quicker recovery of the eggs laid. The sieved soil was, however, quickly replaced by covering the soil surface with paper allowing even quicker recovery and counting of the eggs.

Beetle survival and oviposition were recorded every week. Eggs were kept separately and the newly hatched larvae transferred onto plants of the same species the parents had been placed on to check whether beetles are able to sustain a population on the *Asclepias* spp. All *A. tuberosa* NA and *A. incarnata* NA plants as well as all dead *Vincetoxicum* spp. plants were dissected in October 2011.

RESULTS AND DISCUSSION. Although, again, hardly any feeding occurred on the two *Asclepias* species, females were able to reproduce on both to a certain extent (Table 6). On *A. tuberosa* NA, 122 out of a total of 126 eggs were laid in one of the three replicates and on *A. incarnata* NA, all 24 eggs were laid on one of the three plants offered. Mean number of eggs laid and mean female longevity were higher on the target plants than on *A. tuberosa* NA and *A. incarnata* NA. No adults emerged and no larval survival was recorded in 2011 from a total of 84 larvae and seven larvae transferred on to *A. tuberosa* NA and *A. incarnata* NA, respectively. Survival of one-year-old larvae was relatively high on *Vincetoxicum* spp. Final results will be available after beetle emergence in 2012 and dissection of all plants.

Table 6. No-choice adult feeding and reproduction test for naïve adults of *Eumolpus asclepiadeus* in 2010 and beetle recovery in 2011.

	LT 2008[a]	LT 2009[a]			
	Vincetoxicum rossicum (3 replicates)	*V. rossicum* (3 replicates)	*V. nigrum* (2 replicates)	*Asclepias tuberosa* (3 replicates)	*A. incarnata* (3 replicates)
Mean no. of eggs (± SD)	212 ± 82	127 ± 35	244 ± 193	42 ± 69	8 ± 14
No. of plants with eggs	3	3	2	2	1
Mean female max. longevity (days ± SD)	85 ± 4	80 ± 4	65 ± 23	41 ± 25	26 ± 18
Adult feeding[b]	++	++	++	(+)	(+)
No. of larvae transferred onto host plants in 2010	320	214	450	84	7
No. of adults / larvae recovered in 2011	0 / 13	0 / 11	0 / 62	0	0

[a] Beetles originating from larval transfers (LT) in either 2008 or 2009.
[b] (+)some nibbling; ++ regular feeding.

Table 7. Synopsis of non-target adult feeding and larval development for *Eumolpus asclepiadeus* ex Ukraine (2007–11).

	No-choice tests		Multiple-choice tests	
Plant species[a]	Adult feeding (potted plants)[b]	Maximum percent development to mature larvae / adults[c]	Adult feeding (potted plants)[b]	Oviposition and larval development
Family Apocynaceae				
Subfamily Asclepiadoideae				
Tribe Asclepiadeae				
Subtribe Tylophorinae				
Vincetoxicum hirundinaria	++	25.8%	++	nt
Vincetoxicum nigrum	++	42.6%	++	1.9 ± 2.5 (*N=15*)[i]
Vincetoxicum rossicum	++	36.6%	++	2.4 ± 5.1 (*N=15*)[i]
Subtribe Asclepiadinae				
Asclepias curassavica	0	0	nt	nt
Asclepias fascicularis[a]	nt	7.5%	0	0
Asclepias fruticosa	0	0	nt	nt
Asclepias incarnata[a]	(+)[d]	8.0%	(+)	3.0 ± 3.9 (*N=5*)[g]

	No-choice tests		Multiple-choice tests	
Plant species[a]	Adult feeding (potted plants)[b]	Maximum percent development to mature larvae / adults[c]	Adult feeding (potted plants)[b]	Oviposition and larval development
Asclepias speciosa[a]	nt	18.0%	0	0
Asclepias syriaca[a]	nt	2.2%[e]	nt	nt
Asclepias tuberosa[a]	(+)[d]	5.0%	(+)	0.9 ± 3.2 (*N=12*)[h]
Asclepias viridiflora[a]	nt	2.9%[f]	nt	nt
Subtribe Cynanchinae				
Cynanchum acutum	nt	0	nt	nt
Cynanchum laeve[a]	nt	0	(+)	0
Subtribe Gonolobinae				
Matelea gonocarpos[a] (=*suberosa*)	nt	0	nt	nt
Subtribe Metastelmatinae				
Funastrum angustifolium[a]	nt	4.0%[f]	nt	nt
Subtribe Oxypetalinae				
Araujia sericifera	nt	0	nt	nt
Tribe Ceropegieae				
Ceropegia woodii	nt	0	nt	nt
Stapelia gigantea[a]	nt	0	nt	nt
Tribe Marsdenieae				
Hoya carnosa	nt	0	nt	nt
Subfamily Periplocoideae				
Periploca graeca[a]	nt	0	nt	nt
Subfamily Apocynoideae Tribe Wrightieae				
Nerium oleander	nt	0	nt	nt
Tribe Malouetieae				
Pachypodium lamerei	nt	0	nt	nt
Tribe Apocyneae				
Apocynum cannabinum[a]	nt	0.7%	nt	nt
Trachelospermum jasminoides	nt	0	nt	nt

	No-choice tests		Multiple-choice tests	
Plant species[a]	Adult feeding (potted plants)[b]	Maximum percent development to mature larvae / adults[c]	Adult feeding (potted plants)[b]	Oviposition and larval development
Subfamily Rauvolfioideae				
Tribe Vinceae				
Amsonia tabernaemontana[a]	nt	0	nt	nt
Catharanthus roseus	nt	0	nt	nt
Vinca minor	nt	0	nt	nt
Tribe Plumerieae				
Allamanda cathartica	nt	0	nt	nt
Plumeria rubra	nt	0	nt	nt
Tribe Carisseae				
Carissa macrocarpa	nt	0	nt	nt
Family Loganiaceae	nt	0	nt	nt
Spigelia marilandica	nt	0	nt	nt
Family Gelsemiaceae				
Gelsemium sempervirens	nt	0	nt	nt
Family Gentianaceae				
Centaurium erythraea	nt	0	nt	nt
Family Rubiaceae				
Cephalanthus occidentalis[a]	nt	0.6%	nt	nt

[a] Plant species native to North America.
[b] 0 = no feeding; (+) = nibbling; ++ = regular feeding.
[c] Larval transfer tests carried out 2007–11 at URI and CABI.
[d] Oogenesis and oviposition of fertile eggs occurred although very little adult feeding was recorded.
[e] Based on % survival of one-year-old third instar larvae.
[f] % larval survival after a four month period.
[g] Single-choice adult feeding and oviposition test with *V. hirundinaria* 2008–09.
[h] Multiple-choice adult feeding and oviposition test 2009–10.
[l] Multiple-choice adult feeding and oviposition test 2008–09.
nt Not tested.

2.4. Summary and conclusions

Time to complete one generation is extremely variable in *Eumolpus ascelpiadeus*. In 2010, in contrast to previous observations, the majority of adults (ex Ukraine) emerged from the target *Vincetoxicum* spp. one year after larval transfers. However, in 2011, only a few adults emerged from larval transfers made in 2010.

Adult emergence from dead plants and the recovery of live larvae from dead plants suggests that one-year-old third instar larvae may emerge as adults the following year even in the absence of food if no external mortality factor intervenes.

Previous work at URI has shown that *E. asclepiadeus* can complete larval development on the following North American non-target plants: *Asclepias fascicularis*, *A. incarnata*, *A. speciosa* and *A. tuberosa* (Gassmann *et al*., 2010). Results in 2009–11 have confirmed that *A. incarnata* NA, *A. tuberosa* NA and *A. syriaca* NA are suitable hosts for larval development of *E. asclepiadeus*. Very occasionally larval development may occur on *Apocynum cannabinum* NA and *Cephalanthus occidentalis* NA, perhaps because of a similar chemistry to the Apocanyceae in the latter species. So far, we have observed larval development to the third instar but no adult emergence on *Asclepias viridiflora* NA and *Funastrum angustifolium* NA.

In single-choice tests offering *A. incarnata* NA or *A. tuberosa* NA together with *Vincetoxicum* spp., adult feeding was negligible on the two non-target plants but a few larvae were observed developing on three *A. incarnata* NA the following year (Gassmann *et al*., 2009, 2010).

In multiple-choice field cage tests in 2008–09 and 2009–10, adult feeding was negligible on the non-target plants *A. incarnata* NA*, A. tuberosa* NA, *A. fascicularis* NA, *A. speciosa* NA and *Cynanchum laeve* compared to *Vincetoxicum*, but 11 neonate larvae were recorded on one *A. tuberosa* NA plant in the 2009–10 test indicating that oviposition occurred in the vicinity of this species (Gassmann *et al*., 2009, 2010, 2011).

In 2009, in oogenesis tests using excised shoots, *E. asclepiadeus* females were able to produce fertile eggs on the non-target species *A. incarnata* NA and *A. speciosa* NA, but not *A. fascicularis* NA (Gassmann *et al.,* 2010). In adult starvation tests using excised leaves, the highest survival rate was on *V. rossicum* followed by *A. fascicularis* NA and *V. nigrum* (Gassmann *et al*., 2010).

In 2010, we found that adult feeding on potted non-target plants was much reduced compared to excised shoots: only a little feeding was recorded on *A. incarnata* NA during single-choice adult feeding tests with *A. tuberosa* NA, *A. incarnata* NA, *A. curassavica* and *A. fruticosa* in the absence of *Vincetoxicum* (*E. asclepiadeus* ex France) (Gassmann *et al*., 2011). In a no-choice adult feeding and reproduction test with potted plants, *E. asclepiadeus* naïve females were able to lay fertile eggs on *A. tuberosa* NA and *A. incarnata* NA although only a little adult feeding was recorded on these plants. When neonate larvae were transferred onto the same plant species in 2010, no larval development was recorded. However, the transfer of a larger number of larvae originating from naïve females fed with these two non-target species would be needed before concluding that *A. tuberosa* NA and *A. incarnata* NA are not able to sustain a population of *E. asclepiadeus.*

In summary, a few native North American non-target species in the genus *Asclepias* support complete larval development of *E. asclepiadeus* (Table 7). In the presence of *Vincetoxicum,* oviposition and larval development occasionally occurred on *A. tuberosa* NA and *A. incarnata* NA. Very little or no adult feeding was recorded on potted non-target plants under choice conditions. Adult *E. asclepiadeus* will feed and reproduce when provided with excised leaves of some non-target species. Although little adult feeding occurs on intact non-target leaves (potted plants) in choice or no-choice tests, *E. asclepiadeus* naïve females were able to produce fertile eggs on *A. tuberosa* NA and to a lesser extent on *A. incarnata* NA. Very occasional larval development to the adult stage was also recorded *Apocynum cannabinum* NA and *Cephalanthus occidentalis* NA.

To try to more realistically evaluate potential non-target attack of *E. asclepiadeus* on native NA species supporting larval and/or adult development under no-choice and multiple-choice cage conditions, we suggest that an open-field test or a large cage test should be conducted, where non-target species are placed at different distances from the target plants, since this has been shown to influence attack (Schooler et al. 2003).

3. *Euphranta connexa* (Diptera: Tephritidae)

In spring 2011, Alicia Leroux, University of Manitoba, Winnipeg, Canada, started her Master's degree on the potential biological control agent, *E. connexa* (Diptera: Tephritidae), a seed feeder on *Vincetoxicum hirundinaria* in Europe. Alicia's research is supervised by Dr Neil J. Holliday, University of Manitoba, and at CABI by Drs André Gassmann and Hariet L. Hinz. Alicia's thesis will focus on five main aspects: (i) investigating the timing of *E. connexa* emergence from the overwintering pupal stage, which is a major determinant of time to oviposition, (ii) determining the potential fecundity of *E. connexa* on its natural host and the target weeds, *V. rossicum* and *V. nigrum,* (iii) examining host preference among *V. hirundinaria*, *V. rossicum*, and *V. nigrum,* (iv) examining the oviposition behaviour of *E. connexa* to develop host-range testing methods applicable to this agent, and (v) determining the parasitoid community on *E. connexa*.

Synchronization between adult emergence and the availability of seed pods in an optimal phenological stage for oviposition and larval development is essential. Much effort is therefore directed toward obtaining reproducing test plant species and obtaining active *E. connexa* adults for when test plants become available.

3.1. Post-diapause development

METHODS. To facilitate the synchronization of *E. connexa* adult emergence with target and non-target seed pod production, temperature requirements for post-diapause development and adult emergence time were studied using pupae collected in August 2010 from six sites in southern Switzerland. In October 2010 all pupae were placed in overwintering conditions in an outdoor shelter until 4 February 2011, at which time they were moved to a 3°C incubator. On 15 April, 60 or 72 pupae from each site were placed individually into vials with moistened vermiculite at constant temperatures of 10°C, 15°C, 20°C, 25°C, 30°C and 35°C. Adult emergence was checked 3–4 times a week.

RESULTS. Development time to adult emergence decreased linearly as temperature increased (Fig. 1). The average number of days for adult emergence was 38.1, 45.7 and 76.3 days for 25°C, 20°C and 15°C, respectively. There was no emergence at 30°C and 35°C indicating that these temperatures were too high for development. Although there was noticeable pre-adult development, no adult emergence was recorded at 10°C suggesting that this temperature is very close to the lower threshold for post-diapause development. This study will be continued in 2012 to assess the lower and upper temperature thresholds.

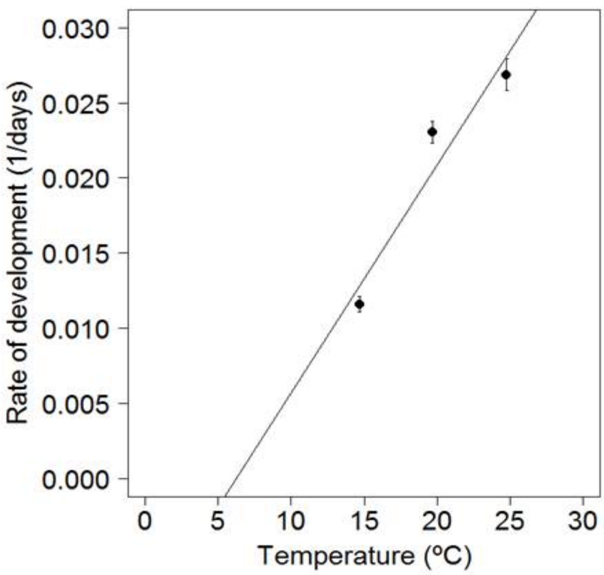

Fig. 1. Effect of temperature on adult emergence time from post-diapausing pupae of *Euphranta connexa.*

3.2. Ovary and egg maturation

METHODS. Tephritid flies emerge as adults without mature ovaries. It is important that time taken for ovary and egg maturation is known to standardize and conduct host-range testing and impact studies. Females emerging from the post-diapause experiment (section 3.1.1) were placed at 20°C in a Petri dish (10 cm diameter, 5.5 cm high) provided with separate portions of sugar, yeast, skimmed milk powder, and milk. Females were randomly assigned a number between one and 20 which corresponded to the number of days each would be kept alive, after which ovaries were dissected and mature eggs were counted. A total of 39 females were used.

RESULTS. The first cycle of ovariole maturation takes about ten days with an average number of eggs of 49 ± 8.0 per female (*N = 18*). All females will be measured in 2012 to check for a correlation between size and egg load. Also, mated females will be given a host plant at day ten and the plants then changed every seven days until female death to assess overall egg production.

3.3. Impact of *Vincetoxicum* spp. on the fecundity of *Euphranta connexa*

In summer 2011, *V. nigrum*, *V. rossicum*, and *V. hirundinaria* were exposed to mated pairs of *E. connexa* within large experimental field cages (200 × 200 × 160 cm). All pods were collected 17 days after release of the adults and held in vials until mature larvae dropped out. The pupae, 56 from *V. nigrum*, 13 from *V. rossicum*, and ten from *V. hirundinaria*, were placed in overwintering conditions until spring 2012. In April 2012, the pupae will be held at 25°C until adult emergence, at which point females will be placed in Petri dishes provided with separate portions of sugar, yeast, skimmed milk powder, and milk. The females will be dissected at ten days of age, as this is when the first cycle of ovariole maturation is complete, to assess fecundity differences when reared from different *Vincetoxicum* species.

3.4. Field collections

Field collections of *V. hirundinaria* fruits nearing maturity were conducted at the end of July 2011 at four sites in southern Switzerland (Plates 1 and 2). The fruits were held in ventilated containers until all pre-pupae had emerged from the fruits. The containers consisted of two cylinders with a mesh screen in between, which allowed the pods to be placed above the mesh and the mature larvae to drop through it and pupate in semi-moist vermiculite in the lower container. Pupae were then placed in Petri dishes with moist vermiculite at ambient temperatures until 27 September, at which point they were moved to an underground shelter for overwintering and then to a 3°C incubator on 2 February 2012. A total of 5654 pupae are currently being overwintered.

Plate 1. Exit hole of *Euphranta connexa* mature larva on *Vincetoxicum hirundinaria.*

Plate 2. Seeds of *Vincetoxicum hirundinaria* have been consumed by *Euphranta connexa* larvae.

4. Discussion

Since 2006 and as of 2011, considerable progress has been made in host-specificity testing of potential biological control agents for invasive *Vincetoxicum* spp. In summary, a few native North American non-target species in the genus *Asclepias* support complete larval development of *Eumolpus asclepiadeus*. Females of *E. asclepiadeus* will occasionally oviposit in the vicinity of non-target plants even in the presence of *Vincetoxicum* resulting in occasional larval development on some non-target plants in the genus *Asclepias*, which still needs further investigation. Very occasional larval development to the adult stage was also recorded on *Apocynum cannabinum* NA *and Cephalanthus occidentalis* NA*.*

We have found that the larval host range of *E. asclepiadeus* on native North American *Asclepias* species is broader than the adult host range. Latex exudation from leaves acts as a strong defence against adult *E. asclepiadeus* herbivory. Although a little adult feeding will occur on intact non-target leaves (potted plants) in choice or no-choice tests, some *Asclepias* species are suitable for occasional nibbling, allowing prolonged longevity and reproduction. Adult *E. asclepiadeus* will feed and reproduce when provided with excised leaves of some non-target species.

The populations of *E. asclepiadeus* from Ukraine and France are genetically quite different but neither population seems to be more host specific than the other.

In 2012, work will focus on monitoring the emergence of *E. asclepiadeus* and on maintaining a colony of the beetle population from Ukraine.

Study of the biology of *Euphranta connexa* will also be continued and methods will be developed for testing this candidate agent.

5. Tentative work programme for 2012

- Record emergence of *Eumolpus asclepiadeus* ex Ukraine adults from plants infested in 2010;
- Maintain rearing colony of *E. asclepiadeus*;
- Depending on funding available, carry out a multiple-choice large cage test with critical NA *Asclepias* species;
- Continue the study of the biology of *Euphranta connexa*;
- Start host-range studies for *E. connexa*.

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Distribution list

Ken Bloem
Bernd Blossey
Robert Bourchier
Naomi Cappuccino
Richard Casagrande
Margarita Dolgovskaya
Richard Hansen
Mark Hoddle
Kim Hoelmer
Fran Lawlor
Suzanne Lyon
Peter Mason
Lindsey Milbrath
Mark Schwarzländer
René Sforza
Sandy Smith
Lisa Tewksbury
Roy van Driesche
Ron Weeks
CABI library (2)

contact CABI

europe

CABI Head Office
Nosworthy Way, Wallingford, Oxfordshire, OX10 8DE, UK
T: +44 (0)1491 832111

CABI
Bakeham Lane, Egham, Surrey, TW20 9TY, UK
T: +44 (0)1491 829080

CABI
Rue des Grillons 1, CH-2800 Delémont, SWITZERLAND
T: +41 (0)32 4214870

asia

CABI
C/o Internal Post Box 56, Chinese Academy of Agricultural Sciences,
12 Zhongguancun Nandajie, Beijing 100081, CHINA
T: +86 (0)10 82105692

CABI
2nd Floor, CG Block, NASC Complex, DP Shastri Marg, Opp. Todapur Village,
PUSA, New Delhi – 110012, INDIA
T: +91 (0)11 25841906

CABI
PO Box 210, 43400 UPM Serdang, Selangor, MALAYSIA
T: +60 (0)3 89432921

CABI
Opposite 1-A, Data Gunj Baksh Road, Satellite Town, Rawalpindi-PAKISTAN
T: +92 (0)51 9290132

africa

CABI
ICRAF Complex, United Nations Avenue, Gigiri, PO Box 633-00621, Nairobi, KENYA
T: +254 (0)20 7224450/62

americas

CABI
UNESP- Fazenda Experimental Lageado, Rua: José Barbosa de Barros, 1780
Botucatu – SP, CEP: 18610-307, BRAZIL
T: (14) 3882 - 6300 / 3811 - 7127

CABI
Gordon Street, Curepe, TRINIDAD AND TOBAGO
T: +1 868 6457628

CABI
875 Massachusetts Avenue, 7th Floor, Cambridge, MA 02139, USA
T: +1 617 3954051

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