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Table of Contents

Summary

Work in 2009 focussed on the root-feeding beetle *Longitarsus noricus*, the leaf-feeding beetle *Cassida stigmatica*, the stem-boring beetles *Microplontus millefolii* and *Phytoecia nigricornis*, and the stem-boring moth *Isophrictis striatella*.

In total, approximately 700 adults of *C. stigmatica*, 293 adults of *M. millefolii* and 40 adults of *P. nigricornis* were collected in Russia, Ukraine and Germany. Several hundred dry stems were collected in northern Germany and eastern France for *I. striatella*. *Longitarsus noricus* adults from Russia could not be shipped to Switzerland for logistic reasons and the root-feeding beetle *Meliboeus graminoides* was not found during the 2009 surveys.

Longitarsus noricus adults emerged in May 2009 from larval transfers on potted plants carried out in summer 2008. Beetles emerged from all *Tanacetum vulgare* plants and all of the nine plant species tested except *Coreopsis grandiflora.* The percent emergence of adults ranged from 34.7% to 96.0% on the *T. vulgare* plants from North America. Percent emergence on test plants was generally lower, except on the North American test species *Achillea ptarmica* and *A. alpina*. However, preliminary molecular identification indicates that contamination in the CABI garden by a local *Longitarsus* may have contributed to the survival rates recorded on several plants. The identification of a larger sample of beetles is ongoing.

Cassida stigmatica larval development to the adult stage was recorded on all of the North American and European populations of *T. vulgare*. However, the number of larvae that developed to the pupal stage was low and inconsistent among replicates and beetle populations. Larval development occurred regularly on the native North American species in the *T. huronense–T. camphoratum* complex as well as the European species *T. parthenium* and *T. balsamita.* There was no larval development recorded on any of the other four European *Tanacetum* species tested or on the eight native North American species in the genera *Artemisia*, *Achillea* and *Arctanthemum*. Adult *C. stigmatica* were still alive in late autumn on all *Tanacetum* spp. except *T. camphoratum* of North American origin, *T. corymbosum* and *T. coccineum*. At the same time, adult *C. stigmatica* were still alive on *Achillea millefolium* and *Arcanthemum arcticum* but not on any of the *Artemisia* spp. The longevity of adults was significantly reduced on *Artemisia* spp. compared to *Tanacetum* control species. The unfed beetles all died within ten days.

In 2008, no oviposition was recorded with *Microplontus millefolii* during laboratory rearing. Several rearing trials were set up in 2009. These included using cut shoots and potted plants, single pairs versus several pairs of beetles, and conducting the trials in an outdoor shelter, in the laboratory or in long-day incubators, in either small plastic cylinders, gauze-covered plants or large field cages. There was some oviposition recorded in all of the trials except when freshly collected adults were reared on cut shoots in an outdoor shelter for less than ten days. Usually, oviposition was recorded two to four weeks after set-up. The best results were obtained on potted plants in a greenhouse and in a 'natural' field cage test. The dissections from the field cage tests indicated that the oviposition period lasted several weeks. The

dissection of a couple of females revealed the absence of mature eggs and suggests that additional food sources besides *T. vulgare* may be required for ovary development.

In no-choice tests, oviposition by *P. nigricornis* pairs was successful on gauze-covered plants of *T. vulgare, T. corymbosum, T. macrophyllum* and *T. parthenium.* Based on the number of eggs laid and the length of the test period, *T. parthenium* appears to be the most suitable species for oviposition followed by *T. vulgare* and *T. macrophyllum*. The highest adult longevity was recorded on *T. vulgare* and *T. macrophyllum.*

In 2009, adult emergence *of I. striatella* started during the third week of July, i.e. at approximately the same time as in 2007 and two weeks earlier than in 2008. Little and inconsistent oviposition was recorded on *T. vulgare* of North American origin in no-choice oviposition tests. *Isophrictis striatella* lays its eggs in the flowerbuds of *T. vulgare*. Mature larvae are extremely mobile when leaving the flowerheads in late autumn to complete development in the dry stems. Occasionally, younger larval instars (in particular from northern Germany) can be found in the dry stems. Thus, the damage the species causes to *T. vulgare* is primarily limited to the base of the flowerbuds, including the achenes. We also found that larvae can spend over 12 months in dry stems and that the moth can have a partial two-year life cycle. There is some evidence that the larval biology of *I. striatella* from northern Germany differs from that of eastern France. Larvae from France are slightly larger than those from northern Germany and we never found larvae in newly growing shoots of *T. vulgare* in the former country. Genetic studies are ongoing to determine whether these populations of *I. striatella* are genetically distinct or perhaps even consist of different species.

As part of a PhD study, a common garden experiment with *T. vulgare* populations from 13 different European countries/areas and nine states/provinces in North America was set up in 2008 to determine the variability of chemical and morphological characters within and between these populations. The morphology was found to be highly diverse between, as well as within, common tansy populations of different origins. All morphotypes occur in both continents. The chemical analyses revealed that 44 different chemotypes could be defined according to the two main terpenoid components. The chemotype diversity was found to be slightly higher in Europe than in North America.

In 2010, we will focus work on host-range and biological studies with *L. noricus*, *M. millefolii* and *C. stigmatica* and on finding *Meliboeus graminoides.* Additional studies with *I. striatella* will depend on results of molecular analyses.

1 Introduction

Common tansy, *Tanacetum vulgare*, is an aromatic European plant that was first introduced into North America as a medicinal and culinary herb, but which is now an increasing weed problem in pastures, rights of way, wastelands and riparian areas across the northern USA and Canada. Investigations in the introduced range suggest that seed dispersal and seedling establishment appear to be more important for population expansion than vegetative spread (White, 1997). The plant produces a range of secondary metabolites that are toxic to humans and livestock. Investigations on the chemical profile of common tansy in Canada revealed that there was a wide variation in essential oil profiles among individual plants (McClay et al., 2002).

The overall goal of the project is to identify European insects that can be introduced into North America as biological control agents for common tansy. A major component of the study is the collection of insects from areas that climatically match invaded areas in North America, and host-specificity testing of prioritized agents using an agreed list of test plants, to minimize risks to non-target plant species.

Field and laboratory studies on European insect species that are potential biological control agents for this weed were initiated in 2006 at CABI Europe – Switzerland (CABI E-CH) in Delémont, Switzerland. A literature survey indicated that 169 herbivores, i.e. one mite and 168 insect species, were associated with the genus *Tanacetum* and other species of Asteraceae in Eurasia (Gassmann et al., 2007). Three insect species develop on *T. achilleifolium* or *T. pseudoachillea* whereas the remaining 166 are associated with *T. vulgare*. Twenty-nine species, i.e. 17%, are noted to be restricted to the genus *Tanacetum*. Most of the species that had previously been identified as potential biological control agents were collected from field sites in Germany, Russia and Ukraine in 2008.

Specific goals in 2009 were to extend surveys to the Moscow area in Russia, and to make progress in (1) determining best collecting times and potential sites for mass collecting, and (2) studying life histories and setting up host-specificity tests. In addition, a common garden experiment with *T. vulgare* populations from 13 different European countries/areas and nine states/provinces in North America has been completed as part of a PhD study with the aim of determining the variability of chemical and morphological characters within and between these populations.

A review of the current distribution of *Tanacetum* spp. in Russia and other republics of the former USSR has been prepared by our collaborators in Russia and is presented in Annex 4.

2 Field surveys, collecting and rearing

2.1 Methods

The main aim of the field work carried out in 2009 was to collect adults of the stem-mining weevil *Microplontus millefolii*, the root-boring leaf beetle *Longitarsus noricus*, the stem-mining longhorn beetle *Phytoecia nigricornis* and the leaf-feeding beetle *Cassida stigmatica,* which are considered to be four of the most promising biological control agents of *T. vulgare*. In addition, field trips were made in early spring and autumn to south-western Germany and eastern France in an attempt to gain a better understanding of the biology of the flower-feeding moth *Isophrictis striatella*. Collections in northern Germany were made in spring, approximately three weeks later than in 2008, to obtain adults of *M. millefolii* at the beginning of their reproductive period. Collections in Ukraine and Russia were made over the same period of time as in 2008 with the purpose of mass-collecting insects for host-specificity testing in the laboratory at CABI E-CH. New field sites around Moscow, Russia, were surveyed to check for the presence of the root-boring beetle *Meliboeus graminoides*. At most sites, plants were swept to collect insects and *T. vulgare* stems clipped and brought back to CABI E-CH for dissection.

2.2 Results

In total, 12 survey and collection trips were made during 2009 to south-western Germany, eastern France, western Switzerland, northern Germany, the St Petersburg and Moscow areas in Russia and the Kiev area in Ukraine.

The collections were successful and resulted in: 293 adults of *Microplontus millefolii*, 40 adults of *P. nigricornis* and approximately 700 adults of *C. stigmatica*.

2.2.1 Northern Germany

The survey and collection trips to northern Germany were carried out in mid May. Sweeping at seven sites resulted in the collection of 42 *M. millefolii* adults and approximately 250 *C. stigmatica* adults (Annex 1). No other insect species of interest were recorded. For insect emergence and results of stem dissections, refer to sections 2.2.5 and 7.1 in this report.

2.2.2 South-western Germany, eastern France and Switzerland

There were very few insects of interest collected during spring 2009 at the five field sites visited in this part of Europe. *Cassida stigmatica* became more visible in late April and early May. Results of stem dissections are reported in section 7.1 in this report.

2.2.3 Ukraine

Work in Ukraine was carried out with the collaboration of Prof. Sergei L. Mosyakin and Andrew Mosyakin (M. G. Kholodny Institute of Botany, Kiev, Ukraine).

Four field sites were visited between 24 and 28 May yielding a total of 40 *P. nigricornis*, 101 *M. millefolii*, and 150 *C. stigmatica*. All but one of the *M. millefolii* adults and all the *P. nigricornis* adults were collected from a single site with half-shaded conditions. At all sites, *T. vulgare* was still in the vegetative stage with the dry stems from the previous year still standing. Maximum plant height was approximately 60 cm at the main collection site.

2.2.4 Russia

Work in Russia was carried out with the collaboration of Dr. Margarita Yu. Dolgovskaya, Dr. Mark Volkovitch and Dr. Sergey Reznik (Zoological Institute, Russian Academy of Sciences, St Petersburg) and Dr. Galina Konechnaya (Botanical Institute, Russian Academy of Sciences, St Petersburg).

Between 1 and 5 June, eight sites around St Petersburg were visited resulting in the collection of 85 adult *M. millefolii* and approximately 220 adult *C. stigmatica*. The majority of the *M. millefolii* adults were collected at one single site which was visited again three weeks later in late June. There were no *M. millefolii* adults found at this time but 100 adult *L. noricus* were collected. Unfortunately, no international carrier was found in St Petersburg to send the living insects to Switzerland and, therefore, no work could be completed with *L. noricus* adults in 2009. *Cassida stigmatica* was present at almost all of the sites, but like *M. millefolii* seemed to be more common in early June. *Phytoecia nigricornis* was present in low densities in the field sites visited. For the second year in a row, *Meliboeus graminoides* was not detected.

Work in the Moscow area was carried out between 4 and 5 June by Dr Gena E. Davidyan, Institute of Plant Protection, St Petersburg. Seventy *C. stigmatica* and 65 *Microplontus millefolii* were collected. *Meliboeus graminoides* was also not detected in this area.

At all sites, *T. vulgare* was still in the vegetative stage and most of the dry stems from the previous year were still standing. The maximum plant height was approximately 40–50 cm.

2.2.5 Dissections of field-collected newly growing stems

Methods: Fresh stems were collected from northern Germany, Ukraine and Russia and dissected in the laboratory under a stereomicroscope within a couple of days of collection. The main objective for dissecting newly growing stems of *T. vulgare* collected in spring 2009 was to better understand the life history and phenology of *M. millefolii, P. nigricornis* and *I. striatella*.

Results: The presence of 11 eggs and two larvae of *M. millefolii* in 19 shoots from Kiev, Ukraine, indicates that collections of *M. millefolii* were made at the beginning of their reproductive period in this area (Table 1). Only one weevil egg was found in 37 shoots dissected from Russia and three weevil eggs in 88 stems dissected from northern Germany. In northern Germany and in Russia, a few moth larvae were recorded in the fresh stems of *T. vulgare,* but these have yet to be identified. In Ukraine, *P. nigricornis* was also at the beginning of its reproductive period, indicated by the presence of several eggs in the shoots dissected.

Table 1 Dissection of newly growing shoots of *Tanacetum vulgare* in spring 2009

Collection site	**Country/area**	**Collection date/ dissection date**	**No. of shoots dissected (total shoot length dissected (cm))**	**No. of weevil eggs/larvae**	**Headcapsule width (mm)**	**No. of other eggs/larvae**	**Headcapsule width (mm)**	**Notes**
Vogelsheim (F1)	Eastern France/Alsace	23 April 2009/ 27 April 2009	14 (549)	0	-	0		3 holes in roots
Vogelsheim (F1)	Eastern France/Alsace	8 May 2009/ 9 May 2009	18 (848)	0	-	0		21 *Cassida stigmatica* eggs on foliage
Rendsburg (D1)	Northern Germany	16 May 2009/ 22 May 2009	30 (1277)	2 *Microplontus millefolii* eggs in shoot bud	-	3 Lep larvae		9 holes, sometimes at base of stems; 7 empty mines
Daldorf (D3)	Northern Germany	18 May 2009/ 20 May 2009	28 (893)	0	-	2 live Lep larvae (1 in vegetative shoot tip), 1 dead		Mines 1.5 and 3 cm long; 6 empty mines up to 9 cm long; holes in stems
Neumuenster (D2)	Northern Germany	17 May 2009/ 22 May 2009	30 (977)	1 *M. millefolii* egg in shoot bud	-	1 live Lep larva, one dead		2 long mines
Hotiv (U4)	Ukraine	28 May 2009/ 2 June 2009	19 (429)	11 *M. millefolii* eggs in shoot bud just below leaf node; 2 live larvae	0.408 0.384	*Phytoecia nigricornis*: 6 eggs in stems; 3 live larvae, 2 dead	0.384 0.576 0.576	
Izvara (RU-2008-20)	Russia/St Petersburg	4 June 2009/ 9 June 2004	37 (755)	1 *M. millefolii* egg		10 Lep larvae		

2.3 Conclusion and outlook

Taking into consideration the range of collection times and all the field sites visited in 2009, *M. millefolii* appears to be most prominent in north-eastern Europe as it was found commonly in northern Germany, Ukraine and Russia. In contrast, *P. nigricornis* appears to be more common in central and western Europe as it was found mostly in the Kiev area in Ukraine, and it is known to be relatively abundant in Bonn in western Germany (Schmitz, 1998) which share the same latitude. *Cassida stigmatica* appears to be the most common species found on *T. vulgare* across the whole of Europe while *L. noricus* has the narrowest geographical distribution of all the species recorded during surveys so far and was only found in Russia. Although dry stems have not been sampled and dissected from all sites surveyed this year, *I. striatella* seems to be as common as *C. stigmatica* but this still needs to be confirmed. *Meliboeus graminoides* is a more central-eastern European species (Alekseev, 1988) but was not found during 2009 field collections. For the purpose of mass insect collection, in 2009, it appears that the best collection times for adults of *M. millefolii* and *C. stigmatica* in northern Germany, Russia and Ukraine and *P. nigricornis* in Ukraine were met.

3 *Longitarsus noricus* (Col., Chrysomelidae)

3.1 Background

Longitarsus noricus is a small, bright-brown flea beetle which was found for the first time during field collections at one main site near St Petersburg, Russia, in 2007. It was initially thought that this species might be *L. succineus*, the only flea beetle associated with *T. vulgare* that had been described in the literature at this time, but Ron Beenen (The Netherlands) identified it as *L. noricus*. Adults of this species have been frequently misidentified as *L. succineus,* but they can be distinguished by differences in the aedaeagus and spermatheca. According to Leonardi (1976), the geographical range of *L. noricus* includes northern Italy, Austria, Germany, Poland, the Czech Republic, Hungary, Romania, the former Yugoslavia and possibly the former USSR.

3.2 Host-range studies, 2008–2009

Methods: The 99 adults collected in Russia in July 2008 were placed in groups of 20 in five transparent plastic cylinders and offered a cut shoot of *T. vulgare* inserted into a moist block of florist sponge. Cylinders were kept in a rearing chamber at 22±2°C. Adult *L. noricus* fed on the cut *T. vulgare* shoots and immediately started to lay eggs into the florist sponge. Eggs were regularly collected by dissecting the sponge material and were then transferred to Petri dishes and stored at room temperature. Newly hatched larvae were transferred onto six different populations of *T. vulgare* collected from North America, and one population of *T. vulgare* from Europe (Table 2). Larvae were also transferred onto nine test species, mostly plants native to North America, forming a total of 77 plants and 1,908 larvae transferred. The number of larvae per replicate (plant) was usually 25. The plants were monitored over the summer for signs of adult emergence. All potted plants were kept in an unheated greenhouse between October 2008 and April 2009 when the plants were brought into the rearing chamber to monitor adult emergence. Plants which were dead in spring 2009 were not included in the results.

Table 2 No-choice larval transfer tests with *Longitarsus noricus* in 2008 and adult emergence in 2009

Plant species[a]	**No. valid replicates**	**Total no. larvae transferred (2008)**	**No. of replicates with adults emerged**	**No. of adults emerged in 2009 (% emergence)**
Tanacetum vulgare EU	2	50	2	26 (52.0)
T. vulgare Big Bay	5	125	5	45 (36.0)
T. vulgare Carlton	4	100	3	41 (41.0)
T. vulgare Ely area	2	50	2	48 (96.0)
T. vulgare Parkland	5	125	4	47 (37.6)
T. vulgare Calgary	3	75	3	26 (34.7)
T. vulgare Edmonton	4	100	3	39 (39.0)
T. camphoratum	4	100	3	16 (16.0)
T. parthenium	4	100	4	21 (21.0)
T. cinerariifolium	2	50	2	3 (6.0)
Achillea alpina NA	1	25	1	17 (68.0)
A. ptarmica NA	2	50	2	37 (74.0)
Artemisia frigida NA	2	50	2	4 (8.0)
Arctanthemum arcticum NA	3	58	2	18 (31.0)
Coreopsis grandiflora NA	2	50	0	0
Arnica chamissonis NA	2	50	1	3 (6.0)

[a], Carlton, the Ely area and Big Bay are locations in Minnesota, USA; Parkland County, Calgary and Edmonton are locations in Alberta, Canada; EU = material from Europe; NA = species native to North America.

Results: *Longitarsus noricus* adults emerged in May 2009. Beetle larvae were able to develop to the adult stage on all *T. vulgare* plants and test plant species except *Coreopsis grandiflora.* The percent emergence of adults ranged from 34.7% to 96.0% on the *T. vulgare* plants from North America. The percent emergence on the test plants was generally lower; however, it was very high on the North American test species *Achillea ptarmica* and *A. alpina*. Preliminary molecular identification indicates that contamination in the CABI garden by a local *Longitarsus* may have contributed to the survival rates recorded on several plants. To confirm this, a large subsample of beetles that emerged in 2009 has been sent for genetic identification with results expected in early winter.

3.3 Conclusion and outlook

Because of the contamination of our garden plants by other *Longitarsus* species, it is premature to draw any conclusions on the physiological host range of *L. noricus*. Logistic problems prevented additional host-range studies with this species in 2009. These problems will be solved in 2010. *Longitarsus noricus* is easy to rear and the main focus in the coming season will be on larval transfer tests. On 20 October 2009, all plants being used in the *Tanacetum* project were treated with a contact and ingestion pesticide, diazinon (Alaxon® EW 600g/l) which will be repeated in spring 2010. In addition, all plants to be used in the host-range studies for *L. noricus* will be protected in field cages in spring 2010 to avoid further contamination. Samples of beetles that emerge in 2011 from larval transfers carried out in 2010 will be identified with molecular tools.

4 *Cassida stigmatica* (Col., Chrysomelidae)

4.1 Overwintering, 2008–2009

All beetles were overwintered in an unheated greenhouse either in cages containing potted *T. vulgare* plants or on potted, gauze-covered plants of various origins. As of early March 2009, 45.7% of all *C. stigmatica* adults reared during 2008 had overwintered successfully. None of the 2008 field-collected Russian beetles survived the winter but 15.4% of those collected in Ukraine did (Table 3).

Table 3 Overwintering of *Cassida stigmatica* adults in 2008–2009

Origin of beetles and status	No. adults (2008)	Host plant[a]	Adult survival (2009)
		Cages	
Ukraine; reared 2008	73	*Tanacetum vulgare* Carlton and *T. vulgare* Parkland	31 (42.5%)
Ukraine; field-collected (spring 2008)	13	*T. vulgare* Carlton and *T. vulgare* Parkland	2 (15.4%)
Russia; field-collected (spring 2008)	12	*T. vulgare* Carlton and *T. vulgare* Parkland	0
		Individual potted plants	
Mixed Germany and Russia; reared 2008	6	*T. vulgare* NA	3 (50.0%)
Mixed Germany and Russia; reared 2008	13	*T. vulgare* EU	5 (38.5%)
Mixed Germany and Russia; reared 2008	7	*T. huronense* NA	4 (57.1%)
Mixed Germany and Russia; reared 2008	6	*T. camphoratum* NA	5 (83.3%)

[a], EU = material from Europe; NA = material from North America.

4.2 Fecundity

Methods: Mating *C. stigmatica* pairs from France, northern Germany, Ukraine and Russia were placed in Petri dishes and raised on leaves of *T. vulgare* Carlton and *T. vulgare* Edmonton first in an outdoor shelter and then in an indoor rearing chamber. Adult survival and oviposition were recorded three times weekly.

Results: The highest mean fecundity was observed with beetles collected from northern Germany and eastern France in early to mid May (Table 4). When ovipositing females only are considered, the fecundity of field-collected females from Ukraine was similar to those from northern Germany and eastern France (data not shown). One possible explanation for the non-ovipositing females is that they belonged to the new generation of beetles hatched in 2009 (although it seems too early in the season for this to be likely). Over two-thirds of all *C. stigmatica* pairs were still alive in late summer. Beetles are being overwintered in an outdoor shelter.

Table 4 Mean fecundity of *Cassida stigmatica* by individual pairs in 2009

Origin of beetles (set-up date)	No. females (pairs)	Mean fecundity (± SD) (*n*)	Mean duration of egg-laying period (±SD) (*n*)	No. ovipositing females	Beetle longevity
Eastern France, field collected on 7 May 2009 (13 May 2009)	5	168.5±113.4 (*5*)	47.3±34.3 (*5*)	5	3 pairs still alive mid September
Northern Germany, field collected on 16-18 May 2009 (21 May 2009)	13	169.6±144.3 (*13*)	47.3±27.4 (*13*)	13	11 pairs still alive mid September
Ukraine, reared 2008 (23 May 2009)	4	102.8±72.3 (*4*)	33.8±14.7 (*4*)	4	3 pairs still alive mid September
Ukraine, field collected on 25-28 May 2009 (2 June 2009)	16	97.6±107.1 (*16*)	21.0±18.8 (*16*)	11	10 pairs still alive mid September
Russia, field collected on 1-2 June 2009 (6 June 2009)	3	44.0±28.8 (*3*)	20.0±12.2 (*3*)	3	2 pairs still alive mid August

4.3 Egg development

Methods: *Tanacetum vulgare* leaves containing *C. stigmatica* eggs were placed in Petri dishes and held in a 20°C incubator until hatching. Every day, eggs were observed and larval emergence recorded.

Results: Approximately 4,500 *C. stigmatica* eggs hatched out of the 7,000 laid, resulting in a 64.9% hatch rate. There was a large degree of variation in hatch rates among different populations of *C. stigmatica* ranging from 30% in those collected from eastern France in early spring to over 90% from the population collected in Ukraine in spring 2009.

4.4 Host-range studies

4.4.1 No-choice larval development tests

Methods: Between early June and late July, ten newly hatched larvae were randomly transferred onto potted *T. vulgare* plants from North America and Europe as well as 16 test plant species. Larval survival was recorded 14 and 25 days after transfer until all pupae had been collected. All plants were covered with a gauze bag and kept in an unheated greenhouse.

Results: *Cassida stigmatica* larval development to the adult stage was recorded on all of the North American and European populations of *T. vulgare* (Table 5), however, the number of larvae that developed to the pupal stage was low and inconsistent among replicates and beetle populations. Larval development occurred regularly on the native North American species in the *T. huronense–T. camphoratum* complex as well as the European species *T. parthenium* and *T. balsamita.* There was no larval development recorded on any of the other four European *Tanacetum* species tested or on the eight native North American species in the genera *Artemisia*, *Achillea* and *Arctanthemum*.

Table 5 Results of no-choice larval development tests with *Cassida stigmatica* in 2009

Plant species[a]	No. replicates	Total no. larvae transferred	No. replicates with larval development	Total no. pupae	Percent larval development to pupal stage
Cassida stigmatica from northern Germany					
Tanacetum vulgare Carlton	5	50	3	16	32.0
T. vulgare Finland	5	50	1	5	10.0
T. vulgare northern Germany	3	30	3	14	46.7
T. vulgare Ukraine	4	40	1	4	10.0
T. vulgare Switzerland	5	50	3	13	26.0
T. vulgare Edmonton	5	50	2	7	14.0
T. vulgare British Columbia	5	50	2	4	8.0
T. vulgare California A	5	50	3	7	14.0
T. vulgare California B	4	40	3	9	22.5
T. vulgare Minnesota	5	50	3	11	22.0
T. huronense NA	5	50	3	4	8.0
T. parthenium	5	50	2	2	4.0
Artemisia frigida NA	2	20	0	0	0.0
Cassida stigmatica from Ukraine					
Tanacetum vulgare EU	7	70	3	10	14.3
T. vulgare Big Bay	7	70	6	9	12.9
T. vulgare Ely area	7	70	4	13	18.6
T. vulgare Calgary	8	80	6	13	16.3
T. vulgare Parkland	8	80	3	14	17.5
T. camphoratum NA	70	70	0	0	0.0
T. huronense NA	7	70	3	9	12.9
T. balsamita	6	60	5	11	18.3
T. cinerariifolium	5	50	0	0	0.0
T. coccineum	5	50	0	0	0.0

Plant species[a]	No. replicates	Total no. larvae transferred	No. replicates with larval development	Total no. pupae	Percent larval development to pupal stage
T. corymbosum	7	70	0	0	0.0
T. macrophyllum	8	80	0	0	0.0
T. parthenium	6	60	5	12	20.0
Artemisia campestris NA	5	50	0	0	0.0
A. dracunculus NA	5	50	0	0	0.0
A. frigida NA	5	50	0	0	0.0
A. ludoviciana NA	5	50	0	0	0.0
A. tridentata NA	5	50	0	0	0.0
Achillea alpina NA	5	50	0	0	0.0
A. millefolium NA	5	50	0	0	0.0
Arcanthemum arcticum NA	5	50	0	0	0.0
Cassida stigmatica from Russia					
Tanacetum vulgare EU	3	30	3	12	36.7
T. vulgare Big Bay	3	30	1	4	13.3
T. vulgare Ely area	3	30	1	1	3.3
T. vulgare Calgary	3	30	1	4	13.3
T. vulgare Parkland	3	30	0	0	0.0
T. camphoratum NA	3	30	0	0	0.0
T. huronense NA	3	30	1	4	13.3
T. balsamita	3	30	2	2	6.7
T. cinerariifolium	3	30	0	0	0.0
T. coccineum	3	30	0	0	0.0
T. corymbosum	3	30	0	0	0.0
T. macrophyllum	3	30	0	0	0.0
T. parthenium	3	30	1	4	13.0

[a], NA = material from North America; EU = material from Europe.

4.4.2 Adult starvation tests

Methods: Five adult *C. stigmatica* beetles collected in 2009 were transferred onto one leaf of a test plant in a 10-cm Petri dish and kept in a rearing chamber at 22°C and 14 h daylight. A total of 115 beetles were used. The test was set up between 9 and 12 June 2009 and beetle survival recorded twice each week. Leaves were usually changed once a week. Beetles that were still alive on 4 November 2009 were placed in a 5°C incubator for overwintering. Survival will continue to be recorded throughout the winter.

Results: Adult *C. stigmatica* were still alive as of 4 November 2009 on all *Tanacetum* spp. except *T. camphoratum* of North American origin, *T. corymbosum* and *T. coccineum* (Table 6). At the same time, adult *C. stigmatica* were still alive on *Achillea millefolium* and *Arcanthemum arcticum* but not on any of the *Artemisia* spp. The longevity of adults was significantly reduced on *Artemisia* spp. compared to *Tanacetum* spp. The unfed beetles all died within ten days.

Table 6 Adult starvation tests with *Cassida stigmatica* in 2009 (set-up date: 9–12 June 2009)

Plant species[a]	**No. adults**	**Mean longevity ± SD (*N*), 4 November 2009**	**No. adults alive, 4 November 2009**
None (control)	15	5.6 ± 2.4 (*15*)	0
T. vulgare Big Bay	15	74.4 ± 24.5 (*11*)	4
T. vulgare Edmonton	10	79.5 ± 26.0 (*8*)	2
T. camphoratum NA	5	33.8 ± 28.4 (*5*)	0
T. huronense NA	5	125.0 ± 20.9 (*4*)	1
T. balsamita	5	96.7 ± 15.0 (*3*)	2
T. cinerariifolium	5	51.0 ± 73.6 (*3*)	2
T. coccineum	5	13.6 ± 3.1 (*5*)	0
T. corymbosum	5	94.0 ± 10.3 (*5*)	0
T. macrophyllum	5	113.3 ± 31.6 (*3*)	2
T. parthenium	5	59.3 ± 70.8 (*3*)	2
Artemisia campestris NA	5	10.2 ± 3.4 (*5*)	0
A. dracunculus NA	5	14.2 ± 14.7 (*5*)	0
A. frigida NA	5	8.2 ± 1.1 (*5*)	0
A. tridentata NA	5	36.8 ± 20.9 (*5*)	0
Achillea alpina NA	5	55.8 ± 31.0 (*5*)	0
A. millefolium NA	5	82.5 ± 30.4 (*2*)	3
Arcanthemum arcticum NA	5	32.5 ± 26.0 (*4*)	1

[a], NA = material from North America.

4.4.3 Overwintering, 2009–2010

Currently, 392 *C. stigmatica* beetles have been transferred onto potted, gauze-covered *T. vulgare* Carlton plants and are overwintering in an outdoor shelter. Of these, 190 were from 2009 field collections (53 from Ukraine, 50 from northern Germany and 87 from Russia) and 15 were reared during 2008 (Ukrainian material). Another 187 beetles were newly emerged from larval transfer experiments in 2009 (92 from northern German, 73 from Ukrainian and 22 from Russian material).

4.5 Conclusion and outlook

Newly emerged *C. stigmatica* adults do not reproduce until late winter – early spring of the following year. The fecundity of *C. stigmatica* is variable and adults can live much longer than the egg-laying period. A small percentage of each beetle generation survives more than two winters in our rearing conditions and these beetles can remain active during the third year. Larval development tests indicate that the physiological host range is restricted to a few species in the genus *Tanacetum*. All three *C. stigmatica* populations tested survived on native North American species in the *T. huronense–T. camphoratum* complex as well as on the European species *T. parthenium*. Choice adult feeding and oviposition tests will be carried out in 2010.

5 *Microplontus millefolii* (Col., Curculionidae)

5.1 Surveys and collections

In 2009, 42 *M. millefolii* adults were collected from northern Germany, 101 from Ukraine and 150 from the St Petersburg and Moscow areas in Russia. Collections were made at the beginning of the reproductive period at most sites in northern Germany and in Russia and slightly later in Ukraine as indicated by the results of the dissections of newly growing stems (Table 1).

5.2 No-choice oviposition tests on cut shoots or potted plants

In 2008, no oviposition was recorded during laboratory rearing. Several rearing trials were therefore set up in 2009 and are summarized below.

Trial 1 Cut shoots – outdoor shelter; freshly collected beetles; single pairs versus several pairs

Methods: On 25 May 2009, field-collected *M. millefolii* from northern Germany were sexed and individual couples transferred into cylinders (15 cm height; 11 cm diameter) containing cut shoots of various populations of *T. vulgare.* Cylinders were stored in an outdoor shelter. Beetle mortality was recorded weekly, cut shoots were replaced with fresh ones and old shoots were dissected to check for the presence of egg laying until test completion on 9 June 2009.

On 30 May 2009, field-collected *M. millefolii* from Ukraine were sexed and either one or ten couples transferred into cylinders containing cut shoots of various populations of *T. vulgare*, which were then stored in an outdoor shelter. Beetle mortality was recorded twice, cut shoots were replaced with fresh ones and old shoots were dissected to check for the presence of egg laying until test completion on 9 June 2009.

Results: There were no eggs found and no beetle mortality recorded in the four replicates using *M. millefolii* from northern Germany. There were also no eggs found and no beetle mortality recorded in the ten replicates using one pair of beetles from Ukraine. Finally, no eggs and 6.4% beetle mortality was observed in seven replicates using ten pairs of beetles from Ukraine. The surviving beetles were used in a field cage test (see trial 7).

Trial 2 Potted plants – various conditions; freshly collected beetles

Methods: Field-collected *M. millefolii* from northern Germany, Ukraine and Russia were sexed and transferred onto potted, gauze-covered *T. vulgare, T. macrophyllum* or *T. coccineum* plants. Plants were stored in an unheated greenhouse, a long-day incubator (20°C, 18 h light: 12°C, 6 h light) or a rearing chamber (20°C, 14 h light) until the plants were dissected approximately ten days after transfer. *Microplontus millefolii* adults were then moved onto new potted plants and returned to their rearing chamber. These plants were subsequently dissected to check whether oviposition had continued.

Table 7 No-choice oviposition tests on gauze-covered potted *Tanacetum vulgare* plants under various conditions

Plant species[a]	No. replicates (no. pairs /replicate)	Conditions	Approximate time period	Total no. eggs laid (oviposition period)	Percent beetles dead (or missing)
T. vulgare NA	7 (2)	Greenhouse	4-15 June	0	17.9
T. vulgare NA	4 (2)	Greenhouse	4 June - 10 July	15[b] (early July)	50.0
T. macrophyllum	4 (2)	Greenhouse	4-29 June	0	93.8
T. coccineum	4 (2)	Greenhouse	4-18 June	0	100.0
T. vulgare NA	1 (4)	Rearing chamber	10 June - 7 July	8 (late June)	100.0
T. vulgare NA	1 (8)	Incubator	10 June - 7 July	1 (late June)	93.8

[a], NA = material from North America; [b], a few eggs laid in three out of four replicates.

Results: *Microplontus millefolii* eggs were found in the leaf buds of *T. vulgare* shoots, two to four weeks after set-up (Table 7). No oviposition was recorded on *T. macrophyllum* and *T. coccineum*.

Trial 3 Cut shoots – long-day incubator; older beetles

Methods: *Microplontus millefolii* adults previously reared on potted *T. vulgare* plants (see trial 2) were transferred into cylinders containing cut shoots of various populations of *T. vulgare* and reared in a long-day incubator (20°C, 18 h light: 12°C, 6 h). A total of ten males and ten females were exposed to plants from mid June to mid July (four replicates) and from 10 July to 17 July (two replicates). Twice weekly, cut shoots were replaced with fresh ones and old shoots were dissected to check for the presence of eggs.

Results: Only one egg was recorded, on 10 July, laid by a female which had not oviposited in previous tests on potted plants; 20% mortality was recorded during the trial period.

Trial 4 Cut shoots – outdoor shelter; older beetles

Methods: A total of seven female and eight male *M. millefolii* adults previously reared on cut shoots in a long-day incubator (see trial 3) were transferred into an outdoor shelter in mid July. Dissection of cut shoots continued twice a week to check for the presence of eggs until mid September.

Results: One egg was recorded on 10 August; 33% mortality was recorded during the test period.

Trial 5 Cut shoots – long-day incubator; older beetles, several pairs per container

Methods: *Microplontus millefolii* adults from Russia were sexed and five or six pairs transferred into cylinders containing cut shoots of various populations of *T. vulgare* and reared in a long-day incubator (20ºC: 18 h light, 12ºC: 6 h). Beetle mortality was recorded weekly, cut shoots were replaced with fresh ones and old shoots were dissected to check for the presence of eggs. The tests were carried out between 12 June and 17 July with five replicates completed.

Results: Two eggs were recorded three to seven days after set-up in one of the four replicates completed; 29.5% beetle mortality was recorded during the test period.

Trial 6 Cut shoots – outdoor shelter; older beetles, several pairs per container

Methods: In mid July, 16 female and 15 male adult *M. millefolii* from Russia, previously reared on cut shoots in an incubator (see trial 5), were transferred into an outdoor shelter where they continued to be reared on cut *T. vulgare* shoots in a plastic cylinder. Dissection of cut shoots continued twice a week to check for the presence of eggs until late September.

Results: Only one egg was recorded, on 3 August, laid by a female which had not oviposited in previous tests in the incubator; 29% mortality was recorded during the test period.

Trial 7 'Plain' field cage; freshly collected beetles

Methods: On 4 June 2009, eight female and ten male *M. millefolii* from Ukraine were released into an 'artificial' field cage (Plate 1) containing six potted *T. vulgare* plants from various locations in North America. The *T. vulgare* were embedded in sawdust; no additional plants were present. One month after transfer, all *T. vulgare* plants were dissected to check for eggs. One female from the field cage was re-collected on 12 June and dissected to determine if she had mature eggs and was thus capable of laying eggs. A second field cage was established using seven new, potted *T. vulgare* plants from various locations in North America. These were dissected 42 days later to check for oviposition.

Results: On 1 July, 40 *T. vulgare* shoots were dissected from the first field cage yielding two eggs and one living *M. millefolii* larva. There were no eggs or larvae recorded in the 33 shoots dissected on 11 August from the second field cage. No mature eggs were found in the dissected female.

Trial 8 'Natural' field cage; older beetles

Methods: On 12 June, eight pairs of *M. millefolii* from Russia were released into a 'natural' field cage (Plate 2) containing eight potted *T. vulgare* plants from various locations in North America. In contrast to the plain field cage (trial 7), the natural field cage contained two bushes (*Salix* and *Corylus*) and several herbaceous species, thus creating a more natural environment. Five weeks after set-up, all *T. vulgare* plants were removed and partly dissected to determine if *M. millefolii* had laid any eggs. The remaining shoots were dissected 2–3 weeks later.

Plate 1 'Plain' field cage

Plate 2 Inside the 'natural' field cage

Results: Nine *M. millefolii* larvae were found in a total of 30 *T. vulgare* shoots dissected five weeks after set-up and two empty larval cavities were recorded in the remaining 16 shoots dissected seven weeks after set-up. All three larval instars of *M. millefolii* (Figure 1) were found, indicating that weevils exhibited a prolonged oviposition period under these conditions. Oviposition usually occurred at the uppermost leaf node (Plate 3). Longitudinal tissue splitting was often observed near an old oviposition site or in the lowest part of an old mine (Plate 4). The larvae were found in mines that varied in length from 0.5 cm to 15 cm. Mines were found in the flowering stalk often between leaf nodes, travelling upwards. These observations correspond with dissections of fresh plant material collected in the field that showed that oviposition occurs in the uppermost leaf node below the vegetative bud (see section 2.2.5).

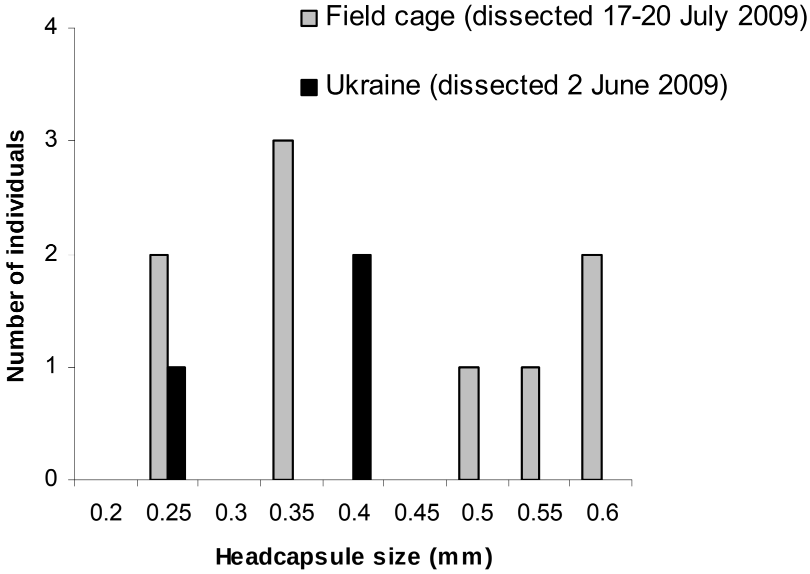

Figure 1 Headcapsule size of larvae of *Microplontus millefolii*

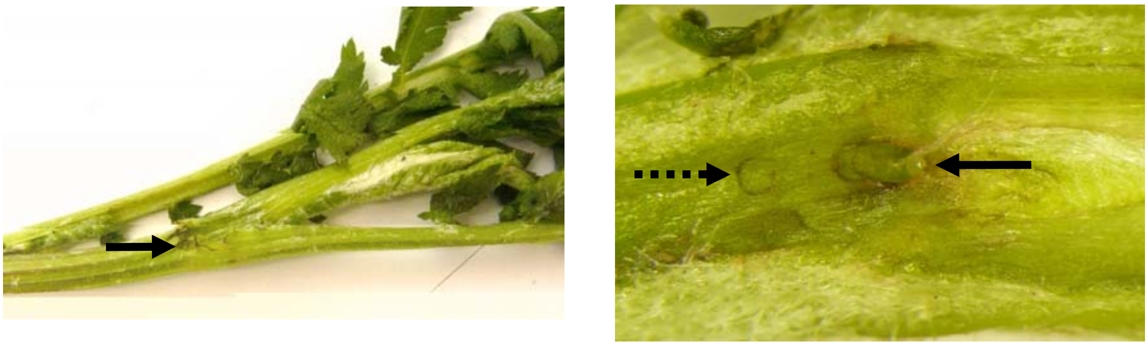

Plate 3 Oviposition site (left, arrow) and oviposition hole with egg (right, full arrow); adult feeding hole (broken arrow)

Plate 4 Tissue splitting at oviposition site (left) and old mine (right)

5.3 Summary of oviposition tests

There was some oviposition recorded in all of the trials except when freshly collected adults were reared on cut shoots in an outdoor shelter for less than ten days (Table 8). Usually, oviposition was recorded two to four weeks after set-up. The best results were obtained on potted plants in a greenhouse and in the 'natural' field cage test. The dissections from the field cage tests indicated that the oviposition period was prolonged. Adults may require additional food sources besides *T. vulgare* for ovary development.

Table 8 Summary of oviposition tests with *Microplontus millefolii* in 2009

	Oviposition on cut shoots		**Oviposition on potted plants**	
	1-2 pairs	Multiple pairs	1-2 pairs	Multiple pairs
Outdoor shelter	**No**: trial 1[c] **Yes**: trial 4[ab]	**No**: trial 1[c] **Yes**: trial 6[ab]	-	-
Greenhouse	-	-	**Yes**: trial 2[a]	-
Long-day incubator	**Yes**: trial 3[ab]	**Yes**: trial 5	-	**Yes**: trial 2[ab]
Rearing chamber	-	-	-	**Yes**: trial 2[a]
Natural field cage	-	-	-	**Yes**: larvae recorded
Plain field cage	-	-	-	**Yes**[a]

[a], oviposition two to four weeks after set-up; [b], only one egg laid; [c], test lasted usually less than ten days.

5.4 Dissection of *Microplontus millefolii* females

Methods: Because few eggs were found in field-collected *T. vulgare* stems and in laboratory oviposition tests, nine adult *M. millefolii* females were dissected on 12 June 2009 to look for maturing eggs in the ovaries. These included one female from northern Germany, two from Moscow, two from St Petersburg and four from Ukraine.

Results: Of the nine *M. millefolii* females dissected, one contained one mature egg (female from St Petersburg) and eight had visible, well-developed ovarioles, but no mature eggs present. Adult *M. millefolii* females will also be checked for *Wolbachia* spp. and other symbionts in collaboration with Dr Jen White, Department of Entomology, University of Kentucky, Lexington, USA, since symbionts are known to interfere with the reproduction of their hosts (Werren, 1997).

5.5 Adult starvation tests

Methods: *Microplontus millefolii* adults were transferred into 10-cm Petri-dishes containing cut leaves of *T. vulgare* from various North American populations, *T. macrophyllum* and a no-food control and stored in an outdoor shelter. Twice weekly, leaves were replaced and weevil survival was recorded.

Results: Three out of five beetles reared on *T. vulgare* of North American origin were still alive as of 14 October 2009. The longevities of beetles reared on *T. macrophyllum* and the no-food control were lower and similar (Table 9).

Table 9 Adult starvation tests with *Microplontus millefolii* in 2009

Plant species[a]	No. replicates (no. adult per replicate)	Mean longevity ± SD (days) (*N*)	No. adults alive, 14 October 2009
None (no-food control)	5 (1)	19.6 ± 6.5 (*5*)	0
Tanacetum vulgare NA	5 (1)	46.5 ± 21.9 (*2*)	3
T. macrophyllum	4 (1)	21.8 ± 7.5 (*4*)	0

[a], NA = material from North America.

5.6 Conclusion and outlook

The best collection time for *M. millefolii* adults has been determined to be late May to early June. In spite of several trials and various new experimental rearing conditions, no major improvement was recorded in egg-laying by *M. millefolii*. Laboratory and field observations suggest that a prolonged feeding period by the adults is necessary before the egg-laying period starts. Adult beetles are long-lived and the oviposition period seems to extend over several weeks. Additional tests with potted plants in greenhouse conditions and field cages tests will be carried out in 2010. In addition, we will establish an open field test in the St Petersburg area in Russia.

6 *Phytoecia nigricornis* (Col., Cerambycidae)

Forty beetles were collected from Ukraine on 26/28 May 2009. The sexing of *P. nigricornis* is very difficult and only pairs for which mating had been confirmed under a binocular microscope were used in preliminary oviposition tests. In these tests, single pairs were reared on cut stems of *T. vulgare* in 13 cylinders in an outdoor shelter between 30 May and 2 June. Cut stems were dissected a few days after set-up to check for the presence of eggs. Eggs (1–6) were found in 12 of the 13 replicates. Those ovipositing females were used in no-choice oviposition tests.

6.1 No-choice oviposition tests

Methods: On 5 June 2009, 11 *P. nigricornis* pairs were transferred onto potted, gauze-covered plants of *T. vulgare, T. corymbosum, T. macrophyllum* and *T. parthenium* and placed in an unheated greenhouse. One week after transfer, plants were dissected to check for the presence of eggs. The surviving couples were put onto new plants and weekly dissections continued until 10 July. At this time there were only three remaining *P. nigricornis* couples and, therefore, they were transferred onto cut shoots of *T. macrophyllum* and *T. parthenium* and stored in boxes in a long-day incubator. Every three days shoots were replaced and dissected to check for the presence of oviposition until the remaining couples had died.

Results: *Phytoecia nigricornis e*ggs and larvae were recorded on all of the plant species tested (Table 10). Based on the number of eggs laid and the length of the test period, *T. parthenium* appears to be the most suitable species for oviposition followed by *T. vulgare* and *T. macrophyllum*.

Table 10 No-choice oviposition tests with *Phytoecia nigricornis* in 2009

Plant species[a]	No. replicates (females)	No. shoots dissected	No. replicates with eggs/larvae	Total no. eggs/ larvae	Total exposure time (days)	No. progeny /day
Tanacetum vulgare NA	3	26	2	14	32	0.44
T. corymbosum	3	29	1	8	68	0.11
T. macrophyllum	3	29	2	25	99	0.25
T. parthenium	2	60	2	102	106	0.96

[a], NA = material from North America.

6.2 Adult starvation tests

Methods: Unsexed *P. nigricornis* adults were transferred individually into cylinders containing cut shoots of *T. vulgare, T. macrophyllum, T. corymbosum,* and a no-food control. The cylinders were stored in an outdoor shelter. Shoots were changed three times a week and adult survival was recorded.

Results: The highest longevity was recorded on *T. vulgare* of North American origin and *T. macrophyllum* (Table 11). Adult longevity was lower on *T.*

corymbosum, a species which was also less suitable for oviposition by *P. nigricornis*. Adult longevity was much lower than on potted plants.

Table 11 Starvation tests with *Phytoecia nigricornis* on cut shoots in 2009

	No of replicates	Mean longevity ± SD (days)
No food control	2	10.0 ± 0.0
Tanacetum vulgare NA	3	56.7 ± 28.3
T. corymbosum	2	27.0 ± 1.4
T. macrophyllum	2	64.0 ± 0.0

[a], NA = material from North America.

6.3 Conclusion and outlook

Although we have not studied host suitability for larval development, the stem-boring beetle *Phytoecia nigricornis* is likely to lack host specificity in no-choice conditions. No work is planned in 2010 but, depending on project development, choice oviposition and larval development tests could be carried out in future years.

7 *Isophrictis striatella* (Lep., Gelechiidae)

Adult *I. striatella* moths emerge in summer between late July and early August. Oviposition tests carried out in 2007 and 2008 showed that egg laying only occurred in the flowerheads of the host (Gassmann et al., 2008, 2009). In early April 2008, 85% of the larvae of *I. striatella* still inhabited the flowerheads of *T. vulgare* collected from northern Germany in early December 2007, and 15% were recorded in the dry stems. In May 2008, almost all larvae were found in the dry stems from a collection made in late April in the same area in northern Germany. In early January 2009, about 50% of the larvae were found in the dry stems and 50% in the flowerheads of *T. vulgare* from a collection made in the same area in late October 2008. Thus, although the proportion of the larvae in the stems and the flowerheads at the beginning of the year fluctuates, it appears that *I. striatella* larvae move from the flowerheads to existing dry shoots during the winter to complete their development in the dry stems. It was also observed that larvae can survive over two winters in the dry stems before the adults emerge.

In spring 2009, a field site in eastern France, close to CABI E-CH, was discovered that contained a small, undisturbed population of *T. vulgare* with a high density of *I. striatella*. This population is being sampled regularly to better understand the biology of this moth.

7.1 Dissections of field-collected dry stems and flowerheads

In 2009, stems and flowerheads of *T. vulgare* were collected from a number of sites in northern Germany and eastern France for dissection and adult rearing. In addition, stems collected in 2007 and 2008 were dissected to check for living *I. striatella* larvae still inhabiting the stems in 2009 and for headcapsule measurements to be taken. The results of all the dissections carried out between 2007 and 2009, from field-collected material, are presented in Annexes 2 and 3. A summary of adult emergence for all collections can also be found there.

The dissection of *T. vulgare* shoots collected from the field in 2009 confirmed results from 2008 indicating that only one *I. striatella* larva matures in the flowerhead at one time but that there can be several larvae developing simultaneously in a single stem. The dissection in April 2009 of shoots collected in autumn 2007 revealed a few living *I. striatella* larvae. No larvae were found in August during dissection of the same material and no adult emergence was recorded during the summer. However, it is possible that emerging adult *I. striatella*, which are very small, were not detected among the bundle of dry stems.

The dissection in April 2009 of shoots collected about 12 months earlier also revealed a few living *I. striatella* larvae, indicating that not all adults emerge during the first year. There was one collection site (Negernbötel, 26 April 2008) where adults emerged in two consecutive summers. Summer dissections of the same material only revealed dead larvae. Thus, larvae are unlikely to survive two summer emergence periods. Living larvae were also found in November 2009 in stems collected at several sites in spring 2009, i.e. after the adult summer emergence period was completed.

According to Freise (1997), there are five larval instars and the median of the headcapsule size of the fifth larval instar is approximately 0.66 mm. Mature *I. striatella* larvae were recorded in the flowerheads of *T. vulgare* plants collected from Vogelsheim in eastern France in late September and mid November 2009 (Figures 2, 3; Annex 3). Our measurements suggest, however, that the larval headcapsules of *I. striatella* from eastern France are slightly larger than those from northern Germany (Figure 4). Larvae leaving the flowerheads in November consisted of mature larvae (Figures 2, 4C).

7.2 Study of larval behaviour

Methods: To investigate when *I. striatella* larvae leave the flowerheads in autumn, 600 flowerheads were removed from senescent stems collected on 24 September 2009 in eastern France and placed in plastic boxes. The containers were checked daily for emerging *I. striatella* larvae, which were then transferred in groups of five onto 15-cm pieces of dry *T. vulgare* stems in plastic boxes. Observations were made daily on the condition and location of the larvae. Ten larvae were transferred into 98% alcohol and their headcapsules were measured. On 18 November, 100 flowerheads were dissected to check for the presence of additional larvae.

On 17 November, another five dry shoots were collected in eastern France. They were dissected on 18 November to compare larval biology in the field with that observed in the laboratory.

Results: From early to mid November 2009, 27 *I. striatella* larvae emerged from the flowerheads. The larval headcapsule measurements indicate that these were mostly mature larvae (Figure 2). Of the 15 larvae placed on pieces of dry stems, one was found dead on 19 November and 14 had tunnelled at both ends of the shoots. The dissection of 100 flowerheads indicated the presence of 37, mostly large, larvae in the flowerheads.

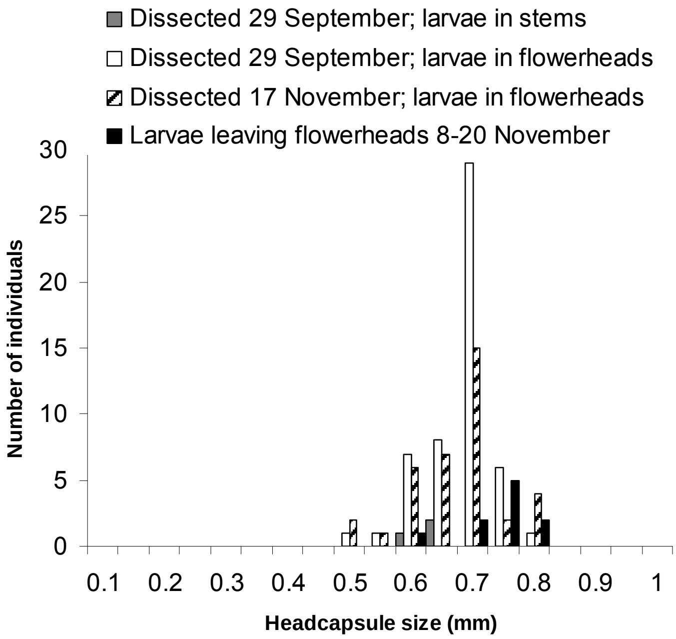

Figure 2 Larval headcapsules of *Isophrictis striatella* collected on 24 September 2009 in eastern France

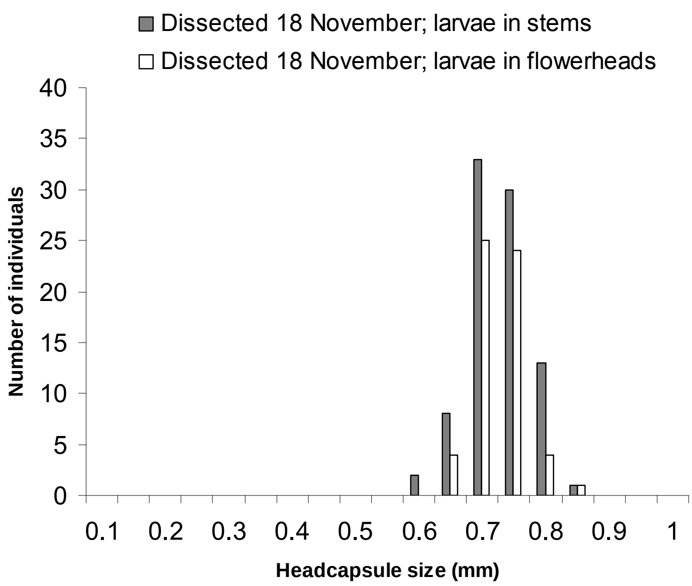

Figure 3 Larval headcapsules of *Isophrictis striatella* collected on 17 November 2009 in eastern France

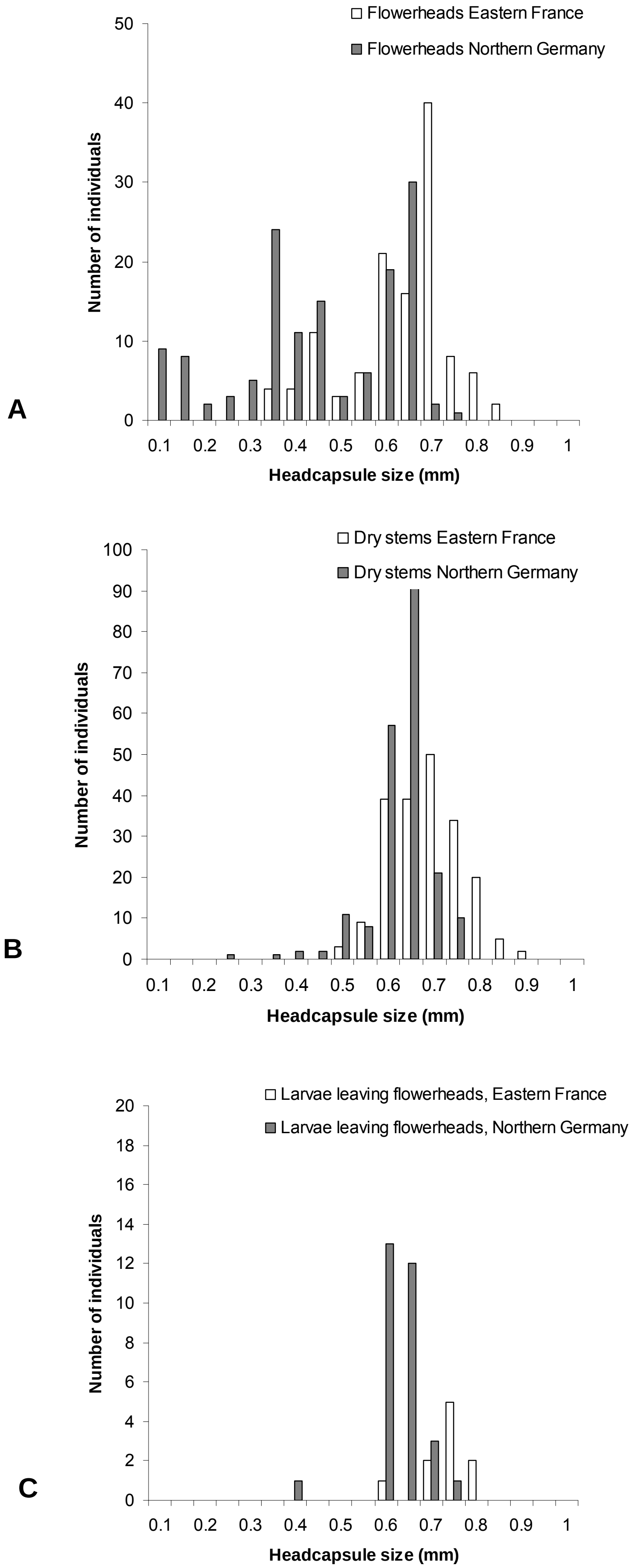

Figure 4 Larval headcapsules of *Isophrictis striatella*; A, in flowerheads; B, in dry stems; C, in larvae leaving the flowerheads in autumn

The dissection of the dry stems collected on 3 September and 24 September indicated that only a few mature larvae had moved by that time to the dry stems (Annex 2; Plate 5). Eight weeks later in mid November, the number of larvae in stems had increased 15-fold. Larval headcapsule measurements revealed only mature larvae in the flowerheads and stems (Figure 3). Thus, in both collections the migration of mature larvae from the flowerheads into the dry stems was ongoing in mid November 2009.

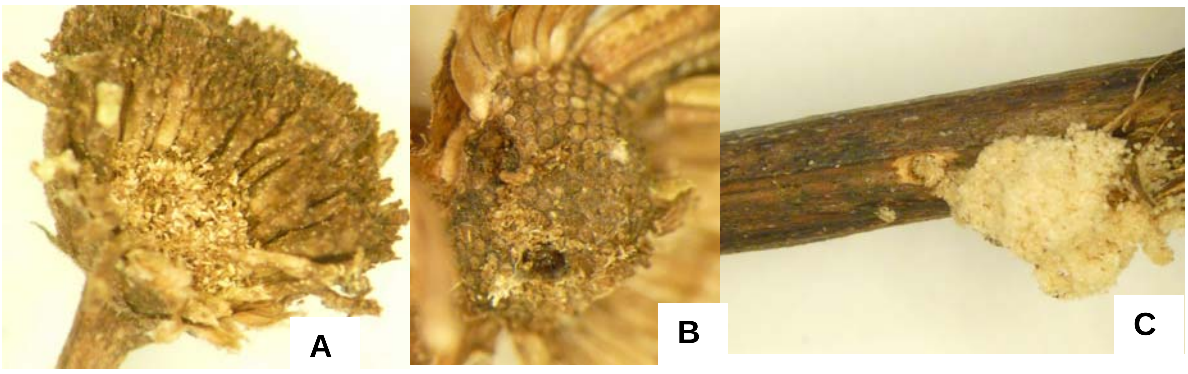

Plate 5 Larval biology of *Isophrictis striatella* on *Tanacetum vulgare* in autumn; A, flowerhead with frass; B, larva in receptacle; C, larval entry hole with external frass in dry stem

7.3 Adult emergence

In 2009, adult emergence started during the third week of July, i.e. at approximately the same time as in 2007 and two weeks earlier than in 2008 (Figure 5).

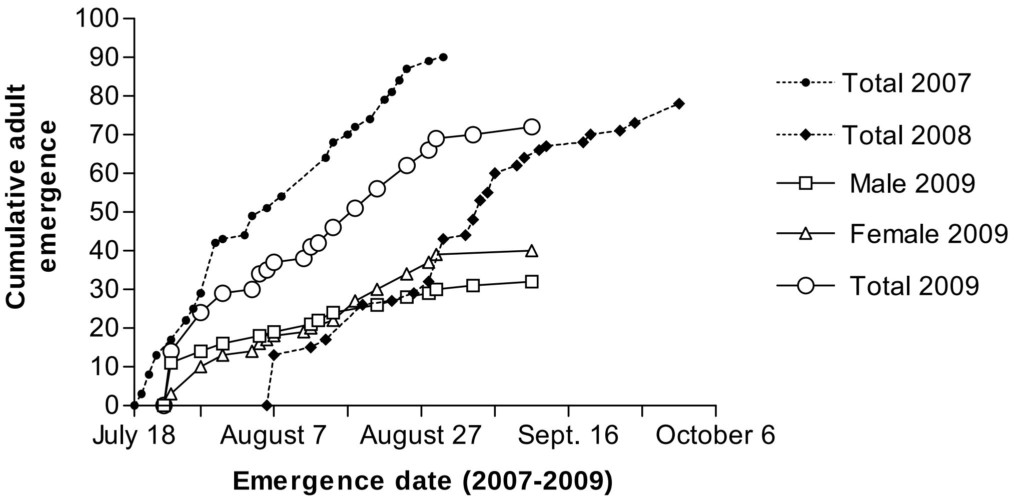

Figure 5 Emergence of *Isophrictis striatella* adults from dry *Tanacetum vulgare* stems in 2007–2009

7.4 No-choice oviposition tests

Methods: *Isophrictis striatella* that emerged between July and August 2009 from dry stems collected in 2008 and 2009 were sexed and then transferred onto potted, flowering *T. vulgare* plants from North America as well as the

North American native species *Achillea ptarmica* and *A. millefolium*. All of the plants were covered with a gauze bag and kept in a ventilated greenhouse until they were dissected approximately two months after set-up to check for the presence of *I. striatella* larvae in the flowerheads or stems.

Results: A few *I. striatella* larvae were recorded in the flowerheads of one of the two replicates using *T. vulgare* Big Bay as well as the one replicate using *T. vulgare* Carlton (Table 12). There was never more than one larva found per flowerhead and no larvae were found in the stems. All but two of the larvae were found dead. Thus, little and inconsistent oviposition was recorded on *T. vulgare* of North American origin.

Table 12 No-choice oviposition tests with *Isophrictis striatella* in 2009

Plant species[a]	No. replicates	No. adults /replicate	No. stems dissected (total length in cm)	No. larvae in stems	No. flowerheads dissected	No. larvae in flowerheads (HC width in mm)[b]
Tanacetum vulgare Big Bay	2	5♀♀/5♂♂ 4♀♀/2♂♂	7 (293)	0	60	2 live larvae (0.408; 0.384); 1 dead larva (0.216)
T. vulgare Ely area	1	5♀♀/3♂♂	2 (94)	0	27	0
T. vulgare Carlton	1	4♀♀/4♂♂	2 (116)	0	19	4 dead larvae (0.168; 0.408)
Achillea ptarmica NA	3	4 ♀♀/4♂♂	11 (252)	0	45	0
A. millefolium NA	2	3♀♀/2♂♂ 1♀/1♂	2	0	192	0

[a], NA = material from North America; [b], HC = headcapsule.

7.5 Conclusion and outlook

The oviposition tests confirm that *I. striatella* oviposits in the flowerheads. Early larval instars of *I. striatella* feed and develop within the receptacle. Later instars can be found mainly in the dry stems.

In contrast to Freise (1997), we did not observe adult emergence as early as late May and oviposition in vegetative shoot buds. Also, according to this author, larvae with a headcapsule size of approximately 0.53 mm leave the dry stems in spring to complete development in newly growing shoots. Our data indicate that the majority of larvae from south-western Germany/eastern France have a headcapsule size greater than 0.65 mm when they leave the flowerheads to move to the dry stems and this migration occurs in winter rather than in spring. On one occasion only, we observed that larvae left dry stems about ten days after a collection made in mid April 2007 in northern Germany. Headcapsule width was in the range 0.9–1.15 mm (Gassmann et al., 2008) but we did not confirm whether these larvae were really *I. striatella*. In contrast to results from south-western Germany/eastern France, we found a relatively high number of younger larval instars in the flowerheads from northern Germany dissected in spring.

Lepidopteran larvae were never found in newly growing shoots collected in May in south-western Germany/eastern France, while shoots from all sites collected in northern Germany and Russia in spring contained a few lepidopteran larvae. We still need to confirm that these are *I. striatella*. However, Freise (1997) also found lepidopteran larvae in newly growing shoots of *T. vulgare* in southern Scandinavia, and these were identified as *I. striatella* after adult emergence.

Schmitz (1998) worked in western Germany south of Bonn on the insect fauna associated with *T. vulgare* and described the biology of *I. striatella* as follows: "*Isophrictis striatella* lays its eggs in the flowerbuds of *T. vulgare*. The larvae live in the base of the flowerbuds up to the fourth larval instar, and then leave in autumn in order to bore into the stem further below. They spend the winter as larvae in the stem. The damage the species causes to *T. vulgare* is primarily limited to the base of the flowerbuds, including the achenes. *Isophrictis striatella* was a common species in the study region, inhabiting sunny locations". These observations are similar to ours but we also found that larvae can spend over 12 months in dry stems and that the moth can have a partial two-year life cycle. Mature larvae leave the flowerheads in late autumn to complete development in the dry stems. Occasionally, younger larval instars (in particular in northern Germany) can be found in the dry stems. How the larvae move from the flowerheads into the stems is not known but it is likely that they move externally and occasionally mine directly into the main stems through the flowerstalks. Our study on larval behaviour in 2009 indicates that the larvae are extremely mobile when leaving the flowerheads and are able to tunnel into cut dry stems.

In summary, there is some evidence that there is a difference in phenology, biology and larval size between populations of *I. striatella* from northern Germany and south-western Germany/eastern France. Interestingly, Bonn in western Germany and Kiev in Ukraine are at the same latitude, and at both sites there was no evidence of *I. striatella* larvae mining into freshly growing shoots in spring. *Isophrictis striatella* larvae from northern Europe (e.g. northern Germany, southern Scandinavia, St Petersburg in Russia), perhaps have a slower development time and the late-developing larvae will move in spring into newly growing shoots to complete development. Molecular studies are ongoing to determine whether the populations of *I. striatella* are genetically distinct or perhaps even consist of different species.

Adult emergence started two weeks earlier than in 2008 and lasted almost eight weeks, until early September. As in previous years, oviposition by *I. striatella* in confinement was not straightforward.

Depending on results of molecular analyses and project development with the other potential biological control agents, we will continue no-choice oviposition and larval development tests on selected native North American plant species.

8 PhD thesis by Vera Wolf (University of Bielefeld, Germany)

In 2008, Vera Wolf, a student at the University of Bielefeld, Germany, started research for a PhD thesis in collaboration with CABI E-CH, studying the different chemotypes of *T. vulgare* and the implications this has for the biological control of the plant. Work in Bielefeld is supervised by Professor Caroline Müller.

8.1 Introduction

Common tansy is a strongly aromatic plant that is characterized by essential oils mainly consisting of monoterpenes and sesquiterpenes such as 1,8-cineole, α- and β-thujone, camphor, borneol, germacrene D and others. Because the qualitative and quantitative composition of these essential oil components varies greatly between individual plants, and this could influence the preference and/or performance of specialist herbivores, we decided to study the diversity and distribution of the different so-called chemotypes of *T. vulgare* and implications for biological control. The different chemotypes of *T. vulgare* can be defined by the different proportions of essential oil components.

8.2 Morphotypes and chemotypes

Methods: To investigate genetically determined differences in chemistry as well as in morphology of native and invasive *T. vulgare* populations, a common garden experiment was set up at CABI E-CH in 2008. Plants from 13 European and nine North American populations were grown under standardized conditions, to exclude different environmental influences. One hundred seeds of each of five mother plants per population were sown. Several growth parameters, such as total biomass, inflorescence biomass, number of stems and stem height, were recorded from three offspring of each mother plant after 3.5 months. In addition, the terpene profile and C:N ratio of four- to five-week-old seedlings were investigated. The terpene spectra were analysed by gas chromatography–mass spectrometry (GC-MS).

Results: The morphology was found to be highly diverse between, as well as within, common tansy populations of different origins (Plate 6). All morphotypes occur in both continents. A continent effect was only found for the number of stems, where it was observed that North American plants had significantly more stems than their European counterparts. For the remaining measured growth parameters, no significant differences could be found between the plants of the two continents. However, these results are only indicative as seed weight as well as latitude, temperature and precipitation in the areas where the plant populations were collected have not yet been included as covariates in the model.

Plate 6 Morphotype variability of *Tanacetum vulgare* of different origins

The chemical analyses revealed that 44 different chemotypes could be defined according to the two main terpenoid components. The chemotype diversity was found to be slightly higher in Europe than in North America (Figure 6, Shannon-Index). The distribution of the chemotypes on both continents was almost homogenous.

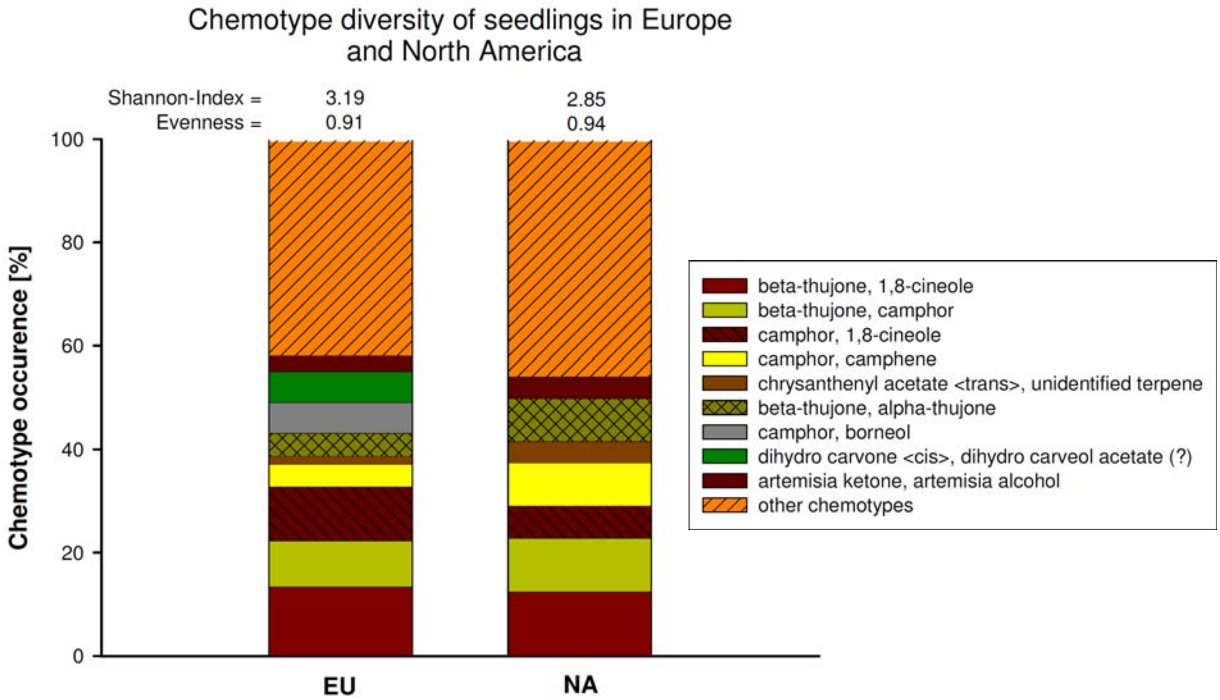

Figure 6 Occurrence and distribution of different *Tanacetum vulgare* chemotypes from Europe (EU) and North America (NA) [%], chemotypes defined by two main components; *n* (EU) = 65; *n* (NA) = 45

8.3 Work with *Cassida stigmatica*

Methods: To assess the implications that the chemical diversity of *T. vulgare* might have on the potential biological control agent *C. stigmatica*, single-choice adult feeding and oviposition tests and larval performance tests were set up on different chemotypes of *T. vulgare* and on the North American species *T. huronense* and *Artemisia frigida* in May 2009. The chemotypes of the test plants were analysed by GC-MS. The single-choice tests were set up in cylinders with cut leaves inserted into a floral sponge, whereas the performance tests were carried out on potted plants covered with a gauze bag (Plate 7). *Cassida stigmatica* collected in 2009 in northern Germany and Russia (St Petersburg) were used for the tests.

Plate 7 Set-up for *Cassida stigmatica* single-choice adult feeding and oviposition tests (left) and larval performance tests (right) on different *Tanacetum vulgare* chemotypes

Results: The sample size for the preliminary *C. stigmatica* single-choice adult feeding and oviposition tests as well as the larval performance tests on different chemotypes was too small and results are therefore not presented. A much larger sample of selected chemotypes will be tested in spring 2010.

9 Discussion

Work in 2009 has highlighted or confirmed a range of unexpected biological features or difficulties with most of the potential candidate agents which have been prioritized. This may delay host-range studies. The leaf-feeding beetle *Cassida stigmatica* and the internally feeding moth *Isophrictis striatella* seem to have partial two-year life cycles, which makes mass rearing more difficult. Whether northern and southern German populations of *I. striatella* are one species will be determined in early 2010. Some progress has been made in the rearing of the stem-boring weevil *Microplontus millefolii.* However, further improvements are necessary to carry out reliable host-specificity tests. Additional field cage tests and an open-field test in Russia are planned in 2010.

From a host-specificity point of view, we have found indications that the physiological host ranges of *C. stigmatica* and of the root-feeding beetle *Longitarsus noricus* are not restricted to the target plant *T. vulgare*. However, preliminary molecular identification indicates that contamination in the CABI garden by a local *Longitarsus* may have contributed to the apparent survival rates of *L. noricus* recorded on several plants. To confirm this, a large subsample of beetles that emerged in 2009 has been sent for genetic identification with results expected in early winter. Opportunistic studies have also shown that the stem-boring beetle *Phytoecia nigricornis* lacks host specificity in no-choice conditions.

Major challenges in future years will include successfully rearing the stem-boring weevil *M. millefolii*. Another challenge will be to synchronize the emergence of *I. striatella* adults with the flowering stage of test plants in order to carry out oviposition tests. It will be important also to find and collect the root-boring buprestid *Meliboeus graminoides.* This species has not yet been found in Germany, Ukraine and northern Russia which indicates that it may occur mostly in central and southern Russia.

Despite the difficulties mentioned above, the complex of candidate agents on *T. vulgare* is promising, since all food niches are filled. Significant adult damage can be expected by all beetle species at high densities. The larvae of *Microplontus millefolii* live in the stems of *T. vulgare* and those of *L. noricus* and *Meliboeus graminoides* feed on the root tissue. Direct seed reduction could be achieved by *I. striatella.*

In 2010, we will focus work on host-range and biological studies with *L. noricus*, *Microplontus millefolii* and *C. stigmatica* (within the framework of the PhD by V. Wolf) and on finding *Meliboeus graminoides.* Some additional studies are also planned with *I. striatella* depending on project development.

10 Work programme 2010

Field surveys and collections

- Survey and collection trip in south-eastern Russia for *Meliboeus graminoides*;
- Collection trips (2x) in the St Petersburg area (Russia) for *Longitarsus noricus*, *Microplontus millefolii* and *Cassida stigmatica*;
- Collection trip in Ukraine (Kiev) for *Microplontus millefolii* and *Cassida stigmatica*;
- Collections of *Isophrictis striatella* in northern and south-western Germany/eastern France.

***Longitarsus noricus* (Col., Chrysomelidae)**

- Depending on results of molecular identification/analyses, continue no-choice larval development tests or conduct single- and multiple-choice oviposition and larval development tests;
- Carry out no-choice adult feeding and survival tests;
- Identify adults emerging from tests using molecular analyses.

***Cassida stigmatica* (Col., Chrysomelidae)**

- Conduct multiple-choice oviposition tests in field cages.

***Microplontus millefolii* (Col., Curculionidae)**

- Further improve rearing methods;
- Carry out host-range studies in ‘natural’ field cages;
- Set up a preliminary field test in Russia.

***Meliboeus graminoides* (Col., Buprestidae)**

- Conduct preliminary biological studies.

***Isophrictis striatella* (Lep., Gelechiidae)**

- Depending on results of molecular analyses and project development with the other biological control agents, continue host-range tests on selected native North American plant species (i.e. no-choice oviposition and larval development tests).

PhD thesis

- Complete chemical analysis of seedlings and mature plants of various populations of *T. vulgare* from Europe and North America;
- Repeat single-choice adult feeding and oviposition tests and larval performance tests for *Cassida stigmatica* with different chemotypes of *T. vulgare* and selected North American test species.

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Annex 1

Summary of field surveys and *Tanacetum vulgare* sites found in 2009

Collection site	Code	Country area	Collection date	Insect species of interest
Volgelsheim I	F1	Eastern France (Alsace)	6 April 2009	Site not mown. *Isophrictis* larvae in dry stems (field dissections); dry stems collected
Volgelsheim II	F2	Eastern France (Alsace)	6 April 2009	Site destroyed; only few plants left
Bremgarten	H25	South-western Germany	6 April 2009	Site partly mown; *Isophrictis* larvae present in dry stems (field dissections).
Grissheim I	H23	South-western Germany	6 April 2009	Site partly mown; no insects recorded
Eschbach II	T12	South-western Germany	6 April 2009	Site completely destroyed
Eschbach II	T13	South-western Germany	6 April 2009	Site partly mown; *Isophrictis* larvae present in dry stems (field dissections); one- and two-year-old dry stems collected
Müntschemier I	CH3	Western Switzerland	14 April 2009	Site completely destroyed
Müntschemier II	CH4	Western Switzerland	14 April 2009	Site completely destroyed
Müntschemier III (new)	CH5	Western Switzerland	14 April 2009	Site not mown; one adult *Cassida stigmatica* recorded
Griessheim I	H23	South-western Germany	15 April 2009	No insects recorded by sweeping; plants still in rosette stage
Eschbach II	T13	South-western Germany	15 April 2009	Two adult *Cassida stigmatica* recorded; plants still in rosette stage
Bremgarten	H25	South-western Germany	15 April 2009	Two adult *Cassida stigmatica* recorded; plants still in rosette stage
Volgelsheim I	F1	Eastern France (Alsace)	15 April 2009	No work done. Plants still in rosette stage
Griessheim I	H23	South-western Germany	23 April 2009	No insects recorded by sweeping.
Eschbach II	T13	South-western Germany	23 April 2009	No insects recorded by sweeping
Bremgarten	H25	South-western Germany	23 April 2009	One adult *Cassida stigmatica* recorded
Volgelsheim I	F1	Eastern France (Alsace)	23 April 2009	One mating pair *Cassida stigmatica*; plants started shooting; old dry and newly growing shoots collected
Griessheim I	H23	South-western Germany	7 May 2009	No insects recorded by sweeping
Bremgarten	H25	South-western Germany	7 May 2009	No insects recorded by sweeping
Eschbach II	T13	South-western Germany	7 May 2009	No insects recorded by sweeping
Volgelsheim I	F1	Eastern France, Alsace	7 May 2009	15 *Cassida stigmatica* collected; old dry and newly growing shoots collected
Rendsburg	D1	Northern Germany	16 May 2009	Old stems still present; 140 adult *Cassida stigmatica* and 6 adult *Microplontus millefolii* collected by sweeping; dry old and newly growing shoots collected
Neumuenster	D2	Northern Germany	17 May 2009	Old stems still present; 40 adult *Cassida stigmatica* and two adult *Microplontus millefolii* collected by sweeping; dry old and newly growing shoots collected
Negernbötel	D4	Northern Germany	17 May 2009	Old stems partly still present; ten adult *Cassida stigmatica* and three adult *Microplontus millefolii* collected

Wahlstedt	D5	Northern Germany	17 May 2009	One adult *Cassida stigmatica* recorded
Trappenkamp	D12	Northern Germany	17 May 2009	25 adult *Cassida stigmatica* and 16 adult *Microplontus millefolii* collected
Daldorf	D3	Northern Germany	18 May 2009	Old stems still present; 50 adult *Cassida stigmatica* and eight adult *Microplontus millefolii* collected by sweeping; dry old and newly growing shoots collected
Fehrenbötel	D13	Northern Germany	18 May 2009	25 adult *Cassida stigmatica* and seven adult *Microplontus millefolii* collected
Hydropark	U2	Ukraine (Kiev)	24 May 2009	Vegetative stage; three adult *Cassida stigmatica* collected
Trukhuniv	U3	Ukraine (Kiev)	25 May 2009	Vegetative stage; 50 adult *Cassida stigmatica*, one adult *Microplontus millefolii* and one *Longitarsus* sp. collected
Hotiv	U4	Ukraine (Kiev)	26/28 May 2009	Vegetative stage; 100 adult *Cassida stigmatica*, 100 adult *Microplontus millefolii* (mostly in shaded areas), 40 *Phytoecia nigricornis* (mostly in shady areas) and two *Longitarsus* sp. collected
Moscow		Russia	1-2 June 2009	40 adult *Cassida stigmatica* and 57 adult *Microplontus millefolii*
Smolensk		Russia (near Moscow)	4-5 June 2009	30 adult *Cassida stigmatica* and eight adult *Microplontus millefolii*
Kalitino	RU-2008-19	Russia (St Petersburg)	1 June 2009	*T. vulgare* in vegetative stage; 40 adult *Cassida stigmatica* collected; a few eggs of *C. stigmatica* recorded; *Microplontus millefolii* possibly present
Dyatlitsky	RU-2008-5	Russia (St Petersburg)	2 June 2009	*T. vulgare* in vegetative stage; 40 adult *Cassida stigmatica* collected; *Microplontus millefolii* possibly present
Izvara (Volosovo)	RU-2008-20	Russia (St Petersburg)	1-2/4 June 2009	140 adult *Cassida stigmatica* and 81 adult *Microplontus millefolii* collected; two adult *Phytoecia nigricornis* recorded; empty mines recorded in dry stems; 30 newly growing shoots collected
Pizhma	RU-2009-1	Russia (St Petersburg)	3 June 2009	A few adult *Cassida stigmatica* and one adult *Microplontus millefolii* collected
Pudomyagi	RU-2009-2	Russia (St Petersburg)	3-4 June 2009	A few adult *Cassida stigmatica* recorded
Kalitino	RU-2009-3	Russia (St Petersburg)	5 June 2009	One adult *Microplontus millefolii* collected. One egg of *M. millefolii* (?) recorded in one of ten fresh stems dissected
Kokkorevo	RU-2009-4	Russia (St Petersburg)	5 June 2009	A few adult *Cassida stigmatica* recorded, two adult *Microplontus millefolii* collected
Murmansk road	RU-2009-5	Russia (St Petersburg)	5 June 2009	Adult *Cassida stigmatica* recorded; empty mines recorded in dry stems; no mines recorded in ten fresh stems dissected
Izvara (Volosovo)	RU-2008-20	Russia (St Petersburg)	25 June 2009	About 100 adult *Longitarsus noricus*; no *Microplontus millefolii* collected
Volgelsheim I	F1	Eastern France (Alsace)	3 September 2009	2009 dry shoots collected
Volgelsheim I	F1	Eastern France (Alsace)	24 September 2009	2009 dry shoots collected
Volgelsheim I	F1	Eastern France (Alsace)	17 November 2009	2009 dry shoots collected

Annex 2

Dissections of dry stems of *Tanacetum vulgare* in 2008–2009 (* see Annex 3 for dissection of flowerheads)

Collection site; date of collection	No. of adults emerged	Date of adult emergence	Dissection date	No. of shoots dissected	No. of entry/exit holes	No. of Lep larvae	Headcapsule width (mm)
Year of collection: 2007							
Daldorf (D3); 7 December 2007*	12	4 September - 13 October 2008					
			7-9 April 2008*	39	Not counted	19	0.432; 2 x 0.504; 0.528; 0.576; 4 x 0.6; 2 x 0.624; 6 x 0.648; 2 x 0.672
			12-15 September 2008	20	Not counted	8	2 x 0.576; 3 x 0.648; 1 x 0.696; 2 x 0.672
			22 April 2009	30	38	3 alive; 17 dead	0.624; 0.672
			18 August 2009	24	14	0	-
Eschbach II (T13); 6 September 2007	0	---					
			10 September 2008	50	Not counted	4	0.6; 0.624; 2 x 0.648
			21 April 2009	20	121	3 alive; 2 dead	0.624; 0.672
			17-18 August 2009	64	34	0	-
Year of collection: 2008							
Daldorf (D3); 26 April 2008*	13	6 August - 3 September 2008					
			19 May 2008*	30	Not counted	19	0.360; 0.552; 12 x 0.648; 0.672; 0.696; 2 x 0.720; 0.768
			22 April 2009	19	5	4 alive; 1 dead	2 x 0.624; 0.696; 0.72; 0.744
			25 August 2009	9	5	1	0.624
Daldorf (D3); 30 October 2008*	0	---					
			6-12 January 2009*	90*	Not counted	26 alive; 1 dead	1 x 0.504; 2 x 0.552; 20 x 0.6; 5 x 0.648
			24 April 2009	12	3	4	0.648; 0.672
			26 Aug. 2009	7	2	2	0.696; 0.648
			5 November 2009*	10	14	5 dead	0.504; 0.624; 0.696; 0.720; 0.768

Collection site; date of collection	No. of adults emerged	Date of adult emergence	Dissection date	No. of shoots dissected	No. of entry/exit holes	No. of Lep larvae	Headcapsule width (mm)
Rendsburg (D1) 26 April 2008*	15	6 August - 24 September 2008					
			30 April-15 May 2008*	30	Not counted	22 alive; 6 dead	0.384; 5 × 0.528; 2 × 0.576; 0.624; 16 × 0.648; 2 × 0.672; 0.72
			22 April 2009	20	23	5 alive; 6 dead	2 x 0.576; 0.624; 0.648; 0.744
			25 August 2009	24	10	2 dead	-
			6 November 2009	14	15	12 dead	-
Neumünster (D2) 26 April 2008	9	6 August - 17 September 2008					
			7-28 May 2008	28	Not counted	8 alive; 1 dead	0.264; 0.504; 0.552; 0.624; 3 x 0.648; 0.672; 0.72
			22 April 2009	19	13	1 alive	0.624
			27 August 2009	26	16	0	-
Negernbötel (D4) 26 April 2008*	14 ----------------- 1	6 August - 30 September 2008 ----------------------- 6 August 2009					
			28 May 2008*	13	Not counted	21 alive; 1 dead	19 x 0.648; 3 x 0.672
			21 April 2009	13	5	7 alive; 6 dead	0.432; 0.528; 0.6; 0.624; 2 x 0.648; 0.672; 2 x 0.696; 2 x 0.72
			25 August 2009	24	18	0	-
Wahlsted (D5) 26 April 2008	10	6 August - 9 September 2008					
			19 May 2008	14	Not counted	5	0.408; 4 x 0.648
			22 April 2009	28	22	5 dead	-
			26 August 2009	29	3	0	-

Collection site; date of collection	No. of adults emerged	Date of adult emergence	Dissection date	No. of shoots dissected	No. of entry/exit holes	No. of Lep larvae	Headcapsule width (mm)
Trappenkamp (D12) 27 April 2008	6	13 August - 30 September 2008					
			24 April 2009	17	1	2 alive; 5 dead	0.624; 0.768
			20-27 August 2009	27	8	1 alive	0.768
Year of collection 2009							
Eschbach II (T13) 6 April 2009	0	---					
			16 April 2009	10	3	12 alive; 2 dead; 4 empty pupae	2 x 0.48; 3 x 0.528; 2 x 0.552; 1 x 0.576; 1 x 0.6; 1 x 0.624; 1 x 0.648; 1 x 0.672; 2 x 0.696
Daldorf (D3) 9 March 2009	3	17 August - 18 September 2009					
			17 April 2009	12	6	2 alive	0.696
			28 August 2009	8	10	1 dead	-
Daldorf (D3) 18 May 2009	3	20-27 August 2009					
			26 May 2009	15	16	5 alive	0.576
			28 August 2009	21	9	2 dead	-
			6 November 2009	10	8	1 alive 6 dead	
Rendsburg (D1) 16 May 2009	34	23 July - 27 August 2009					
			25 May 2009	14	14	10 alive	0.552; 0.576; 0.624; 0.648; 0.672; 0.696; 0.744
			27 August 2009	7	7	1 alive	0.744
			6 November 2009	10	7	1 alive	0.720

Collection site; date of collection	No. of adults emerged	Date of adult emergence	Dissection date	No. of shoots dissected	No. of entry/exit holes	No. of Lep larvae	Headcapsule width (mm)
Neumünster (D2) 17 May 2009	9	23 July - 20 August 2009					
			25 May 2009	10	24	18 alive	0.552; 2 x 0.576; 3 x 0.624; 3 x 0.6; 0.72
			27 August 2009	10	12	0	-
			6 November 2009	11	14	1 alive 1 dead	-
Vogelsheim I (F1) 6 April 2009	0	---					
			16 April 2009	10	92	174 alive; 5 dead	4 x 0.528; 0.552; 9 x 0.576; 6 x 0.6; 6 x 0.624; 9 x 0.648; 11 x 0.672; 20 x 0.696; 7 x 0.72; 10 x 0.744; 16 x 0.768; 7 x 0.792; 9 x 0.816; 0.84; 2 x 0.864; 2 x 0.936
Vogelsheim I (F1) 23 April 2009	9	17 August - 18 September 2009					
			24 April 2009	4	30	57 alive	2 x 0.576; 2 x 0.60; 2 x 0.624; 2 x 0.648; 2 x 0.672; 8 x 0.696; 2 x 0.72; 4 x 0.744; 2 x 0.816
			28 August 2009	1 (partly)	13	4 alive	0.648; 0.864
			6 November 2009	1	0	7 alive 1 dead	-
Vogelsheim I (F1) 7 May 2009	13	23 July - 28 August 2009					
			8 May 2009	2	18	48 alive	0.605; 2 x 0.624; 3 x 0.648; 6 x 0.672; 5 x 0.696; 2 x 0.72; 3 x 0.744; 0.816; 0.840
			28 August 2009	1	5	5 alive	0.624; 0.768; 3 x 0.696
			6 November 2009	1	8	3 alive 2 dead	0.574; 0.6; 0.648; 0.696; 0.792; 0.864
Vogelsheim I (F1) 3 September 2009*	---	---					
			4 September 2009*	11*	9	17 alive	-

Collection site; date of collection	No. adults emerged	Date of adult emergence	Dissection date	No. shoots dissected	No. entry/exit holes	No. Lep larvae	Headcapsule width (mm)
Vogelsheim I (F1) 24 September 2009*	---	---					
			29 September 2009*	10*	11	6 alive	0.648; 0.624; 0.648
Vogelsheim I (F1) 17 November 2009*	---	---	18 November 2009*	5*		115 alive	2 x 0.624; 3 x 0.648; 5 x 0.672; 9 x 0.696; 20 x 0.720; 12 x 0.744; 18 x 0.768; 10 x 0.792; 2 x 0.816; 1 x 0.84

Annex 3

Dissections of dry flowerheads of *Tanacetum vulgare* in 2008-2009 (* see Annex 2 for dissection of dry stems)

Year of collection: 2007						
Collection site; date of collection	**No. of adults emerged**	**Date of adult emergence**	**Dissection date**	**No. of flowerheads dissected**	**No. of Lep larvae**	**Headcapsule width (mm)**
Daldorf (D3); 7 December 2007*	12	4 September - 13 October 2008				
			7-9 April 2008*	325	98	9 x 0.12; 2 x 0.144; 2 x 0.216; 2 x 0.264; 2 x 0.288; 3 x 0.312; 9 x 0.336; 4 x 0.384; 6 x 0.408; 0.432; 7 x 0.456 ; 2 x 0.48; 0.504; 0.528; 3 x 0.552; 8 x 0.576; 8 x 0.6; 2 x 0.624; 25 x 0.648; 3 x 0.672; 0.768
Year of collection: 2008						
Collection site; date of collection	**No. of adults emerged**	**Date of adult emergence**	**Dissection date**	**No. of flowerheads dissected**	**No. of Lep larvae**	**Headcapsule width (mm)**
Rendsburg (D1) 26 April 2008*	15	6 August - 24 September 2008				
			30 April – 15 May 2008*	364	3 alive; 1 dead	0.096 (dead); 0.144; 2 x 0.168
Daldorf (D3); 26 April 2008*	13	6 August - 3 September 2008				
			19 May 2008*	90	2 alive; 1 dead	0.168; 0.384 (dead); 0.648
Negernbötel (D4) 26 April 2008*	14 ----------------- 1	6 August - 30 September 2008 ----------------------- 6 August 2009				
			28 May 2008*	110	3 alive	2 x 0.168; 0.264
Daldorf (D3); 30 October 2008*	0	---				
			6-12 January 2009*	~200*	22 alive; 6 dead	15 x 0.36; 7 x 0.48; 0.528; 0.552; 0.6; 0.672; 2 x 0.720
			5 November 2009*	40	-	-

Year of collection: 2009						
Collection site; date of collection	**No. of adults emerged**	**Date of adult emergence**	**Dissection date**	**No. of flowerheads dissected**	**No. of Lep larvae**	**Headcapsule width (mm)**
Vogelsheim I (F1) 3 September 2009*	0	---				
			4 September 2009*	>700*	318 alive; 1 dead	2 x 0.336; 2 x 0.36; 2 x 0.384; 2 x 0.408; 4 x 0.432; 7 x 0.456; 3 x 0.48; 2 x 0.528; 2 x 0.552; 2 x 0.576; 7 x 0.6; 5 x 0.624; 2 x 0.648; 3 x 0.672; 8 x 0.696; 6 x 0.72; 2 x 0.744; 2 x 0.792; 3 x 0.816; 0.84; 0.864
Vogelsheim I (F1) 24 September 2009*	0	---				
			29 September 2009*	335*	143 alive	0.528; 0.552; 6 x 0.6; 1 x 0.624; 5 x 0.648; 6 x 0.672; 15 x 0.696; 11 x 0.72; 3 x 0.744; 3 x 0.768; 0.816
			17 November 2009	100	37 alive	0.504; 0.528; 0.576; 5 x 0.6; 0.624; 5 x 0.648; 8 x 0.696; 2 x 0.672; 7 x 0.72; 1 x 0.744; 4 x 0.792; 1 x 0.768
Vogelsheim I (F1) 17 November 2009*	0	---	18 November 2009*	478*	84 alive; 2 dead	0.648; 9 x 0.696; 3 x 0.672; 11 x 0.72; 15 x 0.768; 4 x 0.792; 8 x 0.744; 0.84

Annex 4

Occurrence and distribution of *Tanacetum* species in Russia and other republics of the former USSR

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Thirty-five *Tanacetum* species have been recorded in the former USSR (Union of Soviet Socialist Republics). Most of them occur in the eastern European part of Russia (11 species), the Caucasus (15 species), western Siberia (12 species) and the Central Asian part of Russia (19 species) (Figure A4.1; Table A4.1). Three species occur each in eastern Siberia and the Far East. *Tanacetum bipinnatum* (L.) Sch. Bip., which is known to be native in North America, has been recorded in four of the six phytogeographical regions of the former USSR. The species is widely distributed in the subarctic and arctic zones of the Northern Hemisphere. In Russia, it grows on sandy dunes, river banks and lowland meadows.

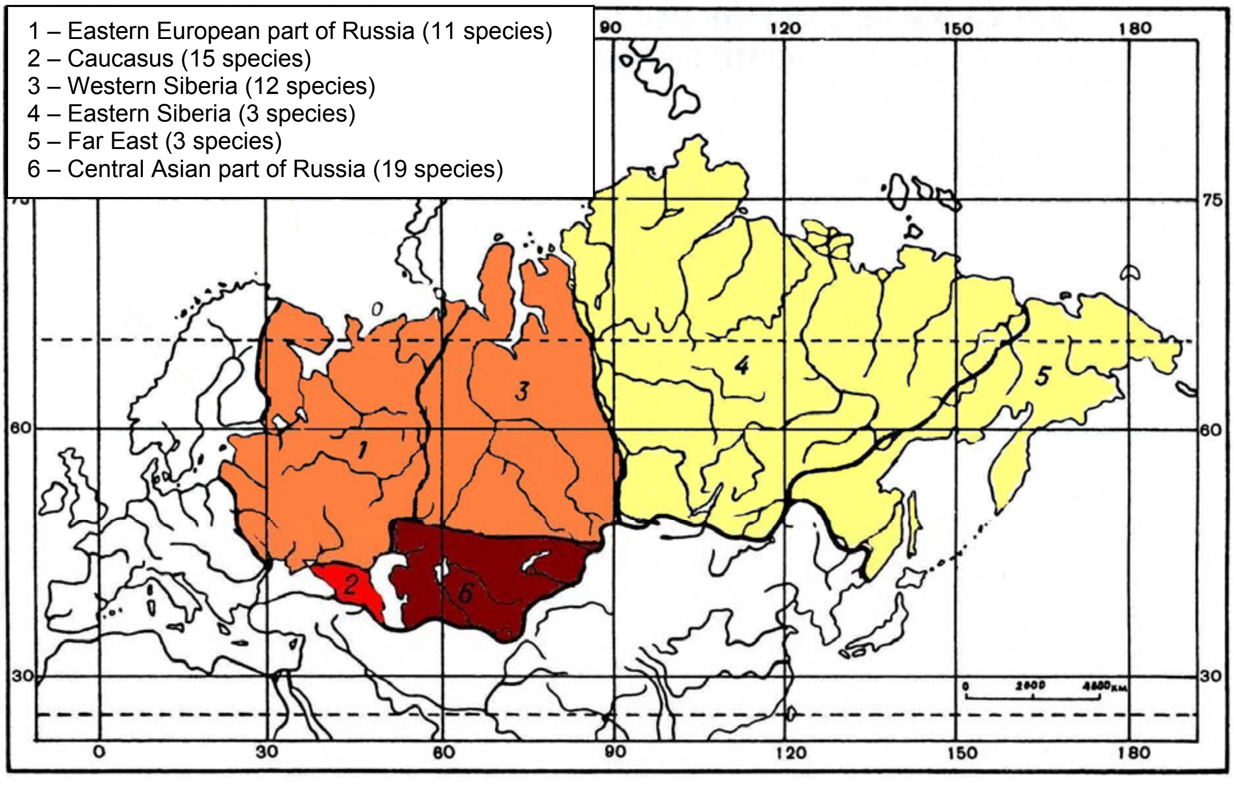

Figure A4.1 Occurrence of *Tanacetum* species in the six main phytogeographical regions of the former USSR

Given the occurrence and distribution of the *Tanacetum* species in the former USSR, it is possible that the centre of species diversity and perhaps the centre of origin of the genus are in the Caucasus region, which is the case for many Russian plant and animal genera.

Tanacetum vulgare is the only *Tanacetum* species which has been recorded in all main phytogeographical regions of the former USSR. The species is also fairly common in all districts of Belorussia, Ukraine and Moldavia and it occurs sporadically in all districts of the Baltic States. It usually grows in meadows and thickets of bushes, along reservoir shorelines, frequently along roads and in ditches, and in woodland glades and edges. *Tanacetum vulgare* prefers sandy and loamy-sandy, carbonate soils.

In the tundra zone, *T. vulgare* is recorded only as a rare alien plant along roads and in valleys of large rivers. In the forest zone (taiga), it grows in open places such as wet and dry meadows, sandy and gravelly coastal areas around rivers and lakes, woodland edges, open birch woods, clear-cut areas, thickets of bushes, deposits, field edges, wasteground, roadsides, and railway embankments. In the steppe zone, the species can be found in meadow steppes, on riverbanks and along roads.

Generally, *T. vulgare* is most abundant in the forest-steppe zone where it is now considered as a relatively important agricultural weed (Figure A4.2). It is frequently found in field margins, and on fallow and abandoned land, and in crops, especially perennial grasses.

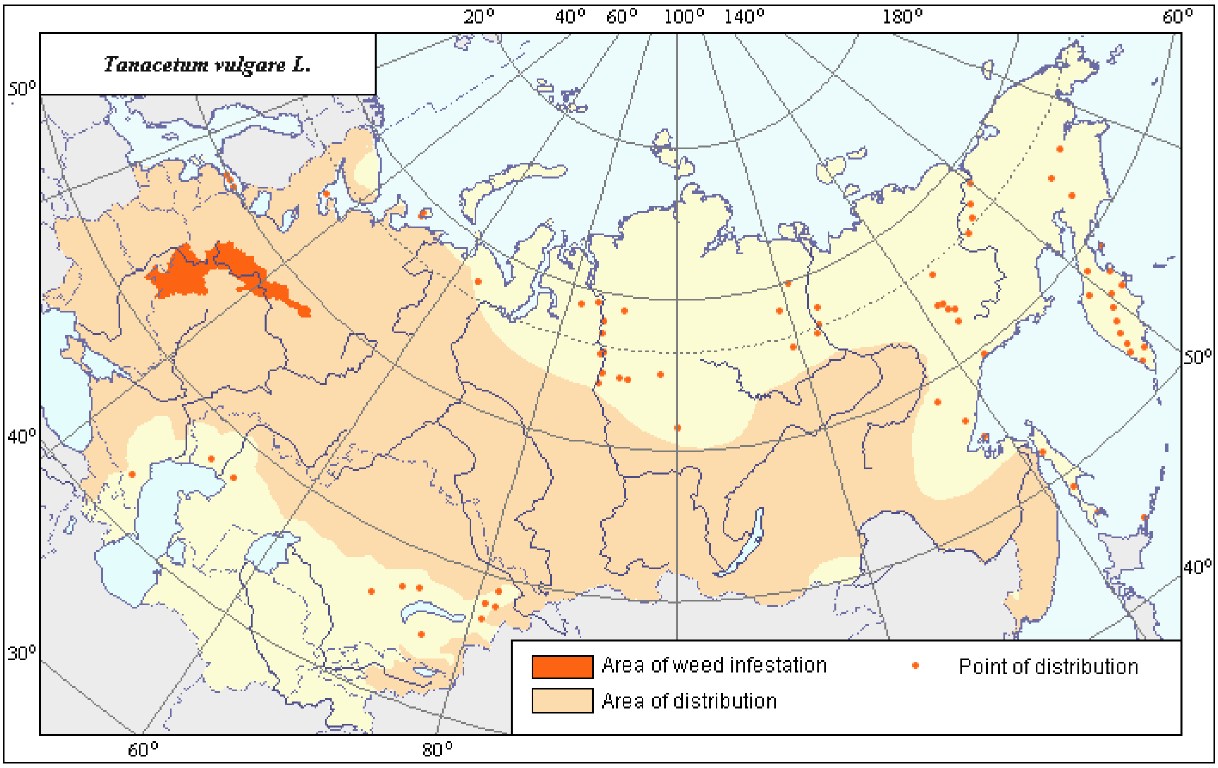

Figure A4.2 Geographical distribution and weediness of *Tanacetum vulgare* in Eurasia

Table A1 Distribution of *Tanacetum* species in the main phytogeographical regions of the former USSR

***Tanacetum* species**	**Eastern European part of Russia**	**Caucasus**	**Western Siberia**	**Eastern Siberia**	**Far East**	**Central Asian part of Russia**
T. abrotanifolium (L.) Druce	-	+	-	-	-	-
T. achilleifolium (Bieb.) Sch. Bip.	+	+	+	-	-	+
T. akinfiewii (Alexeenko) Tzvel.	-	+	-	-	-	-
T. argyrophyllum (C.Koch) Tzvel.	-	+	-	-	-	-
T. bipinnatum (L.) Sch. Bip.	+	-	+	+	+	-
T. boreale Fisch.ex DC.	-	-	+	+	+	+
T. canescens DC.	-	+	-	-	-	-
T. chiliophyllum (Fisch. et C.A.Mey.) Sch.Bip.	-	+	-	-	-	-
T. crassipes (Stschegl.) Tzvel.	-	-	+	-	-	+
T. duderanum (Boiss.) Tzvel.	-	+	-	-	-	-
T. heterophyllum Boiss.	-	-	-	-	-	+
T. karelinii Tzvel.	-	-	+	-	-	+
T. kittaryanum (C.A.Mey.) Tzvel.	+	-	+	-	-	+
T. kokanicum Krasch.	-	-	-	-	-	+
T. longipedunculatum (Sosn.) Tzvel.	-	+	-	-	-	-
T. millefolium (L.) Tzvel.	+	+	+	-	-	+
T. mindshelkense Kovalevsk.	-	-	-	-	-	+
T. odessanum (Klok.) Tzvel.	+	-	-	-	-	-
T. oligocephalum (DC.) Sch.Bip.	-	+	-	-	-	-
T. paczoskii (Zefir.) Tzvel.	+	-	-	-	-	-
T. pseudoachillea C.Winkl.	-	-	-	-	-	+
T. santolina C.Winkl.	+	-	+	-	-	+
T. saxicola (Krasch.) Tzvel.	-	-	-	-	-	+
T. sclerophyllum (Krasch.) Tzvel.	+	-	-	-	-	-
T. tabrisianum (Boiss.) Sosn. et Takht.	-	+	-	-	-	-
T. tamrutense (Sosn.) Sosn.	-	+	-	-	-	-
T. tancetoides (DC.) Tzvel.	-	-	+	-	-	+

Tanacetum species	Eastern European part of Russia	Caucasus	Western Siberia	Eastern Siberia	Far East	Central Asian part of Russia
T. tenuissimum (Trautv.) Grossh.	-	+	-	-	-	-
T. turcomanucum (Krasch.) Tzvel.	-	-	-	-	-	+
T. turlanicum (Pavl.) Tzvel.	-	-	+	-	-	+
T. ulutavicum Tzvel.	-	-	-	-	-	+
T. uniflorum (Fisch. et C.A.Mey.) Sch.Bip.	-	+	-	-	-	-
T. uralense (Krasch.) Tzvel.	+	-	+	-	-	+
T. vulgare L.	+	+	+	+	+	+
T. walteri (C.Winkl.) Tzvel.	-	-	-	-	-	+
Total number of species	**10**	**15**	**12**	**3**	**3**	**19**

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