Biological Control of BucktHorns (*Rhamnus cathartica* and *Frangula alnus*)

Report 2004-05

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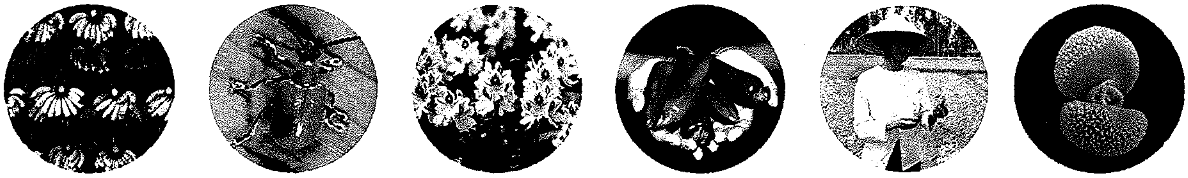

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Summary

The objectives of this study were 1) to carry out targeted surveys for *Philereme vetulata*, *P. transversata*, *Triphosa dubitata*, *Sorhagenia janiszewskae*, *Synanthedon stomoxiformis*, *Oberea pedemontana*, *Trichochermes walkeri* and seed feeding insects, 2) to study some aspects of the biology of these species and 3) to carry out preliminary host specificity studies for biological control of *Rhamnus cathartica and Frangula alnus*. We focused our efforts on *R. cathartica* because of the large number of arthropods associated with common buckthorn in Europe and the lack of genus specific biological control candidates on *F. alnus* in the areas surveyed.

Work carried out in 2004-05 has highlighted some unexpected biological traits for *Synanthedon stomoxiformis* and *Sorhagenia janiszewskae*: the former species does not have a strictly biennial life cycle, but has a partial annual life cycle, and the later species overwinters in the egg stage. For fruit feeding biological control agents, the highlight from 2004 was the discovery of an important population of the midge *Wachtiella krumbholzi* in the fruits of *R. cathartica* in Serbia, which is also probably present in other areas such as western Switzerland and southern Germany.

Preliminary host range studies with several insect species confirm that species in the genus *Frangula* are clearly separated from species in the genus *Rhamnus*. For example, *Frangula alnus* and *F. caroliniana* were not suitable hosts for the leaf feeding geometrid *Triphosa dubitata,* which has not been found on *F. alnus* in our surveys. In contrast both *Rhamnus* and *Frangula* species are suitable hosts for the leaf feeding tortricid *Ancylis apicella,* which may have a slight preference for *F. alnus* in Europe. Larval development on *F. alnus* and *F. caroliniana* was very low for the congeneric species *A. derasana,* which has a clear preference for *R. cathartica* in Europe.

Preliminary host range studies with *Synanthedon stomoxiformis*, which is known from both target plants in Europe, indicate that most species in the genera *Rhamnus* and *Frangula* are suitable hosts for larval development. In contrast, very few eggs were laid on non-host plants by *Trichochermes walkeri*, which is considered to be monospecific on *R. cathartica* in Europe.

The North American species *R. alnifolia* is a suitable host for larval development of most insect species tested and thus it seems to be closely related to *R. cathartica.* In contrast, European *R. alpina* is probably more distant to *R. cathartica* as indicated by the results of the preliminary screening tests and a recent phylogenetic work on buckthorns the main result of which supports the generic separation of *Frangula* as a well-supported monophyletic sister clade to the rest of *Rhamnus* in its widest sense.

Results of preliminary host range studies with several species, progress in the knowledge of the biology of some species and the discovery of the seed

feeding gall midge *W. krumbholzi* suggest redefining priorities for biological control of *R. cathartica* in North America. The selection of biological control agents which attack both *R. cathartica* and *Frangula alnus* in their native range will undoubtfully increase potential non-target impacts. Therefore, it may be necessary in the future to consider primarily biological agents that are associated with only one of these species in their native range. For biological control of *R. cathartica*, it is recommended to give priority in future screening tests to the shoot-boring moth, *Sorhagenia janiszewskae*, the leaf feeding moth *Philereme vetulata*, the leaf margin curled gall psylloid *Trichochermes walkeri* and the seed feeding midge *Wachtiella krumbholzi*.

Biological control of *F. alnus* with species or genus specific agents will undoubtfully be more difficult. Additional field surveys are required before prioritising potential biological agents for *F. alnus*.

1 Introduction

Rhamnus cathartica L. (common buckthorn) and *Frangula alnus* Miller (glossy buckthorn) (Rhamnaceae) are shrubs and small trees of Eurasian origin which have become invasive in North America. *Rhamnus cathartica* was introduced to North America as an ornamental shrub in 19th century and was originally used for hedges, farm shelter belts, and widlife habitats (Gourley 1985; Randall and Marnelli 1996; Gale 2001). It has spread extensively and is currently found in most Canadian provinces (Nova Scotia to Saskatchewan) and 27 states predominantly in the north central and northeastern portion of the United States (Gourley 1985; Gale 2001; USDA/NRCS 2001).

In Europe, *R. cathartica* prefers mesic to mesic-dry warm open or half-shaded habitats. It grows best in calcareous alkaline or neutral soils but it can also be found occasionally in humid or swampy areas (Rameau et al. 1989).

Frangula alnus was imported to North America prior to the 1900s as horticultural stock for landscape plantings and has become naturalized in the northeastern USA and southeastern Canada (Catling and Porebski 1994; Randall and Marnelli 1996; Haber 1997). Currently, *F. alnus* occurs from Nova Scotia to Manitoba, south to Minnesota, Illinois, New Jersey and Tennessee incorporating 23 states in the USA (Converse 2001; USDA/NRCS 2001).

In Europe, *F. alnus* has a slightly wider distribution than *R. cathartica* extending from northern Scandinavia in the boreal zone to the Mediterranean mountain range (Medan 1994; Hampe et al. 2003). Hampe et al. (2003) distinguished 21 different *F. alnus* haplotypes and three lineages that occupied different regions after the Holocene expansion of *F. alnus* across Europe. High genetic differentiation was observed within Iberia (i.e. the Iberian Peninsula south of the Pyrenees and northernmost Morocco) and Anatolia (i.e. Turkey and the western Caucasus) but not in temperate Europe (i.e. from the Pyrenees to West Siberia). Several haplotypes were restricted to Iberia and to the endemic subspecies *F.a. baetica*. A few others were restricted to Anatolia and to the endemic subspecies *F.a. pontica*. The nominate subspecies in temperate Europe, which has been sampled during this study, derives from the Quaternary Balkans refugium and is largely dominated by a single haplotype. In temperate Europe, *Frangula alnus* grows in various open to half shaded habitats. It can be found occasionally in dry calcareous stands but it is usually a widespread woody pioneer species on acid, moist soils and can build up large populations on fens, clearcuttings or at forest edges, which are later overgrown and replaced by forest vegetation (Hampe et al. 2003). The haplotypes of *F. a. alnus* in North America are not yet known.

In North America, common buckthorn invades woodlands and savannas mainly, although it may also be found in prairies and open fields. *Frangula alnus* is most problematic in fens and other wetlands but also can invade uplands and sandy soil forests. Both species are very adaptable, forming dense thickets that shade the ground, inhibiting the growth of native forbs, shrubs, and tree seedlings (Heidorn 1991; Randall and Marnelli 1996). Common buckthorn has also been linked to increased predation in songbird populations (Schmidt and Whelan 1999). Common and glossy buckthorn are alternate hosts for the fungus *Puccinia coronata* that causes oat rust disease (Harder and Chong 1983; Leonard 2003). These two trees are also the overwintering host for the Asian soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), a pest of soybean, *Glycine max* (L.) Merrill which was first recorded in North America during summer 2000 (Voegtlin et al. 2005).

Linnaeus described *Rhamnus cathartica* and *R. frangula* in 1753. In 1754, Miller described the genus *Frangula*, and in 1768, the species *Frangula alnus*. The generic recognition of *Frangula* has been disputed and for many years glossy buckthorn has gone under the name *Rhamnus frangula* L. A recent molecular study by Bolmgren and Oxelman (2004) supports generic recognition of *Frangula*. The global sample of *Frangula* used in their study represents a well defined monophyletic sister clade to the rest of *Rhamnus* in its widest sense. Some easily recognizable morphological differences in the character complex separating *Frangula* from *Rhamnus* are the absence of bud scales and thorns, the presence of bisexual flowers instead of unisexual flowers, flower parts in 5s, as opposed to 4s, and the absence of grooves on the seeds. In this report, we use the nomenclature in the PLANTS Database (USDA/NRCS 2001), i.e. *Rhamnus cathartica* L. for common buckthorn and *Frangula alnus* P. Miller for glossy buckthorn.

Research to develop biological control for buckthorns was started in 1964 on behalf the Entomological Research Institute at Belleville, Ontario (former Agriculture Canada). Surveys for potential agents were carried out in 1964 and 1965 and preliminary screening tests in 1966-1967 (Malicky et al. 1970). The Minnesota Department of Natural Resources initiated a new programme in 2001 to reassess the potential of biological control of buckthorns in the light of the work carried out by Malicky et al. (1970) and the increasing importance of non-target impacts of biological control agents. A literature review was carried before field surveys were started and apparently specific arthropods identified, although the information on the host plants are often limited to the genus level and *F. alnus* often identified as *R. frangula*. The aim of the field study was to identify the specialized arthropods on *R. cathartica* and *F. alnus*, their field host preference and the way in which they use the plants.

In addition to *R. cathartica* and *F. alnus*, two other European buckthorn species have been surveyed and sampled: *Rhamnus alpina* is a rather thermophilous and heliophylous montaneous pioneer species which grows in open woods, on dry calcareous rocky soils. It is likely to be sympatric with *R. cathartica* in slightly more organic and moister soil conditions. *Rhamnus saxatilis* (subsp. *saxatilis*) is a heliophylous to half-shade south and s-central European species growing in warm dry conditions and rocky calcareous soils. It appears to be an uncommon species in the areas surveyed. *Rhamnus saxatilis* and *R. cathartica* can grow sympatrically in slightly more organic soils. A few *Rhamnus saxatilis* (subsp. *tinctorius*) have been found in Serbia in the warm sandy area of the Deliblatski Pescara region, growing within a very large stand of *R. cathartica*.

Work carried out in 2002-03 in some 75 sites in several European countries identified collection sites and the best collection time for most of the promising biological control agents of buckthorns. A database was developed with information on collection sites, the target trees and their natural enemies. Dr. Luke Skinner, Minnesota Department of Natural Resources, project manager of this project, reviewed the project during his visit at the CABI Bioscience Switzerland Centre, in June 2003. On this occasion, priorities were agreed upon for 2004-2005 and several insect species were selected for host specificity studies based on their food niche, period of attack, potential availability and likely specificity. Work carried out in 2004 has been reported in our Annual Report 2004 (Gassmann et al. 2005). In March 2005, A. Gassmann attended the Symposium on the Biology, Ecology, and Management of Garlic Mustard (*Alliaria petiolata*) and European buckthorn (*Rhamnus cathartica*) organised by L. Skinner at the University of Minnesota, St. Paul, Minnesota (Skinner 2005). Work carried out between 2002-04 was presented and discussed, and priorities for 2005 re-defined in the light of the most recent results.

The overall goal of the work carried out in 2004-05 was 1) to carry out targeted surveys for selected biological control candidate agents; 2) to continue to study the distribution of potential natural enemies of buckthorns in Europe, i.e. to locate and collect further potential natural enemies of *R. cathartica* and *F. alnus*, e.g. seed feeders; and 3) to study the biology and to carry out preliminary screening tests with selected species with focus on biological control of *R. cathartica*.

This report summarizes the results of the field surveys carried out between 2002-2004 and details work carried out in 2004-05 on several arthropod species. It shows also how some project priorities have changed following initial results and new findings.

2 The specialized entomofauna associated with *R. cathartica* and *F. alnus* in Europe

Surveys have been made in 97 buckthorn sites, including 17 sites in Serbia. *Rhamnus cathartica* and *Frangula alnus* have been found together in only 19 collection sites/areas (Table 2). At 8 sites, one species was much less common than the other, while in 5 sites both were uncommon (<10 individuals). The two species were thought to be equally abundant in two large mesic-dry riparian pine forests on the Adriatic coast in northern Italy, as well as in two sites each in North-Eastern Austria and in western Switzerland. *Rhamnus cathartica* and *F. alnus* trees were found growing contiguously only occasionally. In summary, the two target species grow in different habitats and co-occurrence of large populations in Europe is rare. In temperate Europe, the large-scale climate regions seem to play a much smaller role in defining the distribution of common and glossy buckthorns than local conditions and soil type.

Table 2. Number of sites discovered and sampled in 2002-04 (Austria, Germany, Italy, Czech Republic, Switzerland and Serbia)

Number of sites with:

R. cathartica	*F. alnus*	*R.c. + F.a.*	*R. alpina*	*R.a. + R.c.*	*R. saxatilis*	*R.s. + R.c.*
36	29	19	3	1	3	6

In total, 39 specialized arthopods are known from *R. cathartica* and *F. alnus* in Europe (Gassmann et al. 2005) (Annex 1). A few species such as *Menesia bipunctata* (Zoubk.) (Col.; Cerambycidae) the host plant range of which is doubtful, have not been included in this list. Lepidoptera (22 species) dominate, followed by Hemiptera (8 species), Diptera (4 species), and Acarina (4 species). There is only one specialised beetle known on these two buckthorn species in Europe. The commonest feeding guild on *R. cathartica* and *F. alnus* is leaf chewing phytophages (18 species), followed by sap feeding species (9 species) and flower or fruit feeders (6 species). According to the literature, four species of gall midges induce galls either in the flowers or fruits of buckthorn. There are very few internal feeders. The larvae of *Sorhagenia janiszewskae* (Lep., Cosmopterigidae) develop in the shoot–tips; those of *Oberea pedemontana* (Col., Cerambycidae) in the branches, and the larvae of *Synanthedon stomoxiformis* (Lep., Sesiidae) mine the roots.

The main characteristic of the arthropod fauna associated with *R. cathartica* and *F. alnus,* which has been confirmed in our surveys, is that the complex of specialized arthropods is much more diverse on *R. cathartica* than on *F. alnus*. Twenty-two species are known almost exclusively from *R. cathartica*, and other species of *Rhamnus*. Of these 22 species, only seven have also been associated with *F. alnus*. Most of these are literature records (e.g. *Sorhagenia rhamniella* (Lep., Cosmopterigidae), *Triphosa sabaudiata* (Lep., Geometridae), *Calybites quadrisignella* (Lep., Gracillaridae) and *Hysterosia solidiana* (Lep., Tortricidae)), which have not been confirmed during surveys for biological control (Annex 2). Therefore, the occurrence of those species on *F. alnus* still needs to be confirmed. However, geographic variation in host plant use cannot be excluded. For example, *Sorhagenia rhamniella* is known from *F. alnus* in England, an area which was not included during our surveys (Emmet 1969). Three species have been found very occasionally on *F. alnus* during past surveys for biological control of buckthorns: *Trioza rhamni*, *Cacopsylla rhamnicolla* and *Philereme transversata* have been found each only once (< ten individuals) on *F. alnus* by Malicky et al. (1970). It can be noted that Ossiannilson (1992) does not report neither *T. rhamni* nor *C. rhamnicolla* on *F. alnus* in Fennoscandinavia and Denmark. Thus, a few rare records of insect species narrowly associated with *Rhamnus* on *F. alnus* would need to be confirmed. Misidentification of e.g. immature stages or herbivory symptoms, or sampling errors cannot be excluded.

Another eleven species have been recorded on both *R. cathartica* and *F. alnus*. Three species (*Triphosa dubitata* (Lep., Geometridae), *Ancylis derasana* (Lep., Tortricidae), and *Cacopsylla rhamnicola* (Hom., Psyllidae) show a marked preference for *R. cathartica*. Eight species have been found on both target weeds with very few species (e.g. *Ancylis apicella* (Lep., Tortricidae) and *Gonepteryx rhamni* (Lep., Pieridae)) showing a clear preference for *F. alnus*. In Serbia, *Oberea pedemontana* and *Synanthedon stomoxiformis* have been recorded on *R. cathartica* and *F. alnus* but not on *R. saxatilis* and *R. alpina*.

Finally six species are known almost exclusively from *F. alnus*. The jumping plan lice *Zygina suavis* (Hom., Cicadellidae) is the only species which we have found on *F. alnus* but not on *R. cathartica*. However, according to Ossiannilson (1981) *R. cathartica* is a probable host of *Z. suavis* as well. Literature records indicate another five arthropod species specialised on *F. alnus*, i.e. *Contarinia rhamni* and *Dasyneura frangulae* (Dipt., Cecidomyiidae), *Lygocoris rhamnicolla* (Heterop, Miridae), *Aristotelia pancaliella* (Lep., Gelechiidae) and *Eriophyes rhamni* (Acari, Eriophyidae).

The host plant records for 15 insect taxa in 15 sympatric sites with both *R. cathartica* and *F. alnus* confirmed those observed in monospecific buckthorn sites (Table 2). Species known from *R. cathartica* only have not been recorded on *F. alnus*, and species such as *Philereme transversata*, *Trioza rhamni*, *Triphosa dubitata* and *Cacopsylla rhamnicola* have been recorded on *R. cathartica* exclusively. This shows field rejection of *F. alnus* by the majority of arthropods associated with *R. cathartica* and/ or other *Rhamnus* species in Europe.

Table 2. Frequency of occurrence of 15 arthropod taxa in 15 sympatric sites with *R. cathartica* and *F. alnus* (data from 2002-03)

	Arthropod occurrence at sympatric sites No. of sites (%)		
Insect species	***R. cathartica***	***F. alnus***	**Both species**
Philereme transversata	5 (33.3)	-	-
P. vetulata	7 (46.7)	-	-
Gonopteryx rhamni	2 (13.3)	5 (33.3)	3 (20.0)
Sorhagenia janisweskae	-	4 (26.7)	2 (13.3)
Trichochermes walkeri	14 (93.3)	-	-
Trioza rhamni	13 (86.7)	-	-
Triphosa dubitata	5 (33.3)	-	-
Zygina suavis	-	2 (13.3)	-
Ancylis apicella	1 (6.7)	-	2 (13.3)
A. derasana	2 (13.3)	-	1 (6.7)
Bucculatrix frangutella	1 (6.7)	-	2 (13.3)
Cacopsylla rhamnicola	2 (13.3)	-	-
Calybites quadrisignella	2 (13.3)	-	-
Oberea pedemontana	-	-	1 (6.7)
Stigmella sp.	2 (13.3)	-	-

Records of presence/absence of most arthropods associated with *R. cathartica* and *F. alnus* in the areas surveyed in Europe 2002-2004 match well with those from Malicky et al. (1970) (Annex 2). In contrast, the frequency of occurrence of several species differs considerably reflecting a non-random sampling method often foccussing on pre-selected species.

Trichochermes walkeri and *Trioza rhamni* best represent the specific arthropod community associated with *R. cathartica* in Europe. Species like *Philereme vetulata* and *Triphosa dubitata* are also good representative species of common buckthorn in Europe. *Sorhagenia janiszewskae* has been recorded on *F. alnus* and *R. cathartica* in Austria only. In Switzerland and Germany, the occurrence of this species has not been confirmed on the later species, suggesting the possible existence of host races.

Frangula alnus is best represented by *Gonepteryx rhamni* and *Zygina suavis* while *Ancylis apicella*, *Bucculatrix frangutella* and *Oberea pedemontana* are species showing no clear preference for either buckthorn species. The frequency of occurrence of most of the other species is too low to draw clear conclusions on host plant preferences but the good match between our surveys and those conducted by Malicky et al. (1970) indicate that a majority of arthropods associated with buckthorns in Europe usually reject *F. alnus* in the areas surveyed.

3 Surveys and collections

2004: Surveys and collections trips were carried out from April through October (Annex 3). The early and late season field trips were carried out to make observations on the presence and phenology of several species. Collection trips were focussed on *Philereme vetulata*, *P. transversata*, *Triphosa dubitata*, *Sorhagenia janiszewskae*, *Synanthedon stomoxiformis*, *Oberea pedemontana*, *Wachtiella krumbholzi* and *Trichochermes walkeri*. Altogether, 130 field samples were individually processed. Regular surveys were carried out in Serbia between May and July in eight different areas to locate and collect *Synanthedon stomoxiformis*, *Oberea pedemontana* and flower and fruit feeders. Eight sites with *R. cathartica*, 2 sites with *F. alnus*, 2 sites with *R. saxatilis*, 3 sites with both *R. saxatilis* and *R. cathartica* and 2 sites with *R. alpina* were sampled as a result.

2005: The number of collection sites was reduced as work was focussing on a few species. Sixty field samples were treated separately. Work in Serbia was focussed in late May-early June (collection of *Synanthedon stomoxiformis* and *Oberea pedemontana*) and in early July (collection of gall midges on *R. cathartica*).

4 Observations on individual potential arthropod biological control agents

4.1 Lepidoptera

4.1.1 *Sorhagenia janiszewskae* (Lep., Cosmopterigidae)

Collection of mature larvae and adult emergence
The larvae of *S. janiszewskae* mine in the current year's shoots of buckthorns. The frequency of occurrence (i.e. the percentage of positive field sites) was close to 30% on both *R. cathartica* and *F. alnus* during surveys carried out in 2002-03 (Annex 2). Approximately 200 shoots of *F. alnus* apparently attacked by *S. janiszewskae* were collected in south-western Switzerland on 6 May 2004. Another 120 shoots of *F. alnus* and 750 shoots of *R. cathartica* attacked by *S. janiszewskae* were collected in eastern Austria on 13 May 2004. Only 4 adults were obtained from *F. alnus* collected in Switzerland, but 15 from *F. alnus* and 59 from *R. cathartica* in Austria. It appears that the collections were made too early in 2004, in particuliar in Switzerland; larval development was delayed as compared to 2003 as indicated by a much delayed adult emergence in 2004 (Figs. 1 and 2). Thus, the majority of larvae had not completed development when collected and died in the cut material.

In 2005, 70 shoots of *R. cathartica* attacked by *S. janiszewskae* were opportunistically collected in eastern Austria on 9 May. Another 150 shoots of *F. alnus* attacked by *S. janiszewskae* were collected in southwestern Switzerland on 31 May 2005 for adult rearing. No adults emerged from the Austrian material although dissection of another 50 shoots showed a rate of attack of 67%. In due course, 48 females and 31 males emerged from *F. alnus* from Switzerland (Fig. 3).

Irrespective of the number of attacked shoot buds collected (which is difficult to assess without dissecting the shoots) and the number of adults that emerged, figures 1-3 show that a major difficulty with the species is to accurately predict adult emergence and hence best collection time. The year 2003 was exceptionally warm in the whole Europe, thus it appears that during more normal years emergence of *S. janiszewskae* starts in early July and lasts for 2-3 weeks. The number of adults emerged in 2004 was low compared to the number of shoot buds collected, thus, the best collection time is normally during the last week of May.

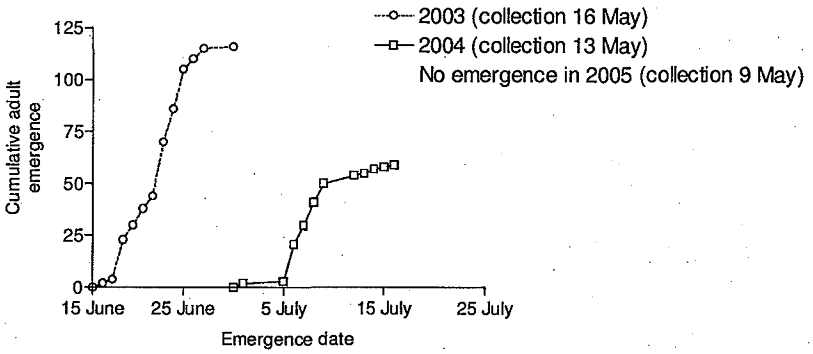

Fig 1 . Emergence of *S. janiszewskae* from *R. cathartica* in Austria (2003-05)

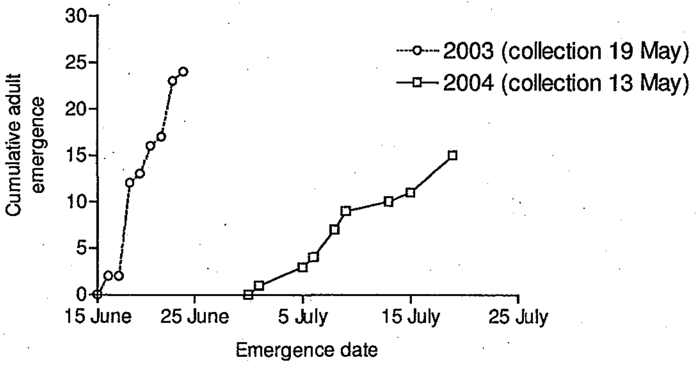

Fig 2 . Emergence of *S. janiszewskae* from *F. alnus* in Austria (2003-04)

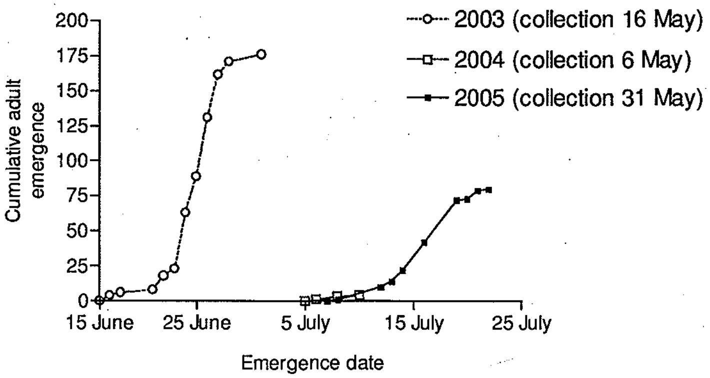

Fig 3 . Emergence of *S. janiszewskae* from *F. alnus* in Switzerland (2003-05)

No-choice oviposition tests in 2004

According to the literature, *S. janiszewskae* has one generation per year and overwinters in the adult stage (Malicky and Sobhian 1971). Because successful adult aestivation could not be obtained in previous years (see Annual Report 2002-03), oviposition tests were carried out to study the possibility that a second generation may occur on young buds or fruits in summer. Five or fewer pairs of *S. janiszewskae* (Table 3) were placed in gauze bags fixed on the top 30 cm of branches of large potted plants. All plants were kept outdoors to avoid the risk of overheating greenhouse conditions during summer time. The tests were set up July 6-9. The fruits, lateral and tip buds were examined and dissected three weeks later.

Forty-nine yellow eggs were found in one replicate but it has yet to be confirmed whether these eggs belong to *S. janiszewskae* (Table 3).

Table 3. No-choice oviposition tests with *Sorhagenia janiszewskae* in summer 2004 (test set-up 6-9 July; dissection: late July).

Cage No.	# adults/ origin	Test plant	# replicates	# fruits dissected	# tip and lateral buds dissected	Eggs / larvae
Cage 1	5 ♀; 5 ♂ from *R. cath.* (Austria)	*F. alnus*	2	61	29	-
Cage 2	5 ♀; 5 ♂ from *R.cath.* (Austria)	*R. cath.*	2	173	67	-
Cage 3	5 ♀; 2 ♂ from *F. alnus* (Austria)	*F. alnus*	1	41	11	49 yellow eggs (*Sorhagenia* ?)
Cage 4	2 ♀; 2 ♂ from *F. alnus* (Switzerland)	*R. cath.*	1	15	95	-

Field observations

Early season field trip in 2004: Having in mind that the species overwinters in the adult stage, a field trip was carried out in Switzerland on April 21-22 to observe the phenology and adult behaviour of *S. janiszewskae.* A total of 200 shoot-tips were randomly sampled at three *F. alnus* sites to try and collect eggs, which could have been used for egg or larval transfer.

Thirty-one yellow L1-L2 larvae, usually in < 0.5 cm mines, were found in the very upper part of the shoot-tips collected. The larvae were used for larval transfers (see below). No adult could be seen at any of the three sites surveyed. Thus, in 2004 the oviposition activity was completed before April 21.

Late season field trip in 2004: With the results of previous surveys and those of the oviposition test carried during summer 2004 (Table 3), we suspected that oviposition might occur in the current year and that the species is overwintering either in the egg stage or in the larval stage. Therefore, a field trip was carried out at the same three sites in Switzerland on 30 October – 1 November 2004. Six samples of 30 top branches 10 cm long were randomly collected at each site. From each sample, 50 tip and 50 lateral buds were dissected in the laboratory, and the branches visually inspected. In addition to the eggs of an unidentified aphid species, 18 yellow eggs, similar to those recorded in the oviposition test (Table 3), were found at dissection.

Early season field trip in 2005: Since no eggs could be found in spring 2004, a field trip was carried out in Switzerland earlier in spring 2005, i.e. April 10-11, to make further observations on the phenology of *S. janiszewskae.* A total of 400 shoot-tips were sampled and dissected from three *F. alnus* sites. No lepidoptera eggs could be found but 83 small yellow larvae were found as in early 2004, of which 21 were transferred onto potted *F. alnus*. This result together with that of the late season field trip in 2004 suggest that oviposition takes place during the year of adult emergence, and that egg hatch occurs in early spring the following year.

Sorhagenia janiszewskae **(?) eggs overwintering and larval transfers (2004-2005)**

A: The 18 yellow eggs collected in the shoot buds of *F. alnus* in Switzerland on 30 October – 1 November 2004 were kept in a 3°C incubator until 8 April 2005 when they were moved to a 15°C incubator. On April 13-14, 14 larvae were found crawling in the Petri dishes and were immediately transferred into individual shoot buds of potted *F. alnus*. Because the shoots were too thin to make a lateral hole near the top of the shoot, shoot tips were cut and a hole prepared at the cut end.

B: The 15 yellow larvae assumed to be L1-2, which were collected in the shoot buds of *F. alnus* in Switzerland on 21-22 April 2004, were transferred in a similar way into individual shoot buds of potted *F. alnus* on 26 April 2004.

C: Again, the 15 yellow larvae assumed to be L2-3, which were collected in the shoot buds of *F. alnus* in Switzerland on 6 May 2004 were transferred into individual shoot buds of potted *F. alnus* on 7 May 2004.

D: Twenty-four yellow L1 larvae collected in the shoot buds of *F. alnus* in Switzerland on 10-11 April 2005, were transferred in a similar way into individual shoot buds of potted *F. alnus* on 13-19 April 2005.

The transfer of newly hatched and young larvae gave poor results (Table 4). Only a few larvae established mines and only four adults emerged. More importantly, however, these larval transfers confirm that *S. janiszewskae* oviposit during the year of emergence and overwinter in the egg stage as indicated by the successful development of a few larvae in the shoot-tips of *F. alnus* from eggs collected during the previous year and the emergence of one adult which has been provisionally identified as *S. janiszewskae*. The typical damage by *S. janiszewskae* is shown in Annex 4, Fig 1.

Table 4. Results of larval transfer with *Sorhagenia janiszewskae*

No. of larvae transferred (date)	Origin	No. of mines	No. of mines with larvae	No. adults emerged (date of emergence)[1]
A: 14 newly hatched L1 (13-14 April 2005)	Eggs collected 30 Oct. – 1 Nov. 2004	8	3	1 (11 July 2005)
B: 15 L1-L2 (26 April 2004)	Larvae collected 21-22 April 2004	8	1	-
C: 15 L2-L3 (7 May 2004)	Larvae collected 6 May 2004	9	3	-
D: 21 L1 (13-19 April 2005)	Larvae collected 10-11 April 2005	8	5	3 (6-10 July 2005)

1) Adults have been provisionnaly identified as *S. janiszewskae*

Single choice field cage oviposition tests in 2005

Considering the results of the larval transfer tests and the likelihood that *S. janiszewskae* overwinters in the egg stage, another mass collection of mature larvae of *S. janiszewskae* was carried out in 31 May 2005 in the nearest site of the laboratory (i.e. on *F. alnus* in Switzerland).

Two 2m x 2m x 2m field cages were set up each with two large potted *F. alnus* and three medium sized potted *R. cathartica* (Annex 4; Fig 2). 12 males and 20 females were released in cage 1 on 11 July, and in cage 2 on 20 July 2005. A total of 639 shoot buds of *F. alnus* and *R. cathartica* from both cages were randomly dissected in 18-20 October 2005 to check for eggs or larvae of *S. janiszewskae*. About half of the shoot buds were left on plants to follow larval development and adult emergence in 2006. In addition, a total of 312 shoot buds of both buckthorn species have been sampled from the plant nursery to check for the natural occurrence of *S. janiszewskae* in CABI's grounds ("control plants").

Results are shown in Table 5. No eggs or larvae of any moth species were found in the shoot buds of "control plants" indicating that no oviposition occurred on test plants in the Center's garden before cages were set-up. A total of 95 eggs of *S. janiszewskae* (?) were found within the shoot buds of *F. alnus* or stuck on the outside. In contrast, only one egg was found on *R. cathartica*. The presence of several ant nests in Cage 2 and damage to the cage could explain the much lower oviposition compared to Cage 1.

No mines or larvae were found within the shoot buds or upper shoots of both plant species. Half of the eggs are being kept in a 3°C incubator and half in an outdoor shelter, for further experiments in 2006.

Table 5. Single choice oviposition tests with *Sorhagenia janiszewskae* from *F. alnus* in 2005 (test set up: Cage 1: 11 July / Cage 2: 20 July; dissection: 18-20 October 2005)

	No. buds dissected	No. yellow eggs
CAGE 1		
F. alnus	140	87
R. cathartica	207	1
CAGE 2		
F. alnus	99	8
R. cathartica	193	0
Plant nursery ("control plants")		
F. alnus	148	0
R. cathartica	164	0

Larval head capsule

Headcapsules of last instar larvae (?) of *S. janiszewskae* from *R. cathartica* collected in Austria on 9 May 2005 and from *F. alnus* collected in Switzerland on 31 May are shown in Fig. 4. Provided all larvae measured are last instar larvae, it would seem that larvae of *S. janiszewskae* from *R. cathartica* are smaller than those of *S. janiszewskae* from *F. alnus*, perhaps another indication of the occurrence of different host races.

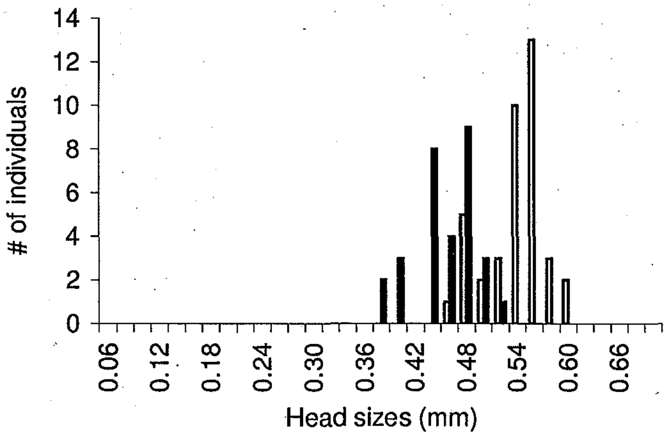

Fig 4. Headcapsule size of mature larvae of *Sorghagenia janiszewskae* from *R. catharica* (Austria) (fill bars) and *F. alnus* (Switzerland) (blank bars) in 2005.

Discussion

Sorhagenia janiszewskae is a rather difficult species to work with. The species is small and fragile. As with many internal feeders or gall makers, larvae must be collected just before pupation, in this case just before they leave the shoots to pupate in the soil; cut shoot-tips decay or dry quickly and this may prevent completion of larval development. Adult aestivation and hibernation was thought to be an insurmountable problem, but we now know that this species most probably does not overwinter in the adult stage but in the egg stage. As indicated by the oviposition experiment carried out in 2004, oviposition most probably occurs within three weeks of adult emergence. The absence of mines and larvae in plants exposed in the oviposition tests in 2005 suggests that there is no second generation and that *S. janiszewskae* overwinters in the egg stage. *Sorhagenia janiszewskae* (?) eggs kept at 3°C hatched within one week when transferred to 15°C. This suggests that post diapause development requires a low number of degree-days. Egg hatch in the field probably occurs in late winter or very early spring. Expected results in spring 2006 should confirm this hypothesis.

The near total absence of eggs of *S. janiszewskae* laid on *R. cathartica* by adults reared from *F. alnus* (Tables 3; 5) supports the hypotheses that *S. janiszewskae* from *F. alnus* and *R. cathartica* are either two different species or two different host races. An alternative hypothesis is that the phenology of *R. cathartica* (e.g. bud size and development) in the test cages was not quite what is needed for oviposition by *S. janiszewskae*. The occurrence of host races or problems in plant phenology of *R. cathartica* might also explain the absence of eggs in three out four no-choice oviposition tests in 2004 (Table 3; cages 1, 2 and 4).

In previous reports and discussions we gave a low priority to *S. janiszewskae* for biological control because of unresolved problems in adult rearing. However, these findings on the biology and host preference of *S. janiszewskae*, which still need to be confirmed in spring 2006, strongly support renewed interest in this species for biological control of *R. cathartica* and *F. alnus* in North America.

The transfer of newly hatched larvae or larvae already established in the shoots gave very poor results, and this cannot be a reliable method for preliminary no-choice larval feeding and survival tests. Therefore, host specificity tests should rely entirely on oviposition tests in choice and no-choice conditions.

Work in 2006 will focus primarily on the populations known from *R. cathartica* in Austria since the occurrence of the species on *R. cathartica* in Switzerland or Germany has not been confirmed so far. This will require mass collections of mature larvae in late May and a field survey later in the season to make observations on the biology of the species and the phenology of its host plant. Work on *S. janiszewskae* from *F.* alnus in Switzerland will continue for comparative host range studies.

4.1.2 *Philereme vetulata* (Lep., Geometridae)

Philereme vetulata is exclusively associated with *R. cathartica* in Europe with the exception of one record on *R. alpina* (Malicky et al. 1965). Although the frequency of occurrence of this species on *R. cathartica* is only about 20%, populations are usually relatively common or abundant where they occur (Annex 2). Larvae of *P. vetulata* feed within folded leaves (Annex 4; Fig 4.).

Collection and adult emergence

A total of 92 males and 85 females were reared from the collection of 336 larvae made in early spring 2004 in Austria, Germany and Switzerland. In 2005, 236 males and 214 females emerged from 620 larvae collected at the same sites in Austria, Germany, Switzerland and one additional new site in western Switzerland. Males emerged on average 4-5 days earlier than females. Difference in emergence patterns between years (Fig. 5) is probably less significant with *P. vetulata* than for *S. janiszewskae* because larval development was completed in ventilated plastic boxes with cut renewed fresh leaf material in an outdoor shelter. However, casual observations showed differences in larval development between sites and these are probably related to plant phenology and local climatic conditions. It is suspected that newly hatched larvae need young small opening leaves to settle and start feeding (Annex 4; Fig 3.). Pupae were kept in ventilated plastic cups half filled with vermiculite and stored in an outdoor shelter for adult emergence. The rate of larval parasitism was very variable, ranging from 1.4% in southwestern Switzerland to 71% in southern Germany in 2004.

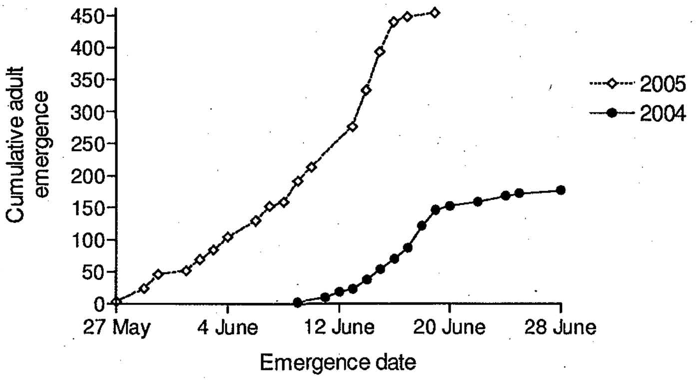

Fig 5. Emergence of *Philereme vetulata* in 2004-05

Adult rearing and oviposition

Cardboard and plastic cylinders with a gauze lid (height 27 cm, diameter 10 cm) as well as cages (about 50x50x60 cm) were used for mass rearing of *P. vetulata* in 2004. The cages were kept outdoors beneath a tarpaulin and the cylinders in an outdoor shelter. The number of adults varied from 2 to 3 pairs in the cylinder and from 10 to 15 pairs in the cages. The moths were fed with honey water, which was renewed twice a week. All rearing containers were sprayed once a day. No plant material is necessary for oviposition since all eggs are laid singly on the bottom of the rearing cages. All rearing containers were checked once a week, the eggs collected and counted, and adult mortality recorded.

On average, the first eggs were laid 10 days after set-up, and a total of 1250 fertile eggs were obtained. The smaller cardboard containers seem to provide more suitable oviposition conditions for *P. vetulata* (67 eggs / female) compared to the rearing cages (28 egg / female). Although female fecundity was higher in the small cardboard boxes in 2004, larger cages were used as well in 2005 because of the unexpected successful larval collection and large number of adults obtained. In 2005, 10,100 eggs were obtained from 239 females (= 42 eggs / female) reared from field collected larval material and from eggs obtained in the 2004 rearing. 52% of those eggs were fertile (22 eggs / female).

Fecundity of individual females (2005): One female and two males were kept each in four cardboard and four plastic cylinders in an outdoor shelter. Mean number of eggs per female was 88 ± 62 (N=8; range 15-177). Mean number of fertile eggs per female was 82 ± 48 (N=7; range 12-137). Thus, 82% of the eggs laid in this test were fertile. Female fecundity and fertility are better when females are reared singly in small containers. Male and female longevity was 24 days and 18 days respectively. Maximum female longevity was 40 days.

Diapause development and post diapause development of the egg of *Philereme vetulata*

Material and methods: *Philereme vetulata* is univoltine and overwinters in the egg stage. Observations made during larval transfer tests during the past two years (see below) indicate that synchronisation between larval hatching and leaf bud development are important for the larvae to settle and start feeding. In order to best synchronize larval hatching and plant phenology for future larval feeding and development tests, diapause development and post diapause development of *P. vetulata* eggs were studied under different regimes of cold temperature and post diapause development temperatures. Eggs were divided into batches of 50 and kept relatively moist with short period of drier conditions during the whole experiment.

Results: Factors inducing egg diapause are not known but photoperiod is likely to be the major seasonal cue controlling the induction of diapause as for most insects from temperate regions that have been studied (Danks 1987).

A cold treatment is necessary for diapause development. Fig. 6A shows that a cold treatment of one month at 3°C followed by a temperature of 20°C allows some egg hatch, i.e the diapause of a few eggs is less intense and requires a shorter exposure to cold treatment. Egg hatch is slightly improved when the cold treatment is of two months at either 3°C or 10°C. A cold treatment of 3 months at either 3°C or 10°C allows complete diapause development. Difference in time to egg hatch is slightly shortened when the cold treatment is prolongued to 4 months at 3°C.

Figure 6B shows egg hatch when the same cold treatment were applied followed by a temperature of 15°C. At 15°C, egg hatch is optimal but time to egg hatch is slightly prolonged as compared to 20°C after cold treatments of 3 and 4 months at 3°C, or 3 months at 10°C. Interestingly, egg hatch is slightly improved at the shortest cold treatment (1 and 2 months at 3°C) indicating that a 15°C temperature contributes to some extent to diapause development. Less favourable conditions for diapause development, i.e. 2 months at 10°C, do not produce the same result.

Figure 6C shows egg hatch after a cold treatment of 1, 2, 3, and 4 months at 3°C followed by a temperature of 10°C. Egg hatch is optimal at all treatment indicating that a 10°C temperature allows both diapause development and post diapause development. However, there is much difference in time to egg hatch: the longer the cold treatment at 3°C, the shorter the time for the eggs to hatch. Also, time to egg hatch after 4 months at 3°C is slightly longer at a post diapause development temperature of 10°C than at 15°C or 20°C. Results are summarized in Fig. 7.

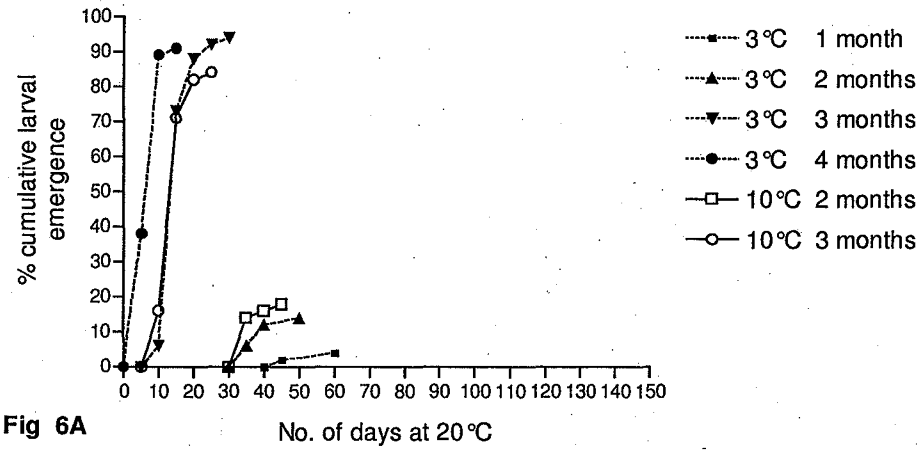

Fig 6A

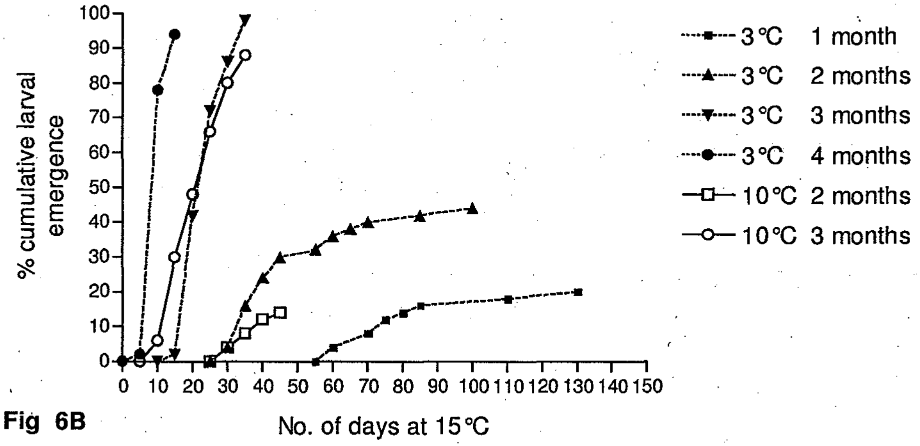

Fig 6B

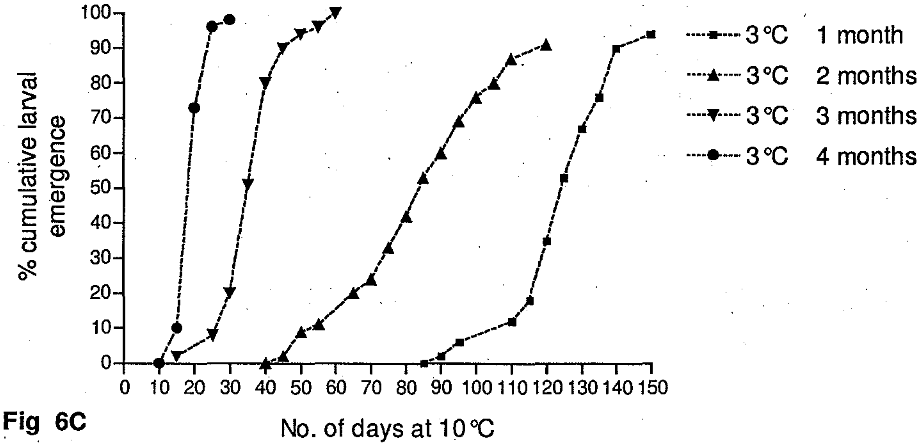

Fig 6C

Figs 6A-C. Diapause development and post diapause development of *P. vetulata* eggs under different temperature regimes

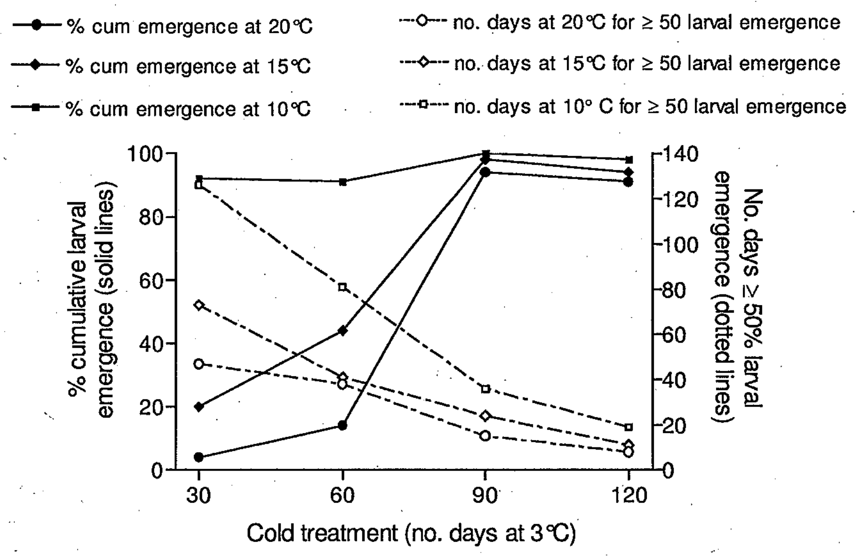

Fig 7. Percent of cumulative larval emergence and time to 50% larval emergence of *P. vetulata* under different temperature regimes

When kept continuously at 10°C, egg hatch started in early February and was completed by late March (Fig 8) demonstrating again that a 10°C temperature allows both diapause and post diapause development. Completion of egg hatch kept at a constant 10°C temperature coincides with the very quick and complete egg hatch in an outdoor shelter (Fig. 8). Our outdoor shelter does not reproduce precisely field conditions, e.g. it does not track rapid temperature increases of eggs exposed directly to sunshine. However, this experiment shows that egg hatch in the field occurs very late in winter, by late March but probably slightly earlier depending on local conditions, i.e. egg hatch coincides with an early stage of leaf bud development. In summary, diapause development requires a 3 months cold period of 10°C or less and a post diapause temperature of 15-20°C for optimal egg hatch. This study also demonstrates that post diapause development occurs at relatively low temperature, i.e. ≤ 10°C. This will help in obtaining best time for egg hatch for future larval feeding and development tests. The study of the egg diapause of *P. vetulata* will be continued in 2005-2006 to better determine temperature threshold for diapause and post diapause development.

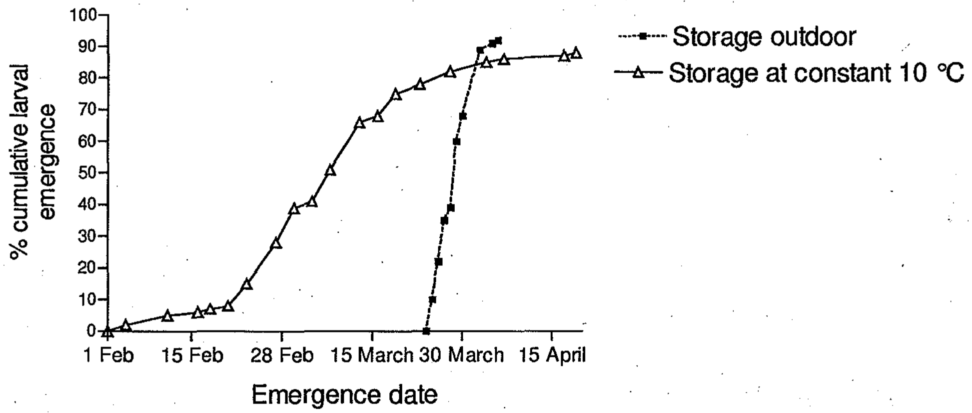

Fig. 8. Percent of cumulative larval emergence of *P. vetulata* at constant 10°C and in an outdoor shelter

Larval feeding and development tests

Potted plants: Between 10 January and 18 February 2005, 180 newly hatched larvae obtained from the egg diapause experiment were transferred onto 11 small and 3 large potted *R. cathartica* plants with newly developed leaf buds. The plants were covered with a gauze bag or kept in cages in a heated greenhouse. By the end of March, 77 mature pupae had been obtained from this material, i.e. 43% of the larvae successfully completed development on the potted plants.

Cut stems/leaves: From 21 February onwards, individual neonate larva was offerred one of four buckthorn species in 50 to 60 replicates in individual Petri dishes (10 cm in diameter) with cut stem/leaves. Test plants were exposed when leaf buds seemed to be at the right phenological stage. Petri dishes were stored in an 15°C incubator. Several variations were tried with *R. cathartica* to improve successful larval establishment and development on cut plant materiel, e.g. using young folded leaves of different sizes, cut leaves of different ages, two or more leaves stick together, etc. Hence, over 150 replicates with *R. cathartica* with very low successful larval development carried out during the second period of the test have been discarded from the results presented below (Table 6). Thus, results can be considered as biaised in favour of the control plant. It must be noted however that all tests with *R. cathartica* and *R. alnifolia* have been carried out during the same period of time. In general, larvae performed better on young folded leaves. Many larvae apparently died because of an excess of humidity in the Petri dishes. In contrast, leaf material often dried very quickly when rearing conditions were kept too dry.

Table 6. Larval survival and development of *P. vetulata* on cut plant material in no-choice conditions in 2005

Test plant	No. replicates	No. pupae (%)	Pupal weight (mg)	Time to pupal stage (days)	No. larvae with incomplete development with feeding (%) (mean no. days alive)
Rhamnus cathartica	55	13 (23.6%)	0.054 ± 0.006	35 ± 2	8 (14.5) (21 days; range 10-39)
R. alnifolia	50	3 (6.0%)	0.039 ± 0.013	41 ± 5	13 (26.0) (10 days; range 4-19)
Frangula alnus	60	0	-	-	0; some nibbling in two replicates
F. caroliniana	60	0	-	-	0; no feeding marks

Frangula alnus and *F. caroliniana* are not suitable host plants for larval development of *P. vetulata*. *Rhamnus alnifolia* seems to be a less preferred host than *R. cathartica* (no. of pupae, pupal weight and time to pupation are less on *R. alnifolia*) but this result will need to be confirmed in future tests. In partial larval feeding tests with medium-sized larvae, Malicky et al. (1970) found consistent feeding on *R. cathartica* and *R. alpina*, but inconsistent feeding on *R. saxatilis*, *R. alaternus* and *F. alnus* in a "short-term test" for the later species. No feeding was recorded on *F. purshiana*. These tests suggest that not all *Rhamnus* species are suitable hosts for *P. vetulata* and confirm that species in genus *Frangula* are not suitable for larval development of *P. vetulata*.

In the field in Europe, *P. vetulata* has been recorded almost exclusively on *R. cathartica*. This species has not been found on *F. alnus* (the single record from 2004 turned out to be a sampling mistake) and only once on *R. alpina* (Malicky et al. 1970). Synchronisation between egg hatch and plant phenology will be one of the main challenges in future tests. Also, it is difficult to provide the best environmental conditions for cut plants and 1st instar larvae in Petri dishes. Thus larval transfer and development tests with newly hatched larvae will be carried out with potted plants when possible and information from the diapause experiments used to match egg hatch and best plant phenological stage. Finally, it is planned to carry out larval feeding and development tests with 2nd instar larvae to avoid some of the constraints related to 1st instar larval establishment on cut plants.

Philereme vetulata is a promising biological control candidate for *R. cathartica* in North America. Likely specific requirements for larval establishment related to plant phenology, stage of developing leaf bud, leaf shape and toughness, etc. will restrict larval development to a few species in the genus *Rhamnus*. The realized host range will be evaluated by choice and no choice oviposition tests which are planned in large field cages in 2007. Eggs are laid on the bark of the host plant in natural conditions. The behaviour of ovipositing females in field cages has not been evaluated yet, so the reliability of such tests is uncertain for the time being.

4.1.3 *Philereme transversata* (Lep., Geometridae)

Collection and adult emergence

According to Malicky (1965), the larvae of *P. transversata* feed on the leaves of buckthorn, but unlike *P. vetulata*, do not web the leaves together. *Philereme transversata* was occasionally found on *R. saxatilis*, *R. orbiculata* and *F. alnus* (Malicky et al. 1970) (Annex 2). The species is univoltine and hibernates in the egg stage.

This species was thought to be rare and so was not included in the list of prioritized species in 2003. This is probably because larvae of *P. transversata* have been opportunistically collected at the same localities and at the same period as those of *P. vetulata*. It turns out that the species is slightly earlier than *P. vetulata* and not as scarce as previously thought. Thus, in 2004, larval collections were made earlier than those of *P. vetulata*, before the larvae leave the plants to pupate in the soil. A total of 57 males and 76 females were reared from the collection of 185 mature larvae made in April 29 and May 4 in southern Germany. In 2005, the species was given a lower priority because of a likely lower specificity than *P. vetulata*. The larvae of *P. transversata* were collected again opportunistically at the same period as those of *P. vetulata*. A total of 35 males and 56 females were reared from about 100 larvae (Fig. 9). As for *P. vetulata*, the emergence of *P. transversata* started about ten days earlier in 2005 than in 2004.

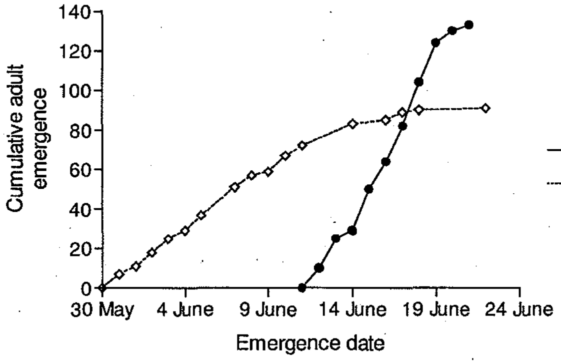

Fig 9. Emergence of *Philereme transversata* in 2004-05

Adult rearing and oviposition

In 2004, eleven cardboard cylinders (height 27 cm, diameter 10 cm) were set up with three pairs of adults each and kept in an outdoor shelter. A further three cages (about 50x50x60 cm) with five pairs each were kept outdoors beneath a suspended tarpaulin. All rearing containers were set up June 11-21. The first eggs were laid 30-40 days after set-up. The rearing of *P. transversata* is not straightforward and no one method seems to be much better than any other. A total of 400 fertile eggs was obtained from 47 females. These eggs were separated into batches of 50 and were kept at different temperature regimes to study diapause development and post diapause development. Larvae have been used for larval transfer tests in spring 2005.

In 2005, proterandry was important and female emergence much more prolonged than male emergence. Consequently, only a few unfertile eggs were obtained in 16 oviposition containers during June 3 – July 11.

Diapause development and post diapause development of the egg of *Philereme transversata*

Material and methods: Diapause development and post diapause development of *P. transversata* eggs were studied under a four months cold temperature of 3°C and post diapause development temperatures of 15°C and 20°C. Eggs were divided into batches of 50 and kept relatively moist with short period of drier conditions during the whole experiment.

Results: As for *P. vetulata*, factors inducing egg diapause are not known but photoperiod is likely to be the major seasonal cue controlling the induction of diapause. A cold treatment is necessary for diapause development. Fig 10 shows that a cold treatment of four months at 3°C followed by temperatures of 15°C and 20°C results in 70% egg hatch. Time to egg hatch was prolongued by about 10 days at 15°C as compared to 20°C. Over 80% egg hatch was obtained when eggs were stored permanently in an outdoor shelter (Fig 11). Egg hatch of *P. transversata* and *P. vetulata* started at the same time in outdoor condition but time to complete egg hatch was longer for the former species. In general, egg hatch was lower for *P. transversata* than for *P. vetulata*.

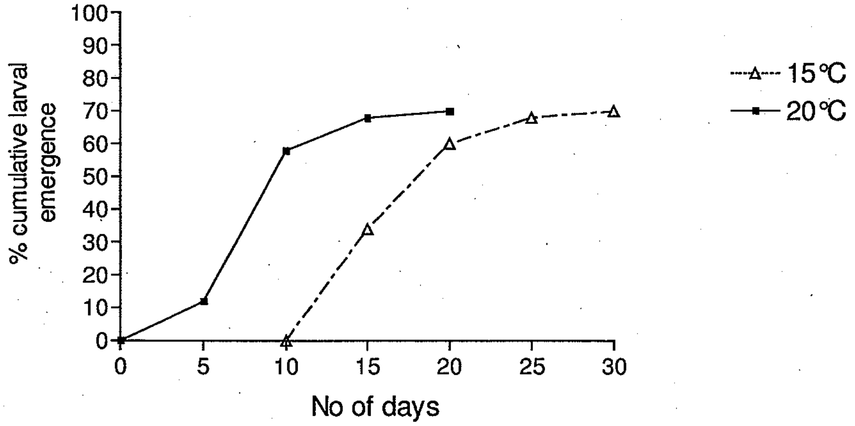

Fig 10. Percent cumulative larval emergence of *P. tranversata* after a cold treatment of four months at 3°C.

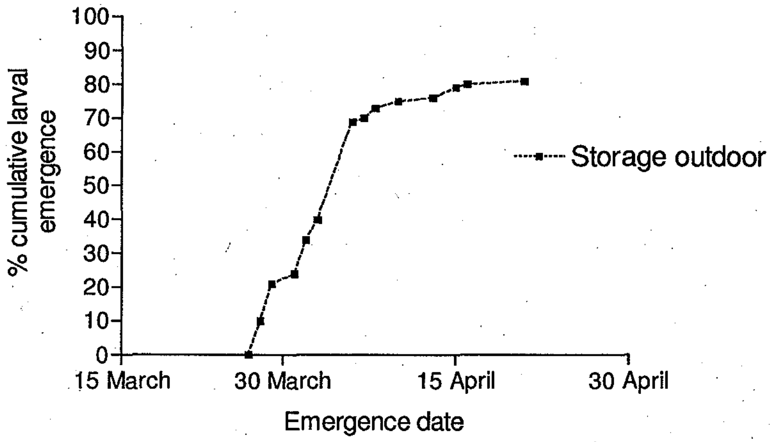

Fig 11. Percent of cumulative larval emergence of *P. transversata* in an outdoor shelter

Larval feeding and development tests in 2004

On 3 March, individual neonate larva were offerred one of four buckthorn species and cut leaves changed twice a week in 20 to 55 replicates in individual Petri dishes (10 cm in diameter). Petri dishes were stored in a 15°C incubator, and feeding and development monitored until completed or death of the individual.

Frangula alnus and *F. caroliniana* are not suitable host plants for larval development of *P. transversata* (Table 7). However, some larval development and consistent feeding were observed in about 50% of the tests with *F. caroliniana*. *Rhamnus alnifolia* seems to be a less preferred host than *R. cathartica* (no. of pupae and pupal weight are less on *R. alnifolia*), but this result will need to be confirmed in future tests. Indeed, ten out of 12 larvae died on *R. alnifolia* just before pupation.

In partial larval feeding tests with medium-sized larvae, Malicky et al. (1970) found consistent feeding on *R. cathartica*, *R. alpina*, *R. alaternus*, *F. alnus* and *F. purshiana*. In the field in Europe, with the exception of one larval record on *F. alnus* (Malicky et al. 1970), *P. transversata* has been found exclusively on *Rhamnus* spp. In the field in Europe, *Philereme transversata* appears to be slightly less specific than *P. vetulata* (Annex 2) and requirements for larval establishment less as well. No further work is planned in 2006 with this species.

Table 7. Larval survival and development of *P. transversata* on cut leaves in no-choice conditions in 2004

Test plant	No. replicates	No. pupae (%)	Pupal weight (mg)	Time to pupal stage (days)	No. larvae with incomplete development with feeding (%) (mean no. days alive)
Rhamnus cathartica	40	9 (22.5)	0.084 ± 0.024	36 ± 3	13 (32.5) (13 days; range 3-37)
R. alnifolia	20	1 (5.0)	0.047	36	12 (60.0) (29 days; range 6-39)
Frangula alnus	55	0	-	-	-
F. caroliniana	40	0	-	-	21 (52.5) (20 days; range 9-39)

4.1.4 *Triphosa dubitata* (Lep., Geometridae)

Triphosa dubitata, which overwinters as an adult in natural caves (Cherix 1976; Jacobi and Menne 1991), was recorded exclusively on *R. cathartica* and *R. alpina* (Annex 2). The species has a high frequency of occurrence on common buckthorn since it was found in 50% of all *R. cathartica* sites surveyed but it was rare or relatively uncommon in the majority of sites. These results are in agreement with those of Malicky et al. (1970) who also found *Triphosa dubitata* occasionally on *F. alnus* and *R. orbiculata*. According to Carter (1987), the host plants of *Triphosa dubitata* are *R. cathartica*, *F. alnus* but also *Prunus padus*. *Frangula alnus* and *R. alpina* were accepted in feeding tests with medium-size field collected larvae, but the native North American *F. purshiana* and two species of *Prunus* were rejected (Malicky et al. 1970). *Triphosa dubitata* is univoltine.

Limited preliminary no-choice larval feeding tests carried out in 2003 with *F. caroliniana* and *R. cathartica* showed that none of the five young field collected *T. dubitata* larvae fed and survived on the native North American *F. caroliniana*. In contrast, four adults were reared from *R. cathartica*.

Triphosa dubitata has not been prioritized for biological control because of the difficulty in aestivating and hibernating the adults until the following season, and the implications for establishing a rearing colony in the future. Because eggs were available in the vicinity of the laboratory, preliminary no-choice larval feeding tests were carried out with field-collected eggs in spring 2004. In total, 160 and 250 eggs of *T. dubitata* have been collected on *R. cathartica* and *R. alpina,* respectively, in the Swiss Jura between 21 April and 19 May. The resultant larvae from the two field hosts were tested separately.

No-choice larval survival and developmental tests in 2004

Individual neonate larvae were offered one of five buckthorn species in a variable number of replicates in Petri dishes (10 cm). The tests were set up 24 April-28 May. Survival to the pupal stage is independent of the field host plant from which the insect was sampled, but there is a significant effect of the test plant (chi-square=16.629, P<0.001 for the final two-way interaction). Larval survival to the pupal stage was higher on *R. alnifolia* than on *R. cathartica* and *R. alpina* in both populations (Table 8). No larvae developed to the pupal stage on *F. caroliniana* or *F. alnus*.

Time to pupation depends significantly on the test plant (two-way Anova, test $\text{plant}_{2,85}$ F=18.97**), but it is does not differ among field host plants (two-way Anova, field $\text{host}_{1,85}$, F=1.185 ns). When the data from the two field host-plants were pooled, time to pupation was significantly higher on *R. alpina* than on *R. cathartica* and *R. alnifolia* (one-way ANOVA first, followed by a Tukey test, $P<0.001$). In contrast, pupal weight was significantly affected by both the test plant (two-way Anova, test $\text{plant}_{2,85}$, F=9.84***) and the field host plant (two-way Anova, field host $_{1,85}$, F=8.02**). The pupal weight of *R. alpina* larvae reared on *R. alpina* was significantly smaller than that of the larvae reared on *R. cathartica* and *R. alnifolia* (Tukey test, $P\leq0.01$).

Table 8. Preliminary larval host range of *Triphosa dubitata* in no-choice conditions (2004)

Test plant	# replicate (L1)	# pupae (%)	Time to pupation (days) (mean±SD) (N)	Pupal weight (mg) (N) (mean±SD) (N)	# adults (%)
From *R. cathartica*					
R. cathartica	25	12 (48.0)	40.2 ± 2.6 (12)^{a}	140.0 ± 19.7 (12)^{a}	11 (91.7)
R. alpina	29	11 (37.9)	46.5 ± 6.7 (11)^{b}	116.5 ± 18.7 (11)^{ab}	10 (90.9)
R. alnifolia	21	17 (81.0)	40.6 ± 4.7 (17)^{a}	135.3 ± 12.5 (17)^{a}	16 (94.1)
From *R. alpina*					
R. cathartica	35	17 (48.6)	40.5 ± 4.4 (17)^{a}	127.7 ± 22.7 (17)^{a}	16 (94.1)
R. alpina	35	13 (37.1)	48.9 ± 6.4 (13)^{b}	100.0 ± 25.1 (13)^{b}	12 (92.3)
R. alnifolia	30	21 (70.0)	41.3 ± 3.8 (21)^{a}	127.4 ± 23.3 (21)^{a}	21 (100.0)
F. caroliniana	19	-	-	-	-
F. alnus	15	-	-	-	-
No food	10	-	-	-	-

Means were compared using Tukey's test (data pooled).

On the basis of these results we conclude that *Rhamnus alnifolia* is a more suitable host for *T. dubitata* from either field host in no-choice larval developmental tests. In contrast, *F. caroliniana* and *F. alnus* were not suitable hosts for this species in laboratory experiments, and *F. purschiana* was also rejected in the tests carried out by Malicky and Sobhian (1971). Species in the genus *Frangula* are therefore much less suitable hosts for *T. dubitata*. Oviposition preference tests would be needed to assess the potential host range of *T. dubitata* in North America but this is not feasible at present because of the adult biology of the species which is excluded from the prime list of potential biological control agents for exotic buckthorn species in North America.

4.1.5 *Ancylis apicella* (Lep., Tortricidae)

Ancylis apicella is a good representative species of *F. alnus* with a frequency of occurrence of 16.7% as compared to 10.9% on *R. cathartica* (Annex 2).

The species was usually rare or relatively uncommon at nearly all buckthorn sites. *Ancylis apicella* was found in most major areas surveyed except in southern Switzerland. Malicky et al. (1970) recorded *A. apicella* on *R. cathartica*, *R. saxatilis*, *R. alaternus* and *F. alnus*. *Ancylis apicella* is bivoltine. Larvae develop singly. *Ancylis apicella* overwinters as larvae in a silk web in the soil.

Preliminary host range studies were carried out in 2003 and partial results presented in the 2002-03 Report (Gassmann et al. 2004, Table 8). Individual neonate larvae were offered one of five buckthorn species in 10-25 replicates in Petri dishes (10 cm). Two series of tests were carried out. All larvae exposed to test plants from June 28, 2003 onwards pupated and the adults emerged during the same year. All larvae offered to test plants from 27 August 2003, onwards pupated in April 2004 and the adults emerged in May-June 2004.

The results in 2004 confirmed those presented in the 2002-03 Report (Gassmann et al. 2004). Larval survival appears to be slightly lower on *R. alpina*, *F. caroliniana* and *F. alnus* than on the other species tested, i.e. *R. cathartica* and *R. alnifolia* (Table 9). However, the pupae reared on *R. alnifolia* weighed significantly less than those reared on *R. cathartica* (Dunnett's test, P<0.05).

Table 9. Summary of preliminary larval host range of *Ancylis apicella* in no-choice conditions (2003-04)

Test plant	# replicate (L1)	# pupae (%)	Pupal weight (mg) (N)
Rhamnus cathartica	25	18 (72)	11.1 ± 1.7 (13)^{a}
R. alpina	10	4 (40)	9.1 ± 2.2 (3)^{ab}
Frangula alnus	15	8 (53)	9.6 ± 1.3 (8)^{ab}
R. alnifolium (NA)	25	19 (76)	8.5 ± 1.5 (15)^{b}
F. caroliniana (NA)	25	10 (40)	9.6 ± 3.3 (10)^{ab}

P < 0.05 (Dunnett' test)

Preliminary no choice tests with neonate larvae indicate that the larval host range of *A. ancylis* is restricted to species in the genera *Rhamnus* and *Frangula*. In the field, *A. apicella* has been recorded on *R. cathartica*, *R. saxatilis*, *R. alaternus* and *Frangula alnus*, but rarely on *R. alpina*. Given the results of the no-choice larval host range tests and field records, *Ancylis apicella* is now excluded from the prime list of potential biological control agents for exotic buckthorn species in North America, because it is likely to attack indigenous *Rhamnus* spp.

4.1.6 *Ancylis derasana* (Lep., Tortricidae)

Unlike *A. apicella*, *A. derasana* prefers *R. cathartica* to *F. alnus* (Annex 2). This species was usually rare or relatively uncommon in most collection sites, exept in Serbia. *Ancylis derasana* and *A. apicella* co-occur on *R. cathartica* in Austria, southern Germany and Serbia where *A. derasana* was more common than *A. apicella* in the study sites. Malicky et al. (1970) recorded *A. derasana* exclusively from *R. cathartica*. *Ancylis derasana* is bivoltine and overwinters in the larval stage. According to Sobhian et al. (1964), the biology of *A. derasana* and *A. apicella* are very similar.

Preliminary host range studies were carried out in 2003 and partial results presented in the 2002-03 Report (Gassmann et al. 2004; Table 9). Individual neonate larvae were offered one of five buckthorn species in ten replicates in Petri dishes (10 cm). Three series of tests were carried out. Any larval mortality occurred usually within two weeks after set-up. The larvae that did not pupate in 2003 stopped feeding by late September and started preparing a silk web for overwintering. Of the overwintering larvae, 38% pupated in April-July 2004 and the adults emerged in May-July 2004. The percentage of pupation was much lower on *R. alpina* (10.0%), *F. alnus* (3.3%) and *F. caroliniana* (6.7%) than on *R. cathartica* (36.7%) and *R. alnifolia* (40.0%) (Table 10). The pupal weight appears to be lower on *F. caroliniana* and *R. alpina* than on the other three buckthorn species, but the number of replicates is too low to test this.

Table 10. Summary of preliminary larval host range of *Ancylis derasana* in no-choice conditions (2003-04)

Test plant		# replicate (L1)	# pupae (%)	Pupal weight (mg)	(N)
Rhamnus cathartica		30	11 (36.7)	9.6 ± 1.6	(11)
R. alpina		20	2 (10)	8.9 ± 3.4	(2)
Frangula alnus		30	1 (3.3)	7.2	(1)
R. alnifolium	(NA)	30	12 (40)	9.3 ± 1.8	(12)
F. caroliniana	(NA)	30	2 (6.7)	4.8	(1)

Preliminary no choice tests with neonate larvae indicate that *F. alnus*, *F. caroliniana* and *R. alpina* are less suitable hosts than *R. cathartica* and *R. alnifolia*. Given the results of these preliminary no-choice larval host range tests, *Ancylis derasana* has not been selected as a candidate biological control agent for exotic buckthorn species in North America, because it is likely to develop on native buckthorn species. In the field, *A. derasana* has been recorded exclusively on *R. cathartica* and *F. alnus*. This suggests that the realized (ecological) host range of *A. derasana* is more restricted than the physiological (larval) host range. Oviposition preference tests would be needed to assess the potential host range of *A. derasana* in North America. *Ancylis derasana* is at present excluded from the prime list of potential biological control agents and no further work is planned for 2006.

4.1.7 *Synanthedon stomoxiformis* (Lep., Sesiidae)

The buckthorn clearwing moth *Synanthedon stomoxyformis* is widely distributed in the Palaearctic area (Doczkal and Rennwald 1992; Stadie 1995; Bittermann 1997). *Synanthedon stomoxyformis* ssp. *riefensthahli* is known from *R. cathartica* and *F. alnus* in southeastern Spain; *S. stomoxyformis* ssp. *amasina* has been recorded on *R. palasii* and *F. grandiflora* in eastern Turkey, Libanon, northern Iran and the Caucasus area; *S. stomoxyformis* ssp. *stomoxiformis* is known from *R. cathartica* and *F. alnus* from central-southern

Europe to Ural. Interestingly, the geographical distribution of these three subspecies matches well the geographical distribution of the three *F. alnus* subspecies. According to Pühringer et al. (1998) and Spatenka et al. (1999), *Sorbus aria* (Rosaceae) and more rarely *Corylus avelana* (Corylaceae) are alternative hosts of *S. stomoxyformis* ssp. *stomoxiformis* in upper Austria.

Synanthedon stomoxiformis is a relatively common species at several sites in the Deliblastiki Pesak and Pescara region in Serbia where its presence has been confirmed by the use of pheromone lures. According to literature, larvae have a biennial life cycle (Stadie 1995; Spatenka et al. 1999). The first year is spent in a shallow tunnel in the stem base or root, and during the second year, the larvae move down, boring into the roots. In autumn, the larva build a long and visible reddish exit tube above the ground made out of scraps, sawdust and silk. Several larvae can be found in the roots of a single *Rhamnus* tree. Pupation occurs by May the second year in the proximal end of the exit tube. The adult flies from late May to late July. The adults feed on flower nectar before mating. Eggs are laid on the trunk and branches. No parasitoids have been reared from some 40 larvae collected in the past two years but predation by small mammals could be important.

Larval feeding and development tests in 2004-05

Trees showing the reddish exit tubes of *S. stomoxyformis* were marked in autumn 2003 to facilitate the collection of mature larvae/pupae in late spring 2004. Twelve females and eight males emerged from rearing 21 mature larvae collected during the first half of June at three sites in Northern Serbia. *Synanthedon stomoxiformis* did not mate in the laboratory. Two females mated under field conditions laid 1060 eggs on 18-20 June 2004. The eggs were sent to Switzerland and the larvae used in no-choice larval feeding and development tests.

Larval feeding and development tests were carried out in replicates of 6 or 12 larvae depending on plant size and plant availability. Larval transfers were carried out 1-3 July 2004. Quite surprisingly, these tests revealed that *S. stomoxiformis* may complete development in one year. On 9-15 June 2005, empty exit tubes and pupae were found on all *Rhamnus* and *Frangula* species tested except *R. cathartica* (Table 11). The examination of *Rhamnus* and *Frangula* plants growing in our plant nursery did not reveal the presence of exit tubes or pupae so that natural infestation of test plants in the Centre's garden can be excluded. Best larval development was observed on *F. alnus* and *R. alpina* (Annex 4; Fig. 5). Larval survival was similar on *R. cathartica*, and the North American species *R. alnifolia* and *F. caroliniana*. Larval development was much faster on *R. alpina* as indicated by the relative number of pupae recorded. The absence of pupae and the size of the larval head capsules indicated that it was slowest on *R. cathartica* (Fig. 12). No larvae were found on any of the other species tested but a few empty mines on *Hippophae rhamnoides*, *Ribes rubrum* and *Sorbus aucuparia* the origin of which still need to be confirmed. It can be noted that *Sorbus aria* is recorded as a host plant of *S. stomoxiformis* in the literature. In conclusion, all *Rhamnus* and *Frangula* species tested are suitable hosts for *S. stomoxiformis* and according to these tests, the species has an entire biennial life cycle on

R. cathartica only. Holes have been drilled in the trunk base of large potted plants and the larvae transferred to follow completion of larval development in 2006 (Annex 4; Fig 6.).

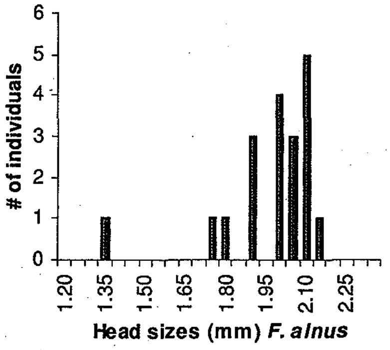

Fig 12 A

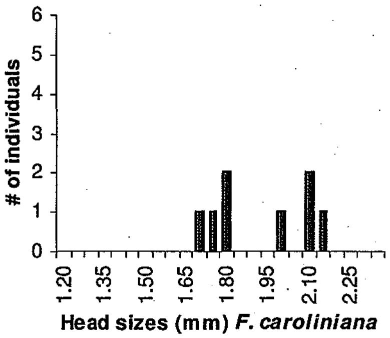

Fig 12 B

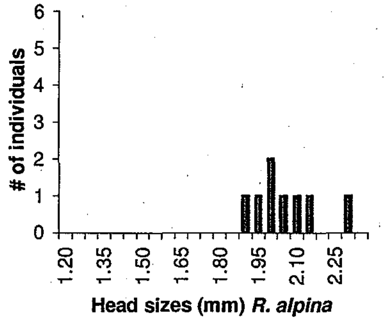

Fig 12 C

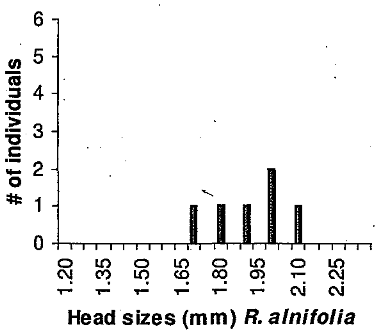

Fig 12 D

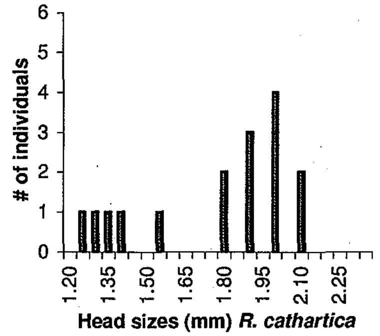

Fig 12 E

Figs 12 A-E. Headcapsule sizes of larvae of *S. stomoxiformis* reared on *F. alnus* (A), *F. caroliniana* (B), *R. alpina* (C), *R. alnifolia* (D) and *R. cathartica* (E) (July 2004 - June 2005)

Table 11. Larval survival and development of *Synanthedon stomoxiformis* in no-choice conditions (2004-05)

	2004			2005							
	No. larvae / replicate	No. replicates	Total no. of larvae	No. pupae empty at dissection (9-15 June)	No. pupae alive	Total no. pupae	No. of larvae	Total % survival	No. plants with larvae/pupae	No. of plants with empty mines**	No. of plants attacked (%)
Rhamnaceae											
Rhamnus cathartica	6	15	90	0	0	0	15	16.7	9	3	12 (80)
R. alpina	6	10	60	8	9*	17	6	38.3	9	-	9 (100)
R. alnifolia NA	6	10	60	6	0	6	6	20.0	7	3	10 (100)
Frangula alnus	6	10	60	5	3*	8	19	45.0	10	-	10 (100)
F. caroliniana NA	6	10	60	3	0	3	9	20.0	3	6	9 (90)
Hovenia dulcis	6	10	60	-	-	-	-	-	-	-	-
Ziziphus ziziphus	6	4	24	-	-	-	-	-	-	-	-
Ceanothus americanus NA	6/ 8/ 15	3	29	-	-	-	-	-	-	-	-
Elaeagnaceae											
Hyppophae rhamnoides	12	5	60	-	-	-	-	-	-	2	2 ? (20)
Elaeagnus commutata	12	5	60	-	-	-	-	-	-	-	-
E. angustifolia	6	3	18	-	-	-	-	-	-	-	-
Vitaceae											
Parthenocissus tricuspidata	12	5	60	-	-	-	-	-	-	-	-
Ampelonpsis aconitifolia	6	5	30	-	-	-	-	-	-	-	-
Grossulariaceae											
Ribes rubrum	15	4	60	-	-	-	-	-	-	1	1 ? (25)
Rosaceae											
Sorbus aucuparia	12	5	60	-	-	-	-	-	-	4	4 ? (80)
Caprifoliaceae											
Lonicera xylosteum	12	5	60	-	-	-	-	-	-	-	-

* No adults emerged from the pupae reared on *F. alnus*; 8 adults emerged from the pupae reared on *R. alpina* (13-27 June 2005)

** The origin of these empty mines needs to be confirmed

Larval feeding and development tests in 2005-06

Eighteen pupae of *Synanthedon stomoxyformis* were collected on *Rhamnus cathartica* at several sites in northern Serbia between 27 May and 15 June 2005. Only two females emerged from this material in June 2005. Both females copulated in field conditions and oviposited a total of 650 eggs. These eggs were sent to Switzerland on June 29. Because of unusal delay of shipment, the majority of the eggs hatched during the transport and the resultant larvae died. The remaining larvae have been used in no-choice larval feeding and development tests in replicates of 6 or 12 larvae (Table 12). Infested plants are being kept in outdoor conditions and will be carefully checked for pupae from April 2006 onwards.

Table 12. Larval transfer tests with *Synanthedon stomoxiformis* in 2005

Test plant	# replicate	# larvae / replicate
Rhamnaceae		
Rhamnus cathartica	2	5
R. alpina	2	5
R. alnifolia NA	2	5
Frangula alnus	2	5
F. caroliniana NA	2	5
Rosaceae		
*Sorbus aria**	2	12
Corylaceae		
*Coryllus avellana**	3	12

* Field records of *S. stomoxiformis* in literature

Discussion

Field records and preliminary no-choice larval feeding and survival tests indicate that most species in genus *Rhamnus* and *Frangula* are suitable hosts for *S. stomoxiformis*. Consequently, it is suggested to redefine the priority level of *S. stomoxiformis* in the list of potential agents for biological control of exotic buckthorns in North America.

4.2 Homoptera

4.2.1 *Trichochermes walkeri* (Hom., Triozidae)

Collections and adult emergence

In Europe, the leaf margin curl gall *Trichochermes walkeri* is known from *R. cathartica* only. *Trichochermes walkeri* is one of the most common insect species on *R. cathartica* in Europe and certainly one of the most conspicuous. It was recorded in previous surveys in nearly 90% of the *R. cathartica* sites surveyed (Annex 2).

In 2004, it was found that it is better to collect the species during the peak of adult emergence, i.e when about 50% of the galls are already empty, and the remaining larvae ready to leave the galls for adult emergence. Also, artificial enlargement of the gall opening allowed better adult emergence. In these conditions, emergence rate was 20-40%. In 2005, the total adult emergence rate was 25% for about 3500 galls collected August 4-10.

In 2004, a male biaised sex ratio of 2.1:1 was recorded for a total of 439 adults emerged. In 2005, the sex ratio was slightly more balanced, i.e. 1.5:1 in favour of the males for a total of 883 adults emerged. The adult emergence pattern was quite similar in 2004 and 2005 (Fig. 13).

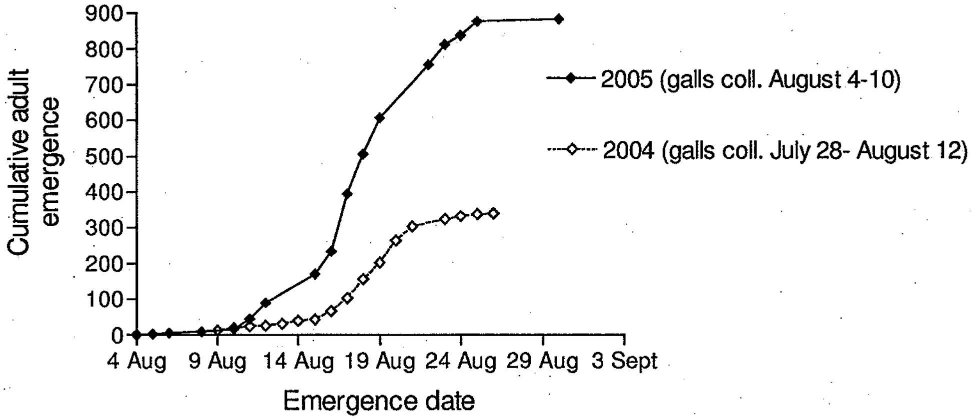

Fig. 13. Emergence of *Trichochermes walkeri* from field collected galls in 2004-05

No choice oviposition test in 2004

Material and methods: Five buckthorn species were individually tested in no-choice tests with 2-5 pairs of *T. walkeri* in small cages or on potted plants covered by a gauze bag. The tests were set up August 13-21. All cages and gauze bages were kept outside beneath a suspended tarpaulin, protected from rain and sun. Preliminary oviposition tests carried out in 2002 had shown that oviposition starts 3-4 weeks after adult emergence. First check for oviposition was therefore carried out 3 weeks after set up (Annex 4; Fig 7.). Thereafter, every cage and gauze bag was checked once a week until the third week of October when all but two females were dead.

Results and discussion: 60% and 62% of all males and females respectively were still alive in the *R. cathartica* cages at the first check, i.e. before any eggs were laid, three weeks after set up. In contrast, with the exception of one female on *R. alnifolia* and one female and one male on *R. alpina*, all adults were dead on test plants within three to four weeks after set-up. Maximum female longevity was 35 days on *R. alnifolia* and 30 days on *R. alpina*. On *R. cathartica*, maximum female and male longevity was 65 and 53 days respectively. Mean longevity of ovipositing females was 44 days (N=26; range: 24-65).

As in 2002, oviposition started 3-4 weeks after set up in all *R. cathartica* cages. Mean fecundity was similar in the two types of container used (Table 14). Mean fecundity was 36 eggs per female (all 42 females included). Mean fecundity per ovipositing female (i.e. females alive when oviposition started; N=26) was 59 eggs. Maximum fecundity per individual female (single observation) was 142 eggs.

Table 14. No-choice oviposition tests with *Trichochermes walkeri* in 2004

Test plants	No. of pairs per replicate	No. replicates	No. replicates with eggs	Total no. of eggs	Mean # eggs/ female (mean range)
Rhamnus cathartica	5	6	6	1024	34 (1-129)
R. cathartica	2	6	5	507	42 (0-127)
R. alnifolia	5	4	0	0	0
R. alnifolia	2	3	0	0	0
R. alpina	5	1	0	0	0
R. alpina	2	3	0	0	0
Frangula alnus	5	1	0	0	0
Frangula alnus	2	3	0	0	0
F. caroliniana	5	4	0	0	0
F. caroliniana	2	1	0	0	0

A total of 1531 eggs were recorded on *R. cathartica.* No eggs were laid on any of the four test plants exposed, i.e. *R. alnifolia* (NA), *R. alpina*, *F. alnus* and *F. caroliniana* (NA).

Branches with eggs were marked with colour threads, and the plants kept outdoors. No empty eggs were observed by late April the following year. By mid-May 2005, about 2/3 of the eggs had hatched and young galls were visible on plants (Annex 4; Fig 8.). In spring 2005, two thirds of the 41 infested plants had nearly the same number of eggs as in 2004, or less, but one third had more eggs indicating that natural oviposition must have occurred on plants in autumn 2004 after the plant had been removed from the test cage. In total, 292 galls have been obtained from 1600 eggs counted on the test plants on 5-7 April 2005.

Host range studies in 2005

No-choice oviposition tests

No eggs were laid in 2004 on any of the test plants exposed to the females of *T. walkeri* in no-choice tests. However, because these preliminary results also indicated that none of the test plant was suitable for adult feeding and adult survival for a period extending much after the oviposition had started, oviposition on non target plants could not be completely excluded. Therefore, in 2005, no-choice oviposition tests were carried out with females, which had been previously exposed to *R. cathartica* for three weeks. All plants used in host range studies in 2005 have been protected from natural infestation by *T. walkeri* from mid-June onwards. All plants used in the tests carrying eggs are being kept in 2m x 2m x 2m field cages until summer 2006 to protect form natural infestation by *T. walkeri* and other herbivores.

Feeding and preoviposition period: Three to six females and at least the same number of males were kept each in 36 ventilated plastic cylinders (Ø 11.0 cm, height 15.0 cm) fixed on branches of potted *R. cathartica* for a period of three weeks (Annex 4; Fig 9.). In addition, 15 females and 20 males were placed in each of two small cages with one *R. cathartica* pot. The containers were kept outside beneath a suspended tarpauline, protected from rain and sun. In total, 217 females and 336 males were exposed to *R. cathartica* during the pre-oviposition period. The pre-oviposition period started on August 5. The last cage was set up on August 25.

A total of 174 eggs were found in 10 out of 38 preoviposition containers of which 80 eggs in one cage only. This confirms that most females start to oviposit about three weeks after emergence or even later. After three weeks 103 females (48%) and 179 males (53%) were still alive and were used in no-choice oviposition tests (see below). These survival rates after three weeks are slightly lower than those observed in 2004

No-choice oviposition tests (after a three weeks feeding and preoviposition period on *R. cathartica*): Seven test plant species were individually tested with one pair of *T. walkeri* in small ventilated plastic cups (Ø 7.0 cm, height 8.5 cm) fixed on branches of potted *R. cathartica* or three pairs of *T. walkeri* in small gauze bags fixed around a branch of *R. cathartica* (Annex 4; Fig 9.). All plants were kept outside beneath a suspended tarpauline, protected from rain and sun. All cups and gauze bags were checked every 5 to 8 days on average until 14 November when all females were dead. Dead males were replaced during the tests.

Results are summarized in Table 15. A total of 1349 eggs were found on *R. cathartica*, i.e. 71 eggs on average per female (N=19). Mean number of eggs laid per ovipositing female (N=15) was 90 eggs since no eggs were found in two replicates. Oviposition rate in 2005 was thus higher than that recorded by ovipositing females in 2004 (59 eggs) and the method and material used for the test reliable. Twenty eggs were laid on *R. alnifolia* and one egg was found on *R. alpina* (Table 15).

Female longevity was 32 days (after a three weeks period on *R. cathartica*) on *R. cathartica* and thus about ten days higher than the total longevity of 44 days recorded in 2004 for ovipositing females. Female longevity was much reduced on all test plants as compared to *R. cathartica* (Table 15), although a slightly longer female longevity was observed occasionaly on *R. alnifolia*.

Table 15. No-choice oviposition tests with *Trichochermes walkeri* in 2005 (after a three weeks feeding and preoviposition period on *R. cathartica*)

Test plants	No. pairs/ replicate	No. replicates	No. replicates with eggs	Total no. of eggs	Mean # eggs / female (test) (range)	Mean female longevity (days) (range)
Rhamnus cathartica	1	10	9	1163	71 (0-229)	32 (9-60)
R. cathartica	3	3	2	186		
R. alnifolia	1	10	-	-	0.8 (0-11)	10 (5-26) *
R. alnifolia	3	5	3	20		
R. alpina	1	10	-	-	0.06	8 (4-10) *
R. alpina	3	2	1	1		
Frangula alnus	1	10	-	-	-	8 (5-10)
Frangula alnus	3	2	-	-		
F. caroliniana	1	10	-	-	-	7 (5-9)
F. caroliniana	3	2	-	-		
Sorbus aria	1	10	-	-		8 (5-9)
Corylus avellana	1	12	-	-		7 (5-10)

* Longevity of the ovipositing females on *R. alnifolia* and *R. alpina* was less than 10 days.

Adult feeding and oviposition tests: Five buckthorn species and two no-food controls were individually tested with one newly emerged pair of *T. walkeri* in small ventilated plastic cups (Ø 7.0 cm, height 8.5 cm) fixed on branches of potted *R. cathartica* (Annex 4; Fig 9.) (i.e. without a three weeks feeding and preoviposition period on *R. cathartica*). Adult mortality and oviposition was checked every 3-5 days. Dead adults were sexed only when oviposition had started and males replaced.

An average of 136 eggs per female were laid on *R. cathartica* in 11 out of 20 replicates (Table 16). Oviposition started about 30 days after female emergence. 45% of the females on *R. cathartica* died before starting to oviposit, i.e. a rate similar to those observed in the previous tests. Female longevity on *R. cathartica* was 41 days on average. Longevity of ovipositing females was 60 days (N=11; range 33-87). Male longevity was 30 days. Adult longevity was five days in the no-food control and six to eight days on *R. alnifolia*, *R. alpina*, *Frangula alnus* and *F. caroliniana*. Longevity was only slightly prolonged on *R. cathartica* shoots without leaves indicating that adults feed mainly on leaves and not on shoot cells. However, one female survived normally in one replicate on a *R. cathartica* shoot without leaves and laid 70 eggs. This was perhaps because of the presence of some inconspicuous leaf material on some of the shoots offered to the female.

Discussion: The two tests carried out in 2005 confirm that of 2004. In summary, *R. alnifolia*, *R. alpina*, *Frangula alnus* and *F. caroliniana* are not suitable plants for adult feeding and oviposition. Occasionnally, *R. alnifolia* can sustain prolonged adult feeding: at the most, females lived up to 35 days and 26 days in 2004 and 2005 respectively, but none laid any eggs. Female survival on non-host plants was not significantly higher when previously fed for three weeks with *R. cathartica*. It seems very unlikely that occasional slightly prolonged female longevity on non hosts plants will lead to significant oviposition.

Oviposition occurred almost exclusively on *R. cathartica*. Over 2900 eggs were recorded on the target plant in 2005 as compared to 20 eggs on *R. alnifolia* and one on *R. alpina*. In the field in Europe, *T. walkeri* has been recorded exclusively on *R. cathartica*. In mixed stands with *R. cathartica* in North America occasional oviposition by straying individuals on other *Rhamnus* species, such as *R. alnifolia* cannot be totally excluded. The number of eggs laid on *R. alnifolia* in the 2005 tests (20 eggs) is probably too low to get significant results as to whether any gall will develop in 2006. Because larval transfer tests do not produce significant results (Gassmann et al. 2004), host specificity of *T. walkeri* will rely entirely on oviposition tests. Sequential no-choice oviposition and single choice oviposition tests with *R. alnifolia* and *R. cathartica* will be carried out in 2006 to further evaluate the suitability of *R. alnifolia* for oviposition by *T. walkeri*. *Trichochermes walkeri* is one of the most promising biological control agents for *R. cathartica* in North America.

Table 16. No-choice adult feeding and oviposition tests with *Trichochermes walkeri* in 2005 (without a three weeks feeding and preoviposition period on *R. cathartica*)

Test plants	No. pairs/ replicate	No. replicates	No. replicates with eggs	Total no. of eggs	Mean # eggs / ovipositing female (range)	Mean no. of days before oviposition started (range)	Mean adult longevity (days) (range)
Rhamnus cathartica	1	20	11	1493	136 (35-311) N=11	30 (22-40)	♂: 30 (3-90) ♀: 41 (6-87) *
R. alnifolia NA	1	15	-	-	-		6 (4-12)
R. alpina	1	15	-	-	-		7 (4-11)
Frangula alnus	1	15	-	-	-		8 (3-15)
F. caroliniana NA	1	15	-	-	-		6 (3-11)
R. cathartica shoot without leaves	1	15	1	70	-	32	12 (3-27)**
No food	1	12	-	-	-		5 (3-8)

* Mean longevity of ovipositing females was 60 days (N=11; range 33-87)

** The female that laid 70 eggs lived 60 days

4.3 Coleoptera

4.3.1 *Oberea pedemontana* (Col., Cerambycidae)

Oberea pedemontana has a restricted geographical distribution, mainly around the Adriatic Sea. The beetle has been recorded in Northern Italy and former Yugoslavia, and less frequently in southern Austria and southern Hungary, western Bulgaria and Rumania and perhaps in Turkey (Baronio et al. 1988). In Italy, *O. pedemontana* was known from *F. alnus* only (Baronio et al. 1988). In Yugoslavia, *O. pedemontana* is reported causing extensive damage to *R. cathartica* (Lekic and Mihajlovic 1976). The beetle has also been reported from *R. alpina* (Sama 1988) and from *Lonicera* species (Horion 1974; Demelt and Franz 1990). However, according to Frisch (1992), the record of *O. pedemontana* on *Lonicera* is probably due to the fact that *Lonicera* can occur in mixed stands with *F. alnus* and therefore adults of *O. pedemontana* have been collected as strays on *Lonicera*. Contarini and Garagnani (1980) observed beetles to infest *F. alnus* and to avoid adjacent *R. cathartica* bushes. The beetle has been found during our surveys on *R. cathartica* and *F. alnus* in two neighbouring sympatric sites in northern Italy in 2002. In Serbia, it has been recorded in seven *R. cathartica* sites and one *F. alnus* site in 2003-05.

According to Baronio et al. (1988), *O. pedemontana* in Italy has a three-year life cycle on *F. alnus*. Eggs are laid in the branch cortical parenchyma. Upon hatching, the larvae make a gallery by boring along the centre axis of the branch, keeping it open laterally in the initial segment. Both the opened and closed parts of the gallery are extended thereafter. The larva removes frass and powdery borings from the lower part of the gallery by pushing them to the upper part, and into the open section. Before the onset of every winter, the larva retires to a section of the gallery that is plugged at either end by pieces of wood fibre. Here, the larva is turned upwards, i.e. upside down with respect to the feeding position. Feeding activity resumes about mid-April onwards, and the mature larvae pupate from the third week in April. The fully grown larva prepares the adult's emergence path by ascending the gallery up to the initial part, which is still blocked with frass and powdery borings and retunnelling through them as far as the base of the opening. The larva then goes down and pupates near the bottom of the gallery. Adult emergence begins by mid-May. Baronio et al. (1988) reported a larval parasitism rate of 54% by *Billaea irrorata* Meig. (Dipt., Tachinidae) and *Dolichomitus messor* (Gravenhorst) (Hym., Ichneumonidae). Our data from 2002 indicates a total larval parasitism rate of 63%.

Work in 2004 was focused in Yugoslavia because of the much higher rate of parasitism of the Italian population of *O. pedemontana* in 2002. Larvae were found in five sites on *R. cathartica* (south of Belgrade) and *F. alnus* in the mountain regions of Central Serbia. Because the sampling of *O. pedemontana* larvae is very destructive, both for the host tree and for the immature beetle larvae, it was decided not to collect larvae but adults.

Five collection trips were made between June 2 and July 11 to collect adult beetles. Beating, sweeping and visual inspection produced no adults although 40 emergence holes had been marked in the sites previously surveyed. This may be due to the cryptic behaviour of the beetle or to high dispersal rate immediately after emergence.

Between 28 May and 7 June 2005, 80 stem pieces of *R. cathartica* infested with *O. pedemontana* were collected in Serbia and shipped to Switzerland on June 13. Quite surprisingly, no adults and only one parasitoid emerged from this material. All stems have been dissected in September. Holes were drilled into large branches and sixteen living larvae were transferred onto large potted *R. cathartica* plants. Another 17 dead larvae were found in the stems.

The collection of *O. pedemontana* will be one of the main challenges in 2006 and it is likely to require much effort and time. Contacts have been made in Croatia to increase chances for better future collections and additional survey and collection trips are considered in Italy. However, given the foreseeable difficulties to collect *O. pedemontana* in workable numbers and its likely host range will include several species in genus *Rhamnus* and *Frangula*, it is suggested to redefine the priority level of *O. pedemontana* for biological control of buckthorns in North America.

4.4. Flower and fruit feeding insects

Wachtiella krumbholzi (Dipt.; Cecidomyiidae)

In 2004, preliminary collections of flowers and fruits of *R. cathartica* were carried out in Austria, Germany, Switzerland and Serbia. The highlight from 2004 was the discovery of an important midge population in the fruits of *R. cathartica* in northeastern Serbia. Ninety midge larvae were obtained from the collection of 750 *R. cathartica* fruits made on July 2. It was found that, in the laboratory, the midge larvae leave the fruits and go into the soil to prepare a larval cocoon made of silk and soil debris (Annex 4; Figs 13, 14). All midge larvae and fruits were put in ventilated boxes with sieved soil and fine vermiculite. This material has been overwintered part in an outdoor shelter and part in a 3°C incubator.

In 2004, midge larvae have also been discovered in the fruits of *R. saxatilis* spp. *tinctorius* in one site where *R. cathartica* also occurs in Serbia, as well as in low numbers in the fruits of *R. cathartica* in western Switzerland and in southern Germany.

Emergence of gall midge adults from *R. cathartica* in Serbia started on 20 May 2005 in the outdoor storage and was completed on June 14 (Fig. 15). On 23 May 2005, gall midge cocoons kept in the 3°C incubator were moved into the outdoor shelter. Adult emergence started two weeks later on 8 June and was completed on June 17 (Fig. 15). In total, 20 males and 19 females were obtained from the larval material collected in 2004 and identified as *Wachtiella krumbholzi* Stelter by Dr. M. Skuhrava. Interestingly, with the exception a few specimens reared from *R. cathartica* in the Czech Republic, M. Skuhrava has not found this species during 50 years of investigations on Cecidomyiidae in 1800 European localities (Simova-Tosic et al. 2000, 2004; Skuhrava et al. 2005). Unfortunately, no adults emerged from *R. saxatilis* spp. *tinctorius* in Serbia and from *R. cathartica* in Switzerland and Germany and this material could not be identified.

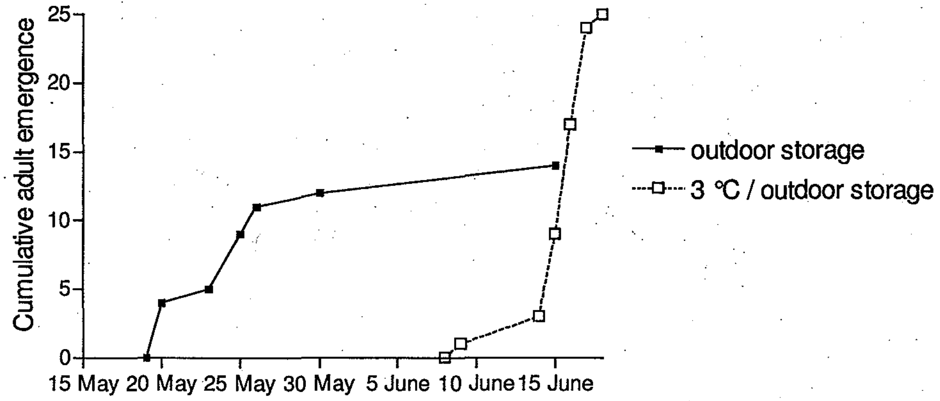

Fig. 15. Emergence of *Wachtiella krumbholzi* in 2005 (larval collection made 2 July 2004)

On 11 July 2005, over 3000 fruits of *R. catharica* apparently attacked by *W. krumbholzi* were collected in Serbia and sent to Switzerland. Fruits were not systematically dissected but casual observations revealed up to 9 midge larvae per fruit and three larvae in one seed. According to Skuhrava (pers. com. 2005), *W. krumbholzi* cannot be considered to be cecidogenous. Field observations in Serbia also suggest that *W. krumbholzi* is a seed feeder rather than a gall maker. The main characteristic of attacked fruits is similar to early fruit maturation with changes in colour. Attacked fruits become dark-red black while healthy fruits are still green (Annex 4; Fig 12). Gall swelling is not visible on damaged fruits. A rough estimation of attack rate per tree varied from 10% to 50% of fruits infested. The southwest parts of trees seem to carry more attacked fruits than less exposed branches.

In contrast, the midge species attacking the fruits of *R. saxatilis* spp. *tinctorius* appears to be a true gall maker. Infested fruits clearly show gall swelling and are bigger than healthy ones. It seems that attacked fruits drop from the trees before larvae leave the galls. The midge species attacking the fruits of *R. saxatilis* spp. *tinctorius* appears about 20 days earlier than *W. krumbholzi* on *R. cathartica*.

280 larvae of presumably *W. krumbholzi* from *R. cathartica* were transferred into Petri dishes with a mixture of sieved soil and vermiculite. In early September, the soil was sieved and 123 larval cocoons recovered (i.e. 44% of the larvae successfully built a larval cocoon for overwintwering). Part of this

material is being kept in an outdoor shelter and the remainder in a 3°C incubator for overwintering.

Two midge species are known from the fruits of *R. cathartica*: *Lasioptera kosarzewskella* and *Wachtiella krumbholzi*. According to M. Skuhrava (pers. comm. 2004), the original material of the former species was found in Ukraine in 1957 and *W. krumbholzi* was found near Rostock in northern Germany. Apart from the host, the biology of both species is unknown and there are very few other records in Europe. The slow moving larvae of *W. krumbholzi* are up to 4 mm long (such a size is considered to be large in the family Cecidomyiidae) and are orange to orange-reddish in colour.

The seed-feeding gall midge *W. krumbholzi* appears to be one of the most promising biological control agent for *R. cathartica* in North America. Limited survey and collection trips are planned on *F. alnus* and other buckthorn species to locate and identify gall midge species known on other buckthorns in the literature.

Others

Only very few moth larvae were collected in 2004 from the fruits of *R. cathartica* and all either died shortly after collection or were parasitized. No adults could be reared from a few specimens of a moth larva feeding in the male flowers of *R. cathartica* discovered in early May in Austria (Annex 4; Fig 10).

A few larvae of an unknown lepidoptera species have been found in the fruits of *R. cathartica* in Switzerland in 2005 (Annex 4; Fig 11). These larvae are being kept in an outdoor shelter for overwintering. Adult emergence is likely to occur in late June 2006.

Additional collections are planned in 2006 mostly in Switzerland and southern Germany.

5 Discussion

Preliminary host range studies with several insect species confirm that species in the genus *Frangula* are clearly separated from species in the genus *Rhamnus*. For example, in our host specificity tests, *Frangula alnus* and *F. caroliniana* were not suitable hosts for the leaf feeding geometrid *Triphosa dubitata,* which has not been found on *F. alnus* during our surveys. In contrast both *Rhamnus* and *Frangula* species are suitable hosts for the leaf feeding tortricid *Ancylis apicella,* which may have a slight preference for *F. alnus* in Europe. Larval development on *F. alnus* and *F. caroliniana* was very low for the congeneric species *A. derasana,* which has a clear preference for *R. cathartica* in Europe.

Preliminary host range studies with *Synanthedon stomoxiformis*, which is known from both target plants in Europe, indicate that most species in genus

Rhamnus and *Frangula* are suitable host for larval development. In contrast, very few eggs were laid on non-host plants by *Trichochermes walkeri*, which is considered to be monospecific on *R. cathartica* in Europe.

The North American species *R. alnifolia* is a suitable host for larval development of most insect species tested and thus it seems to be closely related to *R. cathartica*. In contrast, European *R. alpina* is probably more distant to *R. cathartica* as indicated by the results of the preliminary screening tests and the most recent phylogenetic hypothesis in *Rhamnus* s.l. (Bolmgren and Oxelman 2004). In this regard, it would be interesting to include the North American species *R. crocea* in the test plant list as the species is closely related to *R. alpina*.

The recent phylogenetic work by Bolmgren & Oxelman (2004) supports the generic separation of *Frangula* which represents a well-supported monophyletic sister clade to the rest of *Rhamnus* in its widest sense. Grubov (1949) suggested that *Rhamnus* s.l. consists of almost 200 species, but the 25 species of neotropical *Frangula* listed by him were reduced to 12 by Johnston and Johnston (1978) who treated eight more species not included at all by Grubov, and reduced the total number of *Rhamnus* s.l. to about 125 species. Thus, it is difficult to suggest a reliable accurate number of *Rhamnus* and *Frangula* species worldwide. It appears however, that *Rhamnus* and *Frangula* are predominant in the Old World and New World respectively.

In Europe, the genus *Rhamnus* includes some 13 species and 23 taxa and the genus *Frangula* three species only (Tutin 1968). *Rhamnus cathartica* and *F. alnus* have a broad, rather similar, geographical range in their native range in Europe. Assuming species abundance and resource availability offered by both species being equal in the area surveyed, the phylogenetic distance between the two genera, the number of plant species in each genus, and the probable different centre of genus diversification explain to a large extend the higher species richness of specialised arthropods associated with *R. cathartica* in its native range in Europe as compared to *F. alnus*.

In conclusion, preliminary screening tests with several buckthorn insects confirm host plant use observed in the field and the rejection of *Frangula* spp. by many insect species associated with *Rhamnus* in their native range. The likely geographically separate evolution of *Rhamnus* and *Frangula* has led to specialized diets in *Rhamnus* and *Frangula* species with only very few species specialized on *F. alnus* in its native range in Europe and relatively few species with no clear preference for either buckthorn species.

Work carried out in 2004-05 has also highlighted some unexpected biological traits for *Synanthedon stomoxiformis* and *Sorhagenia janiszewskae*, which will need to be confirmed in 2006 for the latter species. Studies showed that *Synanthedon stomoxiformis* is not purely a biennial species, but partly an annual species and that *S. janiszewskae* most probably overwinters in the egg stage. For fruit feeding biological control agents, the highlight from 2004 is the discovery of an important population of the midge *Wachtiella krumbholzi* in the fruits of *R. cathartica* in Serbia, which is also probably present in other areas such as western Switzerland and southern Germany.

Results of preliminary host range studies with several species, progress in the knowledge of the biology of some species and the discovery of the seed feeding gall midge *W. krumbholzi* suggest redefining priorities for biological control of *R. cathartica* in North America. These are summarized in Table 17. The selection of biological control agents which attack both *R. cathartica* and *Frangula alnus* in their native range will undoubtfully increase potential non-target impacts. Therefore, it may be necessary in the future to consider primarily biological agents that are associated with only one of these species in their native range. An upcoming study on the phylogenic reconstruction and relations between European and North America taxa by Bolmgren (pers.com. 2005) will help to assess the level of risk within each genus.

There is great interest for the few internal feeders known on buckthorns in their native range. However, field observations and laboratory studies showed that the host range of the root boring moth *Synanthedon stomoxiformis* will likely include several species in genus *Frangula* and *Rhamnus* and this is likely to be the same with the stem boring beetle *Oberea pedemontana*. Therefore, it is suggested to discard these two species from the prime list of potential biological control agents. At the end of 2004, rearing problems with the shoot-boring moth *Sorhagenia janiszewskae* prompted us to give a lower priority to this species. However, a better understanding of the biology of the species and the results of preliminary host range studies in 2005, led to a renewed interest in this moth.

It is generally accepted that internal feeding species such as gall formers, leaf miners, and stem and root borers are likely to have a more intimate relationship with their host plants, a suggestion that is supported by their generally narrower host ranges (Strong et al. 1984; Mitter and Farrell 1991; Bucheli et al. 2002). Within Lepidoptera, there is a trend for externally feeding species to be generalists and internally feeding species to be oligogagous specialists (Gaston et al. 1992). Insects on *Rhamnus* s.l. offer a complex situation where the stem-, root-boring species, e.g. *Oberea pedemontana* and *Synanthedon stomoxiformis* are among those species showing the broadest oligophagy. In contrast, three of the four leaf miners (*Stigmella catharticella*, *S. rhamnella* and *Calybites quadrisignella*), and several leaf chewing Lepidoptera (e.g. *Phileremе vetulata*, *P. transversata*, *Triphosa dubitata*, *Sorhagenia lophyrella*) have a more restricted host range. This suggests that secondary metabolites in the green parts of buckthorns are different from that in the branches or trunks leading to different degrees of host specialisation for many insects associated with buckthorns. Although not surveyed in depth, the literature on secondary metabolites in buckthorn species seems to be quite abundant and suggests that these metabolites are not similar in all parts of the plant and that concentration varies over time (e.g. Tsahar et al. 2002).

Among the leaf chewing species, the geometrid *Philereme vetulata* appears to be the most specialised species for genus *Rhamnus*. Therefore, we suggest giving *P. vetulata* the highest priority in the leaf chewing feeding guild of species.

Gall formers and sap-suckers such as *Trichochermes walkeri*, *Trioza rhamni* and *Cacopsylla rhamnicolla* belong to those species having the most restricted host range. Among the species that have been studied so far, the leaf margin gall psylloid, *T. walkeri*, is certainly the most specific and will be given the highest priority.

In North America, the high rate of seed production of *R. cathartica* is an important element contributing to its invasiveness. The seed feeding midge, *Wachtiella krumbholzi* is therefore one of the key species as it could significantly reduce the seed production of common buckthorn in North America.

In conclusion, it is recommended to select the following species for intensive host range and biological studies: the shoot-boring moth *Sorhagenia janiszewskae*, the leaf feeding moth *Philereme vetulata*, the leaf margin gall psylloid *Trichochermes walkeri* and the seed feeding midge *Wachtiella krumbholzi*.

In addition, there are still several species which have not yet been studied and which should be considered for biological control of *R. cathartica* in a further phase of the project. Among those species which are probably specific to *Rhamnus cathartica* or to a few species in the genus *Rhamnus*, it is worth mentionning the psyllids *Cacopsylla rhamnicolla* and *Trioza rhamni*, the leaf miners *Stigmella catharticella*, *Calybites quadrisignella* and *Bucculatrix rhamniella* or the mites *Aceria rhamni* and *Tetra rhamni* (Annexes 1, 2).

Biological control of *F. alnus* with species or genus specific agents will undoubtfully be more difficult. The jumping plant lice *Zygina suavis* is the only species which we have found on *F. alnus* but not on *R. cathartica* although *R. cathartica* is a probable host of *Z. suavis* as well (Ossiannilson 1981). Literature records indicate another five arthropod species known from *F. alnus* only, i.e. the flower gall forming midges *Contarinia rhamni* and *Dasyneura frangulae* (Dipt., Cecidomyiidae), the plant bug *Lygocoris rhamnicolla* (Heterop., Miridae), the leaf chewing moth *Aristotelia pancaliella* (Lep., Gelechiidae) and the mite *Eriophyes rhamni* (Acari, Eriophyidae) (Annex 1).

Genetic analysis suggests that European *Frangula alnus* may originate in the Balkans and have spread into Europe after the last glaciation (Hampe et al. 2003). Only the most common haplotypes of *F. alnus* in Europe have been sampled for arthropods in this study. The endemic populations distributed over the Iberian Peninsula (*F. alnus* ssp *baetica*) and Anatolia (*F. alnus* ssp. *pontica*) are highly differentiated and fixed mainly for a single haplotype. This suggests that the arthropods associated with these two disjunt *F. alnus* populations may have a higher degree of specialization and thus it may be worth to look at potential biological agents in these areas as well as in Scandinavia and north-eastern Europe.

Table 17. Reassessment of potential for biological control of *Rhamnus cathartica*

Species	Field hosts	Experimental host range	Comments	Potential host specificity	Priority level
Internal feeders					
Oberea pedemontana	*R. cathartica* / *F. alnus*	Unknown	Mass collection and mass rearing problematic	*R. cathartica* and *F. alnus*	**Low**. Species unlikely to be specific to *Rhamnus*
Synanthedon stomoxifomis	*R. cathartica* / *F. alnus* / Other buckthorn species	*Rhamnus* spp. and *Frangula* spp. suitable for larval development	Life cycle unclear	*Rhamnus* and *Frangula*	**Low**. Species discarded from the prime list of potential agents
Sorhagenia janiszewskae	*R. cathartica* / *F. alnus*	Population from *F. alnus* showed clear preference for *F. alnus*	Possible occurrence of host races or sibling species; better knowledge of the life cycle allows reliable oviposition host range tests	Specific either to *Rhamnus cathartica* or to *Frangula alnus* ?	**High**. Host range of populations from *R. cathartica* and *F. alnus* should be tested
Leaf chewing feeders					
Ancylis apicella	*R. cathartica* / *F. alnus* / Other buckthorn species	*Rhamnus* spp. and *Frangula* spp. suitable for larval development	-	*Rhamnus* and *Frangula*	**Low**. Species discarded from the prime list of potential agents
Ancylis derasana	*R. cathartica* and *F. alnus* with a preference for common buckthorn	*Rhamnus* spp. and *Frangula* spp. suitable for larval development	-	*Rhamnus* and *Frangula*	**Low**. Species discarded from the prime list of potential agents.
Triphosa dubitata	*Rhamnus cathartica* / *Rhamnus* spp. / *F. alnus* (rare)	*Rhamnus* spp. / *Frangula* spp. unsuitable for larval development	Life cycle makes oviposition tests not feasible	*Rhamnus* spp.	**Low-medium.** Species discarded from the prime list of potential agents.
Philereme vetulata	*Rhamnus cathartica* / *Rhamnus* spp.	*Rhamnus* spp. / *Frangula* spp. unsuitable for larval development	Oviposition tests needed. Larval establishment in the young folded leaf is a limiting factor for host plant suitablity	A few species within genus *Rhamnus*	**High**. Probably the most specific leaf chewing species.
Philereme transversata	*Rhamnus cathartica* / *Rhamnus* spp. / *F. alnus* (rare)	*Rhamnus* spp. / *Frangula* spp. unsuitable for larval development	Oviposition tests needed	*Rhamnus* spp.	**Medium**. Probably less specific than *P. vetulata*
Sap feeders					
Trichochermes walkeri	*R. cathartica*	*R. cathartica* / only a very few eggs were laid on *R. alnifolia* in no-choice tests	Host specificity tests will be based on oviposition tests	*R. cathartica*	**High.** Probably monospecific to *R. cathartica*

Flower and fruit feeders					
Wachtiella krumbholzi	*R. cathartica* but systematic surveys on buckthorn berries species have never been carried out. A similar species has been found on *R. saxatilis*	Unknown	Host range studies might be challenging. Gall midges on buckthorns in Europe are very little known	*R. cathartica* ?	**High.** The impact of *W. krumbholzi* on *R. cathartica* seed production would need to be assessed

The following species have not been studied but they are of interest with regard to their likely specificity *to R. cathartica* or to a few *Rhamnus* spp.:

Cacopsylla rhamnicolla, *Trioza rhamni*, *Stigmella catharticella*, *Calybites quadrisignella*, *Bucculatrix rhamniella*, *Aceria rhamni*, *Tetra rhamni*

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Annex 1 Specialized arthropods associated with *R. cathartica* and *F. alnus* in Europe (* personal records; ** Malicky et al. 1970)

Species	Host plants	Specificity[1]	Food niche	References
Coleoptera				
Cerambycidae				
Oberea pedemontana Chevrolat	*R. cathartica*, F. alnus*, R. alpina, Lonicera* ?	O ?	stem, woodboring	(Horion 1974; Lekic and Mihajlovic 1976; Contarini and Garagnani 1980; Baronio et al. 1988; Demelt and Franz 1990; Frisch 1992)
Diptera				
Cecidomyiidae				
Contarinia rhamni Ruebs.	*F. alnus*	M	gall forming (flowers)	(Houard 1909; Barnes 1951) (Buhr 1965; Zerova et al. 1991)
Dasyneura frangulae Ruebs.	*F. alnus*	M	gall forming (flowers)	(Barnes 1951; Buhr 1965)
Lasioptera kozarzewskella Mar.	*R. cathartica*	M	gall forming (fruits)	(Stelter 1975; Zerova et al. 1991)
Wachtliella krumbholzi Stelter	*R. cathartica**	M	gall forming (fruits)	(Stelter 1975)
Heteroptera				
Miridae				
Heterocordylus erythrophtalmus Hb	*R. cathartica**/ F. alnus***	O	sap sucking	(Gollner-Scheiding 1972)
Lygocoris rhamnicola Reuter	*F. alnus*	M	sap sucking	(Coulianos 1998)
Homoptera				
Aphididae				
Aphis commensalis Stroyan	*R. cathartica*	M	gall forming ? (leaves)	(Buhr 1965; Heie 1986)
Aphis mammulata Gimingh. & HRL	R. cathartica	M	free living	(Heie 1986; Blackman and Eastop 1994)
Cicadellidae				
Zygina suavis Rey	*F. alnus *, R. cathartica*	O	sap-sucking	(Ossiannilson 1981)
Psyllidae				
Cacopsylla rhamnicola (Scott)	R. cathartica*/**, F. alnus**	O	free living	(Ossiannilsson 1992)
Triozidae				
Trichochermes walkeri Foerster	*R. cathartica*/***	M	gall forming (leaves)	(Buhr 1965; Okopnyi and Poddubnyi 1983; Meyer 1987; Zerova et al. 1991; Ossiannilsson 1992; McLean 1993)
Trioza rhamni Schrank	*R. cathartica*/**, F. alnus ***	O	gall forming (leaves)	(Buhr 1965; Ossiannilsson 1992)

Species	Host plants	Specificity[1]	Food niche	References
Lepidoptera				
Bucculatricidae				
Bucculatrix frangutella Goeze	_R. cathartica_*/**, _F. alnus_*/**, _R. alpina_*/**	O	leaf miner	(Hering 1957; Heath and Emmet 1985)
Bucculatrix rhamniella H. S.	_R. cathartica_	M	leaf miner	(Hering 1957; Buszko 1992)
Cosmopterigidae				
Sorhagenia lophyrella Douglas	_R. cathartica_** _R. saxatilis_**	O	leaf roller	(Malicky and Sobhian 1971; Baran 1997)
Sorhagenia janiszewskae Riedl	_R. cathartica_*/**, _R. alpina_**, _F. alnus_*/**,	O	shoot miner	(Malicky and Sobhian 1971)
Sorhagenia rhamniella Z.	_R. cathartica_**, _F. alnus_	M?	flowers	(Emmet 1969; Malicky and Sobhian 1971)
Gelechiidae				
Aristotelia pancaliella Stgr.	_F. alnus_	M	leaves	(Ivinskis et al. 1982)
Geometridae				
Odontognophos dumetata Treitschke	_R. cathartica_	M	leaves	(Forster and Wohlfahrt 1981)
Philereme transversata Hufnagel	_R. cathartica_*/**, _R. saxatilis_**, _R. orbiculata_**, _F. alnus_**	O		(Skinner 1984)
Philereme vetulata Schiff.	_R. cathartica_*/**, _R. alpina_**	O	leaves	(Forster and Wohlfahrt 1981; Skinner 1984)
Triphosa dubitata L.	_R. cathartica_*/**,_R. alpina_*/**, _F. alnus_*/**, _Prunus_?, _Fraxinus_ ?, _Crataegus_ ?	O ?	leaves	(Blaschke 1914; Forster and Wohlfahrt 1981; Skinner 1984; Jacobi and Menne 1991)
Triphosa sabaudiata Dup.	_R. cathartica_**,_R. saxatilis_**, _R. orbiculata_**, _F. alnus, R. alpina_	O	leaves	(Blaschke 1914; Forster and Wohlfahrt 1981)
Gracillariidae				
Calybites quadrisignella Z.	_R. cathartica_*/**, _F. alnus_	M?	leaf miner	(Hering 1957)
Nepticulidae				
Stigmella catharticella Stainton	_R. cathartica_*/**, _R. alaternus_	M?	leaf miner	(Hering 1957; Heath 1976; Speight and Cogan 1979; Puplyasis 1984; Puplesis 1994; Michalska 1996)
Stigmella rhamnella H. S.	R. cathartica*/**, R. alpina*	O	leaf miner	(Hering 1957; Klimesh 1968; Puplesis 1994; Michalska 1996)
Pieridae				
Gonopteryx rhamni L.	_R. cathartica_*/**, _R. orbiculatus_*, _F. alnus_*/**	O	leaves	(Frohawk 1940; Bergmann 1952; Pollard and Hall 1980; Bibby 1983; de Freina 1983; Rippey 1984; Heath and Emmet 1989; Mckay 1991; Gutierrez and Thomas 2000)
Pyralidae				
Acrobasis romanella Mill.	_R. cathartica_*/_R. alaternus_**	O	leaves	(Malicky et al. 1970)
Eurhodope legatella Hübner	_R. cathartica_**,_R. saxatilis_**	O	leaves	(Mihajlovic 1978)

Species	Host plants	Specificity[1]	Food niche	References
Sesiidae				
Synanthedon stomoxiformis Hb.	*R. cathartica*, F. alnus, Sorbus aria* ?, *Coryllus avelana* ?	O ?	root miner	(Doczkal and Rennwald 1992; Stadie 1995; Bittermann 1997; de Freina 1997; Spatenka et al. 1999)
Tortricidae				
Ancylis apicella Den. & Schiff.	*R. cathartica*/**, F. alnus*/**, R. alpina**, Ligustrum* ?, *Cornus* ?, *Prunus* ?	O ?	leaves	(Razowski 2003)
Ancylis unculana Haw. (=*derasana* Hb).	*R. cathartica*/**, F. alnus*, Corylus* ?, *Rubus* ?, *Populus* ?	O ?	leaves	(Razowski 2003)
Ancylis obtusana Haw.	*R. cathartica, F. alnus*	O	leaves	(Razowski 2003)
Hysterosia sodaliana Haw.	*R. cathartica**, F. alnus*	O	fruits	(Hannemann 1964; Razowski 1970)
Acari				
Eriophyidae				
Aceria rhamni Roiv.	*R. cathartica*	M	free living	(Amrine and Stasny 1994)
Eriophyes rhamni (Pgst)	*F. alnus*	M	erineum ?	(Amrine and Stasny 1994)
Phyllocoptes annulatus (Nal.)	*R. cathartica**	M	erineum	(Amrine and Stasny 1994)
Tetra rhamni Roiv.	*R. cathartica*	M	free living	(Amrine and Stasny 1994)Petanovic, pers. com. 2005

[1] M = monophagous, restricted to *R. cathartica* or *F. alnus*, O = oligophagous, restricted to species in the genus *Rhamnus* and/or *Frangula*

Annex 2 Frequency of occurrence of specialized buckthorn insects in 2002-04 (Italy, Austria, Switzerland, Germany, Czech Rep.)

Records from (Malicky et al. 1970)

	Rhamnus cathartica	**Frangula alnus**	**R. alpina**	**R. saxatilis**
# of sites sampled :	46 (%)	42 (%)	2 (%)	4 (%)
# of sites sampled :	214 (%)	83 (%)	5 (%)	30 (%)
# of sites with :				
LEPIDOPTERA :				
Bucculatricidae :				
Bucculatrix frangutella	5 (10.9)	5 (11.9)	2 (100)	-
Bucculatrix frangutella	44 (20.6)	11 (13.3)	1.0 (20.0)	-
Cosmopterigidae :				
Sorhagenia janiszewskae	13 (28.3)	12 (28.6)	1? (50.0)	-
Sorhagenia janiszewskae	9 (4.2)	14 (16.9)	2 (40)	-
Sorhagenia rhamniella	14 (6.5)	-	-	-
Sorhagenia lophyrella	27 (12.6)	-	-	13 (43.3)
Gracillariidae :				
Calybites quadrisignella	3 (6.5)	-	-	-
Calybites quadrisignella	10 (4.7)	-	-	-
Nepticulidae :				
Stigmella catharticella	2 (4.3)	-	-	-
Stigmella catharticella	25 (11.7)	-	-	-
Stigmella rhamnella	1 (2.2)	-	1 (50.0)	-
Stigmella rhamnella	2 (0.9)	-	-	-
Pyralidae :				
Acrobasis romanella	1 (2.8)			
Acrobasis romanella 1)	-	-	-	-
Trachycera legatea	3 (1.4)	-	-	12 (40.0)
Pieridae :				
Gonepteryx rhamni	7 (15.2)	22 (52.4)	2 (100.0)	-
Gonepteryx rhamni 2)	18 (8.4)	22 (26.5)	-	-
Geometridae :				
Philereme vetulata	10 (21.7)	-	-	-
Philereme vetulata	68 (31.8)	-	1,0 (20.0)	
Philereme transversata	4 (8.7)	-	-	-
Philereme transversata 3)	38 (17.8)	1 (1.2)	-	2 (6.7)
Triphosa dubitata	23 (50.0)	-	1 (50.0)	-
Triphosa dubitata 3)	42 (19.6)	3 (3.6)	3 (60.0)	-
Triphosa sabaudiata L.	2 (0.9)	-	-	3 (10.0)
Tortricidae :				
Ancylis apicella	5 (10.9)	7 (16.7)	1 (50.0)	-
Ancylis apicella 1)	2 (0.9)	2 (2.4)	-	1 (3.3)
Ancylis derasana	6 (13.0)	3 (7.1)	-	-
Ancylis derasana	9 (4.2)	-	-	-
Cochylidae :				
Hysterosia sodaliana	14 (6.5)	-	-	-

HOMOPTERA				
Psyllidae :				
Cacopsylla rhamnicola	5 (10.9)	-	-	-
Cacopsylla rhamnicola	6 (2.8)	1 (1.2)	-	-
Triozidae :				
Trichochermes walkeri	40 (87.0)	-	-	-
Trichodermes walkeri	67 (16.8)	-	-	-
Trioza rhamni	36 (78.3)	-	-	-
Trioza rhamni	36 (16.8)	1 (1.2)	3.0 (60.0)	-
Cicadellidae :				
Zygina suavis	-	5 (11.9)	-	-
HETEROPTERA				
Miridae:				
Heterocordylus erythrophtalmus Hb.	6 (2.8)	1 (1.2)	-	-
COLEOPTERA				
Cerambycidae :				
Oberea pedemontana	2 (4.3)	2 (4.8)	-	-
ACARI				
Eriophyidae				
Phyllocoptes annulatus	7 (15.2)	-	-	-

1) Also recorded on *Rhamnus alaternus*
2) Also recorded on *R. orbiculata* and *Frangula rupestris*
3) Also recorded on *R. orbiculata*

Annex 3: Surveys and collections in 2004-05

2004		
Date	**Collection area**	**Main purposes**
14 April	S Germany	To make observations on the presence and the phenology of a number of species, e.g. *Triphosa dubitata*, *Cacopsylla rhamnicola*, *Philereme vetulata*, *P. transversata* (*Rhamnus cathartica*; *Frangula alnus*)
21 April	S Germany	To collect eggs of *T. dubitata* (*R. cathartica*)
21-22 April	S-W Switzerland	To collect eggs of *T. dubitata* (*R. cathartica*); to study and to collect *Sorhagenia janiszewskae* (*F. alnus*)
28 April	S Germany	To collect larvae of *P. vetulata* and *P. transversata* (*R. cathartica*)
30 April	Swiss Jura (lowland)	To collect eggs of *T. dubitata* and larvae of *P. vetulata* (*R. cathartica*)
3 May	Swiss Jura (hills)	To collect eggs of *T. dubitata* (*R. alpina*)
4 May	S Germany	To collect larvae of *P. vetulata* and *P. transversata* (*R. cathartica*)
5-6 May	S-W Switzerland	To collect eggs of *T. dubitata* and larvae of *P. vetulata* (*R. cathartica*); to collect larvae of *S. janiszweskae* (*F. alnus*)
13 May	Swiss Jura (hills)	To collect eggs of *T. dubitata* (*R. alpina*)
11-14 May	Eastern Austria	To collect larvae of of *P. vetulata* and *P. transversata* (*R. cathartica*) and larvae of *S. janiszweskae* (*R. cathartica* and *F. alnus*); to sample flowers and fruits of *R. cathartica* and *F. alnus*
19 May	Swiss Jura (hills)	Final collection of eggs of *T. dubitata* (*R. alpina*)
7 June	Swiss Jura (lowland)	To collect young fruits of *R. cathartica*
8 June	S Germany	To collect young fruits of *R. cathartica* and *F. alnus*
12-13 June	Serbia	To collect larvae of *Synanthedon stomoxifomis* (*R. cathartica*); to survey for *Oberea pedemontana* (*R. cathartica*); to survey *R. saxatilis* ssp. *tinctorius*
22 June	W Switzerland	To locate new buckthorn sites
30 June	S Germany	To collect mature fruits of *R. cathartica* and *F. alnus*
2 July	Serbia	To collect mature fruits and gall midges on *R. cathartica*
28 July	W Switzerland	To observe the phenology of, and to collect galls of *Trichochermes walkeri* (*R. cathartica*)
29 July	S Germany	To observe the phenology of, and to collect galls of *Trichochermes walkeri* (*R. cathartica*)
3 August	W Switzerland	To collect galls of *T. walkeri* (*R. cathartica*)
11 August	Swiss Jura (hills)	To observe the phenology of, and to collect galls of *Trichochermes walkeri* (*R. cathartica*)
12 August	W Switzerland	Final collection of *T. walkeri* (*R. cathartica*)
1 September	Swiss Jura	To locate new populations of buckthorns
31 October-1 November	S-w Switzerland	To study the life cycle of, and to sample *S. janiszewskae* (*F. alnus*)

2005		
Date	**Collection area**	**Main purposes**
10-11 April	S-w Switzerland	To study the phenology of and to collect *S. janiszewskae* eggs or larvae (shoot buds of *F. alnus*)
11 April	W Switzerland	To make observations on *S. janiszewskae* (*F. alnus*), and *P. vetulata* (*R. cathartica*)
26 April	S Germany	To collect larvae of *P. vetulata* (*R. cathartica*) and to make observations on other potential agents
3 May	S Germany	To collect larvae of *P. vetulata* (*R. cathartica*)
5 May	S-w Switzerland	To collect larvae of *P. vetulata* (*R. cathartica*)
5 May	W Switzerland	To collect larvae of *P. vetulata* and to make observations on the presence and phenology of other species
8-11 May	Austria	To collect larvae of *P. vetulata* (*R. cathartica*) and *S. janiszewskae* (*F. alnus*; *R. cathartica*)
9 May	S Germany	To make observations on the phenology of *T. walkeri* and other species
27 May - 7 June	Serbia	To collect pupae of *Synanthedon stomoxiformis* (*R. cathartica*); to collect larvae of *Oberea pedemontana* (*R. cathartica*)
31 May	S-w Switzerland	To collect mature larvae *S. janiszewskae* (shoot buds of *F. alnus*)
31 May	W Switzerland	To collect mature larvae *S. janiszewskae* (shoot buds of *F. alnus*); to make observations on the phenology of *T. walkeri* and other species
11 July	Serbia	To collect mature fruits and gall midges on *R. cathartica*
20 July	W Switzerland	To collect fruits of *R. cathartica* and to make observations on the phenology of *T. walkeri* (*R. cathartica*)
22 July	S Germany	To collect fruits of *R. cathartica* and to make observations on the phenology of *T. walkeri* (*R. cathartica*)
4 August	S-w Switzerland	To collect *T. walkeri* (*R. cathartica*)
9 August	Swiss Jura (Hills)	To collect *T. walkeri* (*R. cathartica*)
10 August	S-w Switzerland	To collect *T. walkeri* (*R. cathartica*)

Annex 4

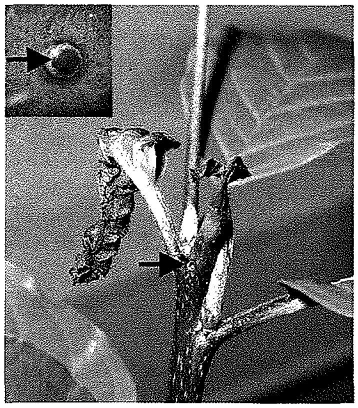

Fig 1. Damage by *Sorhagenia janiszewskae* on *F. alnus* and larval exit hole (arrow); from larval transfer, CABI-CH, 19 May 2005

Fig 2. *Sorhagenia janiszewskae* oviposition field cages (CABI-CH garden, 15 July 2005)

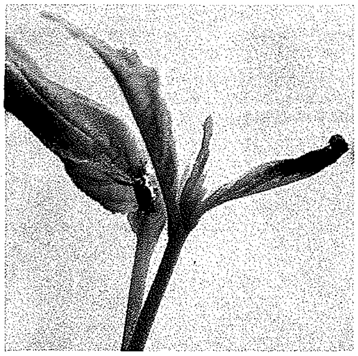

Fig 3. Settlement and first larval instar damage by *Philereme vetulata* on young folded *R. cathartica* leaves (CABI-CH, February 2005)

Fig 4. *R. cathartica* folded leaves with medium to large larvae of *P. vetulata* (Austria, 12 May 2004)

Fig 5. Larval damage by *Synanthedon stomoxiformis* in the shoot-root interface of *R. alpina* (from larval transfer, CABI-CH, 15 June 2005)

Fig 6. Rearing of *S. stomoxiformis* on *F. alnus* (CABI-CH garden, 18 August 2005)

Annex 4 (cont.)

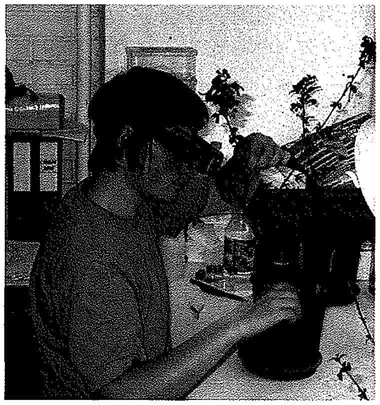

Fig 7. T. Rheinhold looking for *Trichochermes walkeri* eggs on potted *R. cathartica*

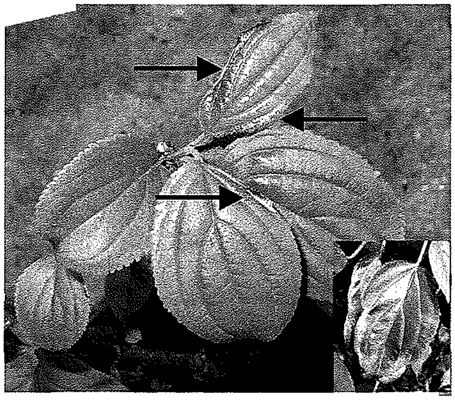

Fig 8. Initial damage by *T. walkeri* on *R. cathartica* leaves and mature galls

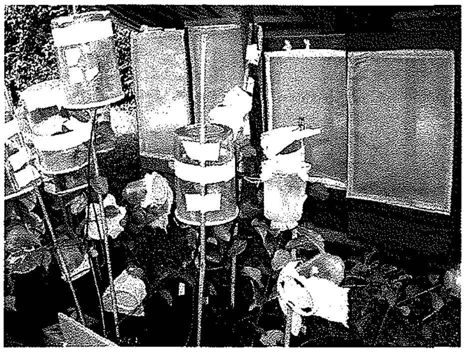

Fig 9. No-choice oviposition tests with *T. walkeri* on potted plants (CABI-CH, 18 August 2005)

Fig 10. Lep larva within *R. cathartica* male flowers with silk and frass (Austria, 13 May 2004)

Fig 11. Feeding damage on fruits *of R. cathartica* by an unidentified Lep larvae (Germany, 22 July 2005)

Fig 12. *R. cathartica* fruits infested with larvae of *Wachtiella krumbholzi* (Serbia, 3 July 2004)

Fig 13. *W. krumbholzi* larvae (Serbia, 12 June 2004)

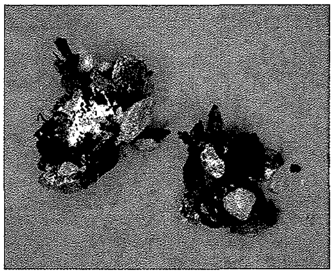

Fig 14. Larval cocoons of *W. krumbholzi* (CABI-CH, 21 October 2004)