Appendix B

Result 1: Investigate potential insects as biological control of European Buckthorn
And
Result 2: Survey of insects on buckthorn in Minnesota

Result 1- Part 1: Investigate potential insects as biological control of European Buckthorn

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

Annual Report 2006

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Summary

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. Work carried out 2002-2005 has lead to the rejection of several species because of their lack of specificity at the genus level: the stem boring cerambycid beetle *Oberea pedemontana*, the root-boring sesiid moth *Synanthedon stomoxiformis* and the leaf feeding tortricid moths *Ancylis apicella* and *A. derasana*. Other species have been discarded because of their likely oligophagy within genus *Rhamnus* and/or the difficulty in establishing a rearing colony in the future, i.e. the leaf feeding geometrid moths *Triphosa dubitata* and *Philereme transversata*.

Work carried out in 2006 confirmed that both *R. cathartica* and *F. alnus* are suitable hosts for the shoot-tip boring moth *Sorhagenia janiszewskae*. 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 is suggested to give *S. janizewskae* a low priority for biological control of buckthorns.

Work in 2006 has also highlighted the difficulty to demonstrate with certainty the absence of consistence oviposition by *Trichochermes walkeri* on non target plants in no-choice conditions. However, the probability for high oviposition rate and gall and larval development on non-target *Rhamnus* species is extremely low. Oviposition choice tests should confirm the high specificity of *T. walkeri*.

Transfer of newly hatched larvae of *Philereme vetulata* on potted plants at the time of leaf bud expansion has produced good results. Species in genus *Rhamnus* appear to be suitable hosts for larval development of this moth although variability in food quality may result in lower pupal weight or longer time for development to pupal stage. The feasibility of oviposition choice tests will be studied in 2007.

Finally progress has been made in mass collection, and rearing to the adult stage of the seed feeding midge *W. krumbholzi*. Oviposition and larval development will be studied in 2007. Growing plants to fruiting stage and the synchronisation between adult emergence and plant phenology will be the main challenges in future tests.

1. Introduction

Rhamnus cathartica L. (common buckthorn) and *Frangula alnus* Miller (glossy buckthorn) (Rhamnaceae) are both 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 the late 1800s and was originally used for hedges, farm shelter belts, and wildlife 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 (Gale, 2001; USDA/NRCS, 2001).

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.

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 2002-2005 has lead to the rejection of several species because of their lack of specificity at the genus level: the stem boring cerambycid beetle *Oberea pedemontana,* the root-boring sesiid moth *Synanthedon stomoxiformis* and the leaf feeding tortricid moths *Ancylis apicella* and *A. derasana.* Other species have been discarded because of their likely oligophagy within genus *Rhamnus* and/or the difficulty in establishing a rearing colony in the future, i.e. the leaf feeding geometrid moths *Triphosa dubitata* and *Philereme transversata.*

In our last report (Gassmann et al. 2006) we recommended to select the following species for further studies and host range testing in 2006: 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*. There are few stem and root borers known on buckthorns and *S. janizewskae* was the only species which might be specific enough. Among the leaf chewing species, *P. vetulata* appeared to be the most specialised species for genus *Rhamnus*. Among the species which have been studied so far, *T. walkeri* is certainly the most specific and *W. krumbholzi* is one of the key species that could reduce the seed production of common buckthorn in North America.

One current constraint in developing biological control of buckthorn is the difficulty to obtain seeds from a number of test plants or to grow plants from seeds. The difficulty is enhanced by the occurrence of the sudden oak death (*Phytophthora ramorum*) on *Frangula californica* and *F. purshiana* in North America and the need

to import in Switzerland cuttings or rootstocks "found free from non-European isolates of *Phytophthora ramorum*".

2. Studies on individual potential arthropod biological control agents

2.1. Lepidoptera

2.1.1 *Sorhagenia janiszewskae* (Lep., Cosmopterigidae)

Background

Sorhagenia janiszewskae, the larvae of which mine in the year's shoots of buckthorns is a difficult species to work with. 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. Thus, time for collection is critical and this can change from year to year depending on climatic conditions in late winter. Adult aestivation and hibernation was thought to be an insurmountable problem. Field observations and the absence of mines and larvae in plants exposed in the oviposition tests in 2004-05 suggest that there is no second generation and that *S. janiszewskae* overwinters in the egg stage. Oviposition most probably occurs within three weeks of adult emergence.

In 2004 we gave a low priority to *S. janiszewskae* because of unresolved problems in adult rearing and the likely lack of host specificity of the moth which occurs on both *R. cathartica* and *F. alnus* in its European range. However, the clear oviposition host preference of *S. janizewskae* reared from *F. alnus* for its field host plant in 2005 renewed interest in this species for biological control of *R. cathartica* and *F. alnus* in North America. It was hypothesized that *S. janiszewskae* from *F. alnus* and *R. cathartica* are either two different species or two different host races.

Collection of mature larvae and adult emergence

The collection of shoot-tips presumably attacked by larvae of *S. janiszewskae* is summarized in Table 1. The shoot tips were placed in an outdoor shelter in ventilated emergence cages / boxes filled with a mixture of sieved soil and vermiculite to allow pupation. Irrespective of the number of adults emerged, adult emergence for all collections sites started July 3-6 and was completed two weeks later (Figs 1, A-C). Emergence pattern in 2006 confirm that during normal years emergence of *S. janiszewskae* starts late June – early July and lasts for 2-3 weeks (see also Gassmann et al. 2006). Adult emergence in 2004-2006 was delayed by about two weeks as compared to 2003 which has been exceptionally warm in the whole Europe.

Table 1 Collections of *Sorhagenia janiszewskae* in 2006

Site	Country	Collection date	Host plant	# shoot tips collected	# adults emerged
Neulengbach	Austria	22 May 2006	*F. alnus*	100	19
Unterwaltersdorf	Austria	22 May 2006	*R. cathartica*	180	73
Purgstall	Austria	22 May 2006	*R. cathartica*	15	7
Truman	Austria	23 May 2006	*R. cathartica*	85	64
Reisenberg	Austria	23 May 2006	*F. alnus*	85	32
Collet Bossy	Switzerland	29 May 2006	*F. alnus*	100	9
Satigny	Switzerland	29 May 2006	*F. alnus*	140	70

As shown in Table 1, one major difficulty with *S. janizewskae* remains to accurately predict adult emergence and hence best collection time. Low rate of adult emergence in a few sites is probably due to local conditions which slow down larval development and immature larvae will not complete development in cut shoots.

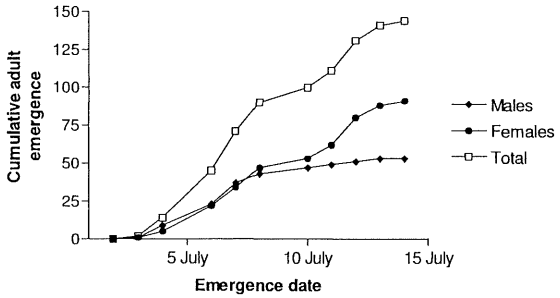

Fig 1A

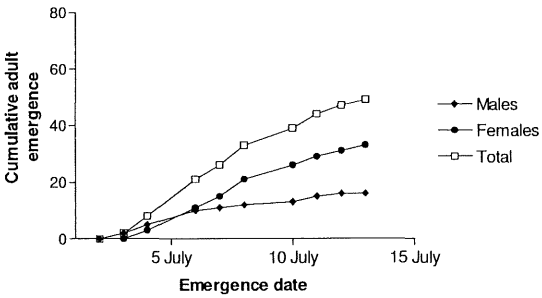

Fig 1B

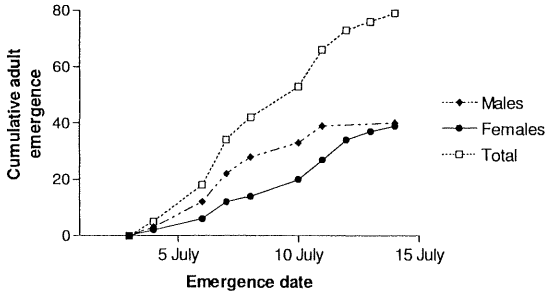

Fig 1C

Figs 1 A-C Emergence of *S. janizewskae* adults in 2006; A. from *R. cathartica* Austria; B. from *F. alnus* Austria; C. from *F. alnus* Switzerland

Egg overwintering and egg hatch (2005-2006)

Sorhagenia janizewskae overwinters in the egg stage. Eggs presumably laid by *S. janizewskae* (reared from *F. alnus*) in single choice oviposition tests in 2005 (Gassmann et al 2006, table 5) were divided into batches of 10 in Petri dishes and kept under different conditions to study post diapause development. Batches of eggs were put at 10°C, 15°C and 20°C on 5 April 2006 after a cold treatment of 4.5 months at 3°C. Egg hatch at 10°C started on April 16 and was delayed by about one week compared to treatments at 15 and 20 °C (Fig 2). Egg hatch in an outdoor shelter started on April 12 and was completed on April 20. The percentage of egg hatch was comprised between 90-100% for all treatments.

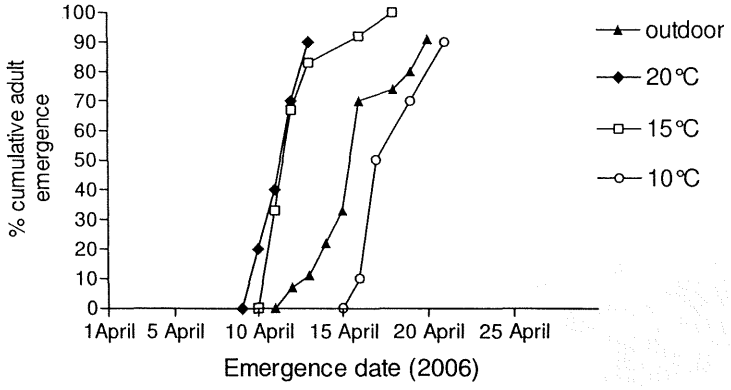

Fig 2 Egg hatch of *Sorhagenia janizewskae* under different temperatures after a cold treatment of 4.5 months at 3 °C (2005-2006)

Larval transfer and development

Larvae obtained from overwintering eggs were transferred into individual shoot buds of potted *R. cathartica* and *F. alnus* during April 12-28. 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. All shoots were cut and dissected during June 9-12, i.e. before larvae left the shoots to pupate in the soil. Results are shown in Table 2.

Table 2 Larval development of *S. janizewskae* from *F. alnus*

Test plant	*# of L1 transferred*	*# of larvae developed (%)*
F. alnus	*29*	*11 (37.9)*
R. cathartica	*37*	*11 (29.7)*

Larval survival and development was quite similar on *F. alnus* and *R. cathartica*. The identification of adults emerged from this material is pending, but this is most certainly *S. janiszewskae*.

At the end of the 2005 field season, it was hypothesized that *S. janiszewskae* from *F. alnus* and *R. cathartica* are two different highly specific species or two different host races. The 2006 larval transfer tests infirm this hypothesis; the physiological host range of the larvae of *S. janiszewskae* from *F. alnus* includes both its field host plant and *R. cathartica*.

Single choice and no-choice field cage oviposition tests in 2006

Considering the results of preliminary single choice field cage oviposition tests in 2005, a large no-choice and single choice oviposition experiment was set-up with nine 2m x 2m x 2m field cages in the Centre's garden (Fig 3). Large 1m-1.5m tall potted *F. alnus* and *R. cathartica* were exposed singly or in combination to *S. janiszewskae* adults freshly emerged from *F. alnus* and *R. cathartica*. All cages were set-up July 4-10. All shoot buds of all plants were dissected in October. Very little oviposition occurred in the whole test (Table 3). The relatively good oviposition rate obtained in the 2005 oviposition test seems to have been exceptional and the best conditions for regular oviposition are still unknown. The field cage experiment carried out in 2006 does not show evidence of oviposition preference of *S. janiszewskae* from *F. alnus* and *R. cathartica*.

Fig 3 Field cage oviposition test with *S. janizewskae* in 2006

No choice oviposition tests in small cages in 2006

Three buckthorn species were individually tested each in two replicates in no-choice oviposition tests with three pairs of *S. janizewskae* from *R. cathartica* (Autrian population) in 80 x 40 x 40 cm cages. All cages were kept outside beneath a suspended tarpauline, protected from rain and sun. The tests were set up July 7-12. All shoot buds were dissected in late September. Results are summarized in Table 4. Again, very little oviposition occurred in the test and there is no evidence of oviposition preference of *S. janizewskae* for its field host plant.

Discussion

The absence of mines and larvae in plants exposed in the oviposition tests in 2005 and 2006 suggests that there is no second generation and confirm that *S. janiszewskae* overwinters in the egg stage. As indicated by the oviposition experiment carried out in 2004, oviposition most probably occurs within three weeks of adult emergence.

Sorhagenia janiszewskae eggs kept at 3°C hatched within one week when transferred to 15°C or 20°C. This suggests that, as for *Philereme vetulata* (This report), post diapause development requires a low number of degree-days and that temperature threshold for diapause development is < 3°C. Egg hatch in an outdoor shelter in 2006 occurred April 12-20. Egg hatch in many field situations is likely to occur slightly earlier when attacked shoot buds are exposed to local higher temperature or direct sunshine. In 2005, only newly hatched larvae were found in shoot buds of *F. alnus* collected 10-11 April at three sites in south-western Switzerland.

Following results obtained in 2005, we were expecting *S. janiszewskae* to oviposit readily in field cages. Preliminary data even suggested the possible occurrence of highly specific species or host races. Data obtained in 2006 are disappointing. First, *S. janiszewskae* females hardly oviposited in confinement. The higher number of eggs recorded in 2004 and 2005 was most probably due to normal oviposition by one or a very few females only, and this can't make oviposition tests reliable. Second, there is no further evidence of the occurrence of oviposition preference of the moth for its field host plant. Finally, larval transfer tests with newly hatched larvae showed that both *R. cathartica* and *F. alnus* are suitable hosts for larval development of *S. janiszewskae* from *F. alnus*. In our 2004-05 report (Gassmann et al. 2006), we were advocating a renewed interest for *S. janizewskae*. Results obtained this year and the difficulty to carry out reliable oviposition tests together with the need to give priority to potential biological control agents which are at least genus specific lead us to recommend discarding *S. janiszewskae* from the prime list of potential biological control agents for buckthorns in North America.

Table 3 Single choice and no-choice field cage oviposition tests in 2006

Cage design	*S. janiszewskae* from *F. alnus* (origin: Switzerland)	*S. janiszewskae* from *F. alnus* (origin: Austria)	*S. janiszewskae* from *R. cathartica* (origin: Austria)	# of shoot buds dissected		# of eggs in shoot buds	
				R. cathartica	*F. alnus*	*R. cathartica*	*F. alnus*
2 *F. alnus*	9 pairs	-	-	-	78	-	0
2 *R. cathartica*	9 pairs	-	-	256	-	0	-
2 *F. alnus*	-	5 pairs	-	-	183	-	1
3 *R. cathartica*	-	5 pairs	-	309	-	0	-
2 *F. alnus* + 2 *R. cathartica*	-	5 pairs	-	201	280	1	0
2 *F. alnus* + 2 *R. cathartica*	9 pairs	-	-	143	102	0	1
2 *F. alnus* + 2 *R. cathartica*	-	-	10 pairs	218	235	1	10
3 *R. cathartica*	-	-	10 pairs	371	-	1	-
2 *F. alnus*	-	-	10 pairs	-	240	7	-

Table 4 No-choice oviposition tests with *S. janiszewskae* from *R. cathartica* (Austrian population) in small cages in 2006

Test plant		# of shoot buds dissected	# of eggs in shoot buds
Rhamnus cathartica	Rep 1	70	3
Rhamnus cathartica	Rep 2	52	0
Rhamnus alnifolia	Rep 1	34	1
Rhamnus alnifolia	Rep 2	32	0
Frangula alnus	Rep 1	51	0
Frangula alnus	Rep 2	50	8

2.1.2 *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 (Gassmann et al. 2006). Larvae of *P. vetulata* feed within folded leaves.

Collection and adult emergence

Following field collections, larvae were reared in ventilated plastic boxes stored in an outdoor shelter, where leaves were kept fresh with moist paper. Pupae were kept in ventilated plastic cups half filled with vermiculite and stored in an outdoor shelter for adult emergence. A total of 63 males and 118 females emerged from the collection of 318 larvae made in early spring in Germany and Switzerland (Fig 4). Another 98 males and 109 females emerged from larvae reared from eggs obtained in 2005. Batches of eggs kept at 3°C were regularly put at 20°C for this purpose. Newly hatched larvae were transferred onto young small folded leaves of potted plants before being transferred two weeks later in ventilated plastic boxes for completion of larval development. Last adult emerged on July 12, i.e. three weeks after the peak of adult emergence from field collected larvae.

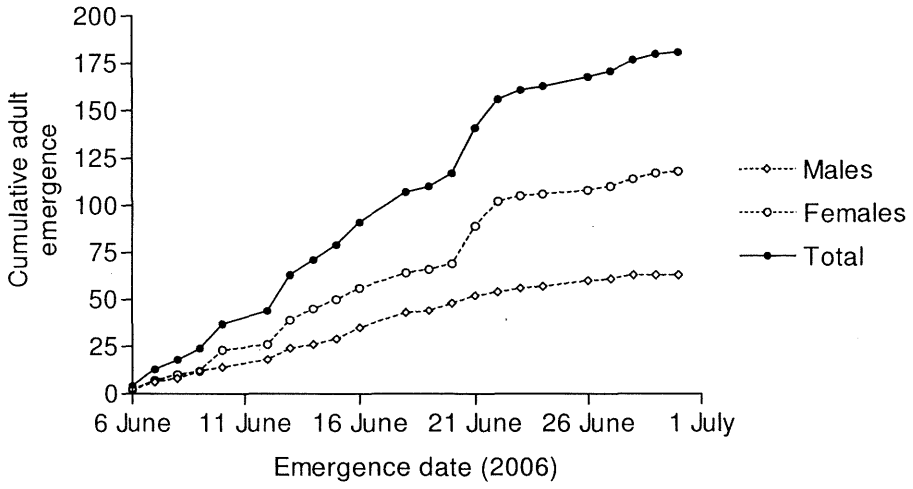

Fig 4 **Emergence of *Philereme vetulata* adults from three field sites in 2006**

Adult rearing and oviposition

A total of 120 females and 120 males were reared in 63 cardboard and plastic cylinders between 6 June and 24 July. Due to the high number of adults available, two

pairs were kept in each cylinder. A total number of 7475 eggs were obtained of which 75% (5603 eggs) were fertile. Average fecundity per female was 62 eggs, i.e. similar to that in 2004 when the females were reared in the same conditions, but lower than that of individual females in 2005 which was 88 eggs per female (Gassmann et al. 2005; 2006). Average fertile eggs per female were 47.

Diapause development and post diapause development of the egg of *Philereme vetulata* (2005-06)

Philereme vetulata is univoltine and overwinters in the egg stage. Observations made during larval transfer tests in previous years indicate that synchronisation between larval hatching and leaf bud development is 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. Studies in 2004-2005 indicated that a cold period at either 3°C or 10°C followed by a temperature of 20°C allowed over 80% of egg hatch within 20 days.

Material and methods: Studies were repeated in 2005-2006 under three different cold treatment (3°C, 10°C and 15°C) and five post diapause development temperature (20°C, 15°C, 12.5°C, 10°C and 7.5°C) in order to define more precisely the temperature thresholds for diapause and post diapause development. Eggs were divided into batches of 50 and kept relatively moist with short period of drier conditions during the whole experiment. Cold treatments were started on 11 November 2005, i.e. three weeks later than in the 2004-05 experiment.

Results: A cold treatment is necessary for diapause development. Fig 5A 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. In contrast to the 2004-05 experiment, a cold treatment of two months at 3 °C allows complete diapause and post diapause development. Time to egg hatch is slightly shortened when the cold treatment is prolonged to 3 and 4 months at 3°C.

Fig 5B shows egg hatch when the same cold treatments at 3°C were applied followed by a temperature of 15 °C. At 15°C, egg hatch is optimal after a cold treatment of 3 or 4 months at 3°C. Again, in contrast to the 2004-05 experiment, a cold treatment of 2 months at 3°C allows nearly complete diapause and post diapause development. Time to egg hatch is however longer than after a cold treatment of 3 or 4 months at 3 °C. 32% egg hatch occurred after one month only of cold treatment confirming that a 15°C temperature allows some diapause development.

Figs, 5C-E shows that egg development is optimal at any cold treatment of 3°C when the cold treatment is followed by temperatures of 12.5°C, 10°C and 7.5°C. Not surprisingly, time to egg hatch is slightly longer at 10°C than at 12.5°C and even longer at 7.5°C. As compared to the 2004-05 experiment, time to egg hatch at 10 °C was noticeably shorter in 2005-06 after a cold treatment of 2 months at 3 °C.

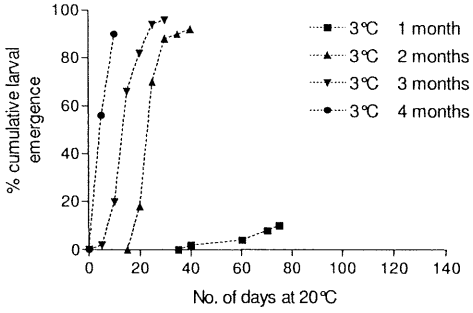

Fig 5A

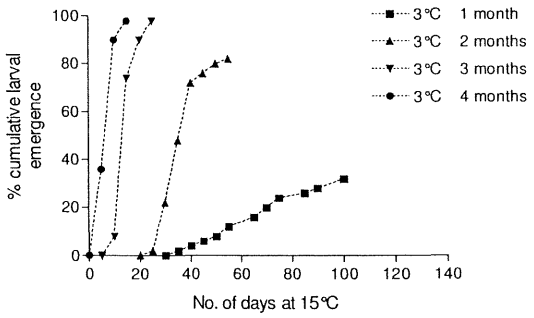

Fig 5B

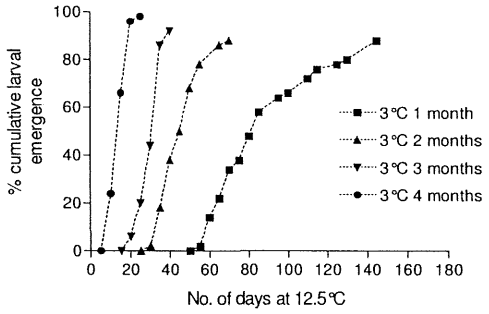

Fig 5C

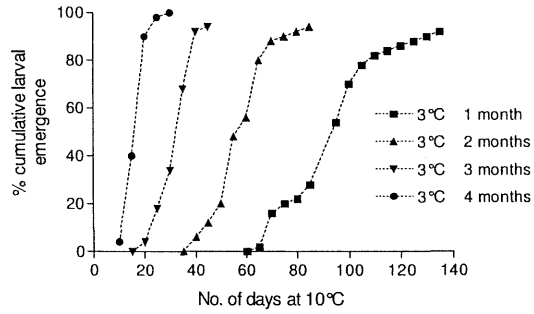

Fig 5D

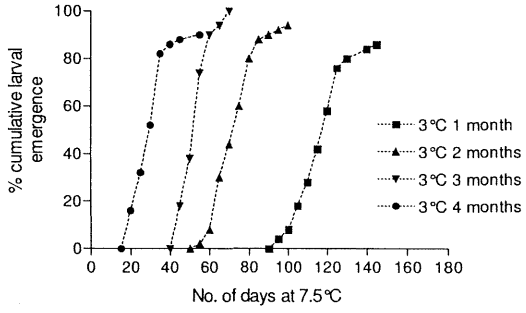

Fig 5E

Figs 5A-E Diapause development and post diapause development of *P. vetulata* eggs under different temperature regimes

Fig 6A shows that a cold treatment of one month at 10°C followed by a temperature of 20°C allows only very little egg hatch. In contrast, a cold treatment of 2 months at 10°C followed by a temperature of 20°C allows a nearly complete diapause development and normal egg hatch. In the 2004-05 experiment, only 18% egg hatch occurred after a 2 months treatment at 10°C. Time to egg hatch was slightly longer with a cold treatment of 2 months at 10°C than with a cold treatment of 3 or 4 months at 10 °C. Larvae started to emerge at 10 °C at the end of the four months cold treatment

Fig 6B shows egg hatch when the same cold treatments at 10°C were applied followed by a temperature of 15 °C. 30% of egg hatch was recorded at 15 °C with a cold treatment of only one month at 10°C. In contrast to 2004-05, almost normal egg hatch was obtained at 15°C after two months cold treatment at 10°C. Time to hatch after 2 months cold treatment at 10°C was only slightly delay compared to longer cold treatment (3 and 4 months at 10°C). In this trial also, larvae started to emerge at 10 °C at the end of the four months cold treatment.

Fig 6C shows egg hatch at 20°C after a cold treatment of 1,2,3 and 4 months at 15 °C. Only few eggs hatched after 1, 2 and 3 months cold treatment at 15 °C.

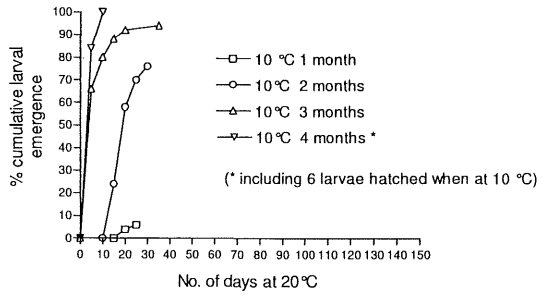

Fig 6A

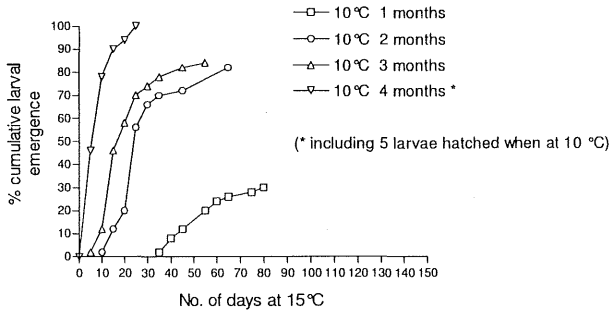

Fig 6B

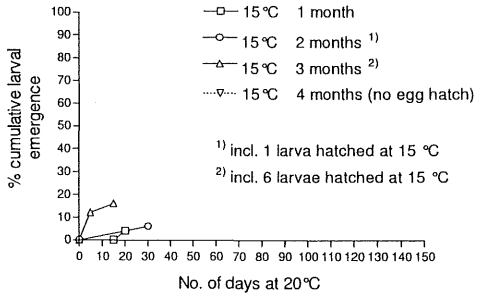

Fig 6C

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

Results are summarized in Fig 7. Normal egg hatch occurred after three months cold treatment at 3 °C and 10 °C. In contrast to the 2004-05 experiment, normal egg hatch occurs also after two months cold treatment at 3°C and nearly so after two months at 10°C. The difference of 20 days in the set-up of the experiments in 2004-2005 and 2005-2006 corresponds roughly to one month cold treatment at 10 °C: the mean daily temperature for the period 21 October – 10 November 2005 was 10.7 °C (meteorological data from FRI, Courtemelon). Thus, eggs have been exposed to additional cold treatment when kept in outdoor conditions in autumn 2005. Not surprisingly, mean time to egg hatch depends on the temperature following the cold treatment. The time to egg hatch and the difference in egg hatch between treatments is reduced with longer cold treatment at 3 °C or 10 °C, indicating that egg development occurs at 3°C and at 10°C.

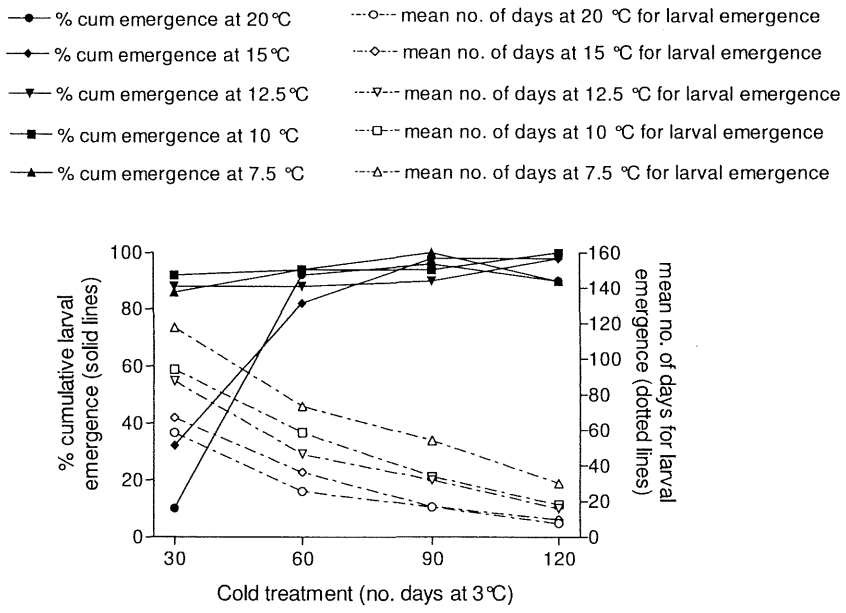

Fig 7 Percent of cumulative larval emergence and mean time for larval emergence of *P. vetulata* under different temperature regimes

Fig 8 shows egg hatch at constant temperature of 3°C, 10°C, 15°C, and 20°C, and in outdoor conditions in 2004-2005 and 2005-2006. No egg hatches at 20 °C and only few at 15 °C, confirming that a temperature of 15°C allows both some diapause and post diapause development. Considering that diapause development may have occurred in part before the set-up of the experiment in autumn 2005, it is hypothesized that the upper limit for diapause development is slightly below 15°C. When kept continuously at 10 °C, egg hatch started in early March, i.e. less than one month later than in the 2004-2005 experiment, but the mean number of days to egg hatch since January 1 was identical in 2005 and 2006, i.e. 137 days. Eggs kept at a 3°C constant temperature started to hatch in mid-May, thus confirming that the

temperature threshold for egg development of P. vetulata is below 3°C (Fig 8). 77% of egg hatch was recorded within 30 days only.

The calculation of the lower threshold for egg development is at least problematic since the 3°C cold treatment allows development. After a cold treatment of two months at 3 °C, the regression line ($r^2 = 0.99$) gives a temperature threshold of 1.6°C and a number of degree-days required for development of 480 (= slope $^{-1}$). After a cold treatment of three months at 3°C, the temperature threshold for development is 1.9°C and the number of degree-days required for development is 279 (= slope $^{-1}$) ($r^2 = 0.85$ for the regression line).

The period of egg hatch in outdoor conditions lasted only 12 days from late March until early-mid April and was similar in 2005 and 2006 (Fig 8). Assuming an hypothetical threshold for egg development of 1°C, the accumulation of temperatures above 1°C from January 1 for egg development in outdoor conditions was similar in 2005 and 2006, i.e. 275 (1 January - 7 April 2005) and 277 respectively (1 January – 20 April 2006).

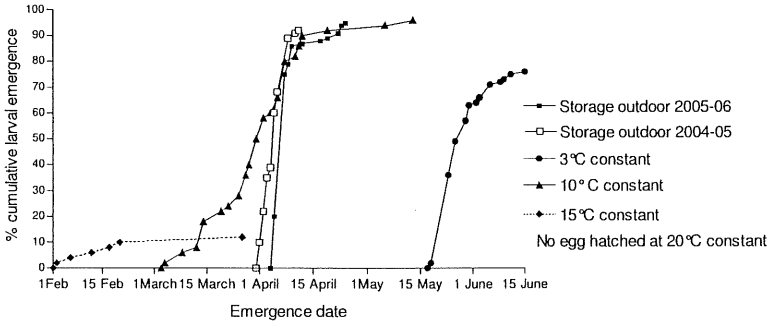

Fig 8 Percent of cumulative larval emergence of *P. vetulata* at constant temperatures and in an outdoor shelter (2005-2006)

Larval feeding and development tests

Introduction and methods: In 2005, it was observed that 43% of newly hatched larvae transferred onto potted plants with newly developed leaf buds successfully completed development. In contrast, a much higher mortality occurred with larvae transferred in individual Petri dishes with cut stem/leaves. Many larvae died because of an excess of humidity in the Petri dishes, or in contrast, young folded leaf material often dried very quickly when rearing conditions were kept too dry. As a result only few larvae established on cut unfolded leaves. Consequently, larval feeding and development tests in 2006 were carried out with potted plants only. Plants were regularly taken from the garden and put in a heated greenhouse to accelerate bud development when necessary. Batches of eggs kept at 3°C were transferred to 20 °C when leaf buds seemed to be at the right phonological stage. Plants with transferred larvae were covered with a gauze bag and kept in an unheated greenhouse for about one week before being transferred in the garden. Most tests were set-up 24 April – 10 May. The

number of larvae transferred onto each potted plant varied from 3 to 12 according to the number of leaf buds per plant and the number of plants available. All plants were dissected 2-3 weeks after set up and the larvae transferred in ventilated plastic boxes kept in an outdoor shelter for completion of larval development. Fresh food was provided to the larvae twice a week or more when necessary. Pupal weight was measured within five days after pupation.

Results and discussion: Results are summarized in Table 5. Survival to pupal stage was similar on *R. cathartica*, *R. alpina* and *R. alnifolia*. However, *R. alpina* and *R. alnifolia* seem to be providing a slightly less optimal food source for *P. vetulata*. The pupae reared on *R. alnifolia* weighed significantly less than those reared on *R. cathartica* and *R. alpina* (Dunnett's test, $P<0.05$), and the time to pupation was significantly shorter on *R. cathartica* than on *R. alnifolia* and *R. alpina* (Dunnett's test, $P<0.05$). No larval establishment and larval damage was observed on *F. alnus* and *F. caroliniana*.

Table 5 Larval survival and development of *P. vetulata* in no-choice conditions in 2006

Test Plant	# L1 transferred (# potted plants)	# larvae alive 2-3 weeks after set-up (%)	# pupae (%)	Pupal weight (mg) (SD)	# days to pupation ± SD	# adults emerged (% of pupae)
R. cathartica	119 (25)	92 (77.3)	86 (72.3)	0.055 ± 0.012 [a]	32.8 ± 3.7 [a]	73 (61.3)
R. alpina	80 (9)	63 (78.8)	48 (60.0)	0.051 ± 0.010 [a]	37.3 ± 3.9 [b]	46 (57.5)
R. alnifolia	58 (5)	41 (70.7)	40 (69.0)	0.046 ± 0.001 [b]	34.5 ± 2.4 [c]	38 (65.5)
F. alnus	80 (12)	-	-	-	-	-
F. caroliniana	75 (10)	-	-	-	-	-

The high rate of larval establishment on the field host plant confirmed the reliability of the method consisting in transferring newly hatched larvae on potted plants with newly developed leaf buds. Pupal weight and time to pupation on *R. alnifolia* are less than on *R. cathartica* but the former species is likely to be a suitable host for larval development. The fecundity of females reared on *R. alnifolia* will be studied in 2007. Species in genus *Frangula* are not suitable hosts for larval development of *P. vetulata*.

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*, but this result will need to be confirmed in future tests with additional *Rhamnus* species. The tests carried out by Malicky et al. (1970) 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). Likely specific requirements for larval establishment related to plant phenology, stage

of developing leaf bud, leaf shape and toughness, as well as habitat requirements will restrict host acceptance and host suitability to a few species in the genus Rhamnus. The realized host range will be evaluated by 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.

2.2 *Homoptera*

2.2.1 *Trichochermes walkeri (Triozidae)*

Collections and adult emergence

430 adults of *T. walkeri* emerged from a total of 3000 leaf margin curl galls collected 25 July in the Jura Mountains and 9 August at another two collection sites in western Switzerland (Fig 9). Galls collected in the Jura Mountains mature earlier and gave a better adult emergence rate (28%) as compared to the galls collected in western Switzerland (8%). The sex ratio was nearly 1:1 at all collections sites.

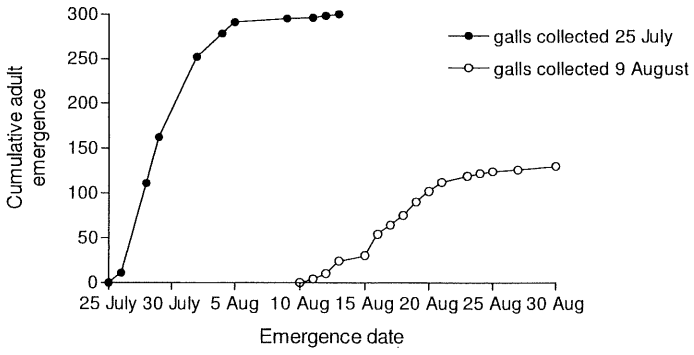

Fig 9 Emergence of adults of *T. walkeri* in 2006

Oviposition and gall development 2005-2006

80 branches of potted *R. cathartica* with 2527 eggs of *T. walkeri* marked with colour threads laid in August-October 2005 were protected from natural oviposition under a large gauze tent in the greenhouse until late November 2005 before being kept outdoors until early July 2006. The number of galled leaves, galls and larvae was counted during the second week of July, i.e. before the larvae leave the galls. A total of 325 galls and 347 mature larvae were obtained from the 2527 eggs laid in 2005 (Table 6). Thus 13.7% of the eggs developed successfully into mature larvae in well developed galls. The number of eggs (31.6 ± 21.9; N=80) and galls per branches (4.1 ± 4.4; N= 80) was much variable. 13 % of attacked leaves had more than one gall, usually no more than two. 59% of all galls contained one larva each but 17% were empty partly because of predatory mites. Two larvae were recorded in 19% of the galls and three larvae in 5%. No galls were found on 20 branches carrying a total of 422 eggs. No galls were recorded on *R. alnifolia.*

Table 6 Oviposition and larval development of *T. walkeri* in 2005-2006

Test plant	No. eggs (2005)	No. of galled leaves (2006)	No. of galls (2006)	No. of larvae (2006)
R. cathartica	2527	285	325	347
R. alnifolia	20	0	0	0
R. alpina	1	0	0	0

Because eggs of *T. walkeri* are too fragile to be removed from their branches, cut branches with were placed in styrofoam boxes to study egg development and for larval transfer. Batches of 50 eggs were kept in a 3°C incubator September 2005 – March 2006 before being transferred to various temperatures (20°C, 15°C, 12.5°C, 10°C and 7.5°C). The material decayed quickly in all storage conditions and no egg hatch was observed. It is strongly suspected that manipulation and storage of eggs on cut plant material is not feasible.

Sequential no-choice oviposition tests

Background: Oviposition by *T. walkeri* started 3-4 weeks after set-up in all tests carried out during previous years. No eggs were laid in 2004 in no-choice oviposition tests carried out with *R. alnifolia*, *R. alpina*, *F. alnus* and *F. caroliniana*. 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 until 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. 71 eggs per female (N=19) on average were laid on *R. cathartica*. Twenty eggs only were laid on *R. alnifolia* by a total of 3 females. However, female longevity was much reduced on all test plants (after a three weeks feeding and preoviposition period on *R. cathartica*) as compared to the field host plant and additional oviposition could not be excluded, had the females survived for a longer period in no-choice conditions (Gassmann et al. 2006). Therefore, sequential no-choice oviposition tests were carried out in 2006.

Materiel and methods: All females and males were exposed to *R. cathartica* for four weeks in groups of 3 pairs each in 20 ventilated plastic cylinders (Ø 11.0 cm, height 15.0 cm) fixed on branches of potted *R. cathartica.* Four test plant species were then 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 plants. The containers were kept outside beneath a suspended tarpauline, protected from rain and sun.
Because no-choice adult feeding and survival tests carried out in 2005 showed that *T. walkeri* usually survives at least 3-4 days on non target hosts, adult survival and oviposition were recorded every 3-4 days and the plant changed sequentially between the test plant and the target plant (*R. cathartica*). The assumption was that females would survive on the test plants before being exposed again to *R. cathartica*, thus allowing them to oviposit on perhaps less preferred but acceptable plant species. Sequential exposure to shorter periods was not feasible because of time constraints. For each test plant, 50% of the replicates started with the test plant and 50% with the target plant.

All sequential no-choice tests were set-up 27 July – 21 August. Branches with eggs were marked with colour threads. All plants used in tests been protected from natural infestation by *T. walkeri* and other herbivores in 2m x 2m x 2m field cages from mid-June until mid November. All plants are overwintering in the Centre's garden and will be put back in 2m x 2m x 2m field cages in April 2007. Gall development will be recorded from mid-May 2007 onwards.

Results and discussion: 60% of males and females survived the four weeks preoviposition period during which 390 eggs have been laid. Results of the sequential no-choice tests are summarized in Table 7. In the *R. cathartica – R. alnifolia* series, a total of 340 eggs and 16 eggs were laid on *R. cathartica* and *R. alnifolia* respectively. In the *R. cathartica – R. alpina* series, 163 and 20 eggs were laid on *R. cathartica* and *R. alpina* respectively. Only 10-12 eggs on average were laid per female on *R. cathartica* in both series. This is much lower than the 71 eggs recorded per female in the no-choice oviposition tests in 2005 (Gassmann et al 2006). However, the number of ovipositing females on *R. cathartica* was relatively similar to what had been observed on average during previous years, i.e. about 50% on average for the two series. The mean total female longevity in the two series was similar to that observed on *R. cathartica* in no-choice tests in 2005. Total longevity of ovipositing females was about 12 days longer than that of sterile females. Almost no eggs were laid in the *R. cathartica – F. alnus* series. All females but one died when first exposed to *F. alnus* for 3-4 days.

The tests carried out in 2006 confirm and refine that of previous tests. Very little oviposition occurred on the non target species in genus *Rhamnus* and no oviposition occurred on *Frangula*. 3-4 days periods of feeding on non target *Rhamnus* species (or the interruption of feeding on the target plant for 3-4 days) do not hamper significantly adult survival but this has a detrimental impact on the reproductive output of the females. Altogether, much fewer eggs were laid on *R. cathartica* but more oviposition attempts on non target hosts have been recorded. In the *R. cathartica – R. alnifolia* and *R. cathartica – R. alpina* series, 25% of all females laid a few eggs on the non target hosts (Table 7).

Perhaps surprisingly, in the *R. cathartica – F. alnus* series, all females except one died during the first exposure to *F. alnus* confirming that *Frangula* is not a suitable host for adult feeding and survival even in the alternate presence of the target host. Thus, if feeding probes occur, it is possible that *F. alnus* is lethal to the adults.
Finally, the possible detrimental impact of repeated manipulations - when transferring adults from one plant to another every 3-4 days - on adult fitness and fertility cannot be excluded.

Discussion: *Trichochermes walkeri* overwinters as eggs which are laid usually on the leaf axil. The manipulation and overwintering of eggs on cut material is not feasible. As shown in 2003, the transfer of first instar larvae or older larvae from young galls onto the leaves of potted plants does not provide conclusive results to assess the physiological host range of *T. walkeri*. Therefore, host specificity tests should rely on oviposition tests and subsequent larval and gall development.
No choice adult feeding tests carried out in 2005 indicate slightly prolonged longevity on non target hosts as compared to the no-food control, suggesting that feeding probes on the non target plants may have occurred. On *F. alnus*, slight feeding is perhaps

even lethal to the adults. Oviposition on non target hosts can be excluded in field situations where *R. cathartica* does not occur since *T. walkeri* females will die within ten days, i.e. much before the oviposition will start, usually 3-4 weeks after adult emergence. In mixed stands, evaluating oviposition behaviour of *T. walkeri* is not straightforward. No-choice tests carried out in 2005 with females, which had been previously exposed to *R. cathartica* for three weeks, resulted in very little oviposition on non target hosts. In these tests also, non target species were not suitable for sustained adult feeding and adult survival for a period extending much after the oviposition had started, thus consistent oviposition on non target plants could not be totally excluded. In 2006, sequential no-choice tests did not reduce adult longevity but resulted in a much lower oviposition rate on *R. cathartica*. This is maybe due to repeated manipulation inherent to the test, to the detrimental impact of slight feeding on insect fitness, or to the interruption of regular feeding on the target plant. Oviposition on non target hosts was low, consisting in 7% of the total number of eggs laid in the test. As in 2005, the number of eggs laid on non target hosts in 2006 will be most probably too low to get significant results as to whether any gall will develop in 2007. Single choice tests will be carried out in 2007 to further evaluate the suitability of *R. alnifolia* for oviposition by *T. walkeri*. New attempts of larval transfer are planned as well.

Table 7 Sequential no-choice oviposition tests with *T. walkeri* in 2006 (after a four weeks feeding and preoviposition period on *R. cathartica*)

	TEST SERIES					
	***R. cathartica* – *R. alnifolia* (N=29)**		***R. cathartica* – *R. alpina* (N=16)**		***R. cathartica* – *F. alnus* (N=11)**	
	R. cathartica	*R. alnifolia*	*R. cathartica*	*R. alpina*	*R. cathartica*	*F. alnus*
Total no. of ♀ / days	290	302	161	143	40	59
Total no. of eggs	340	16	163	20	5	0
Mean no. eggs /♀ (SD)	11.7 (13.1)	0.6 (1.4)	10.2 (22.4)	1.3 (2.7)	0.5 (1.2)	0
Number of ovipositing females (% of total no. females))	17 (59)	7 (24)	6 (38)	4 (25)	2 (18)	0
Mean female longevity in the series (SD)	20.4 (12.7)		19.8 (13.5)		9.0 (4.4)	
Total female longevity (SD)	50.0 (12.1)		47.4 (13.0)		35.4 (7.1)	

2.3 Flower and fruit feeding insects

2.3.1 *Wachtiella krumbholzi (Dipt.; Cecidomyiidae)*

In 2004, preliminary collections of flowers and fruits of *R. cathartica* were carried out in Austria, Germany, Switzerland and Serbia and an important population of the midge *Wachtiella krumbholzi* was discovered in the fruits of *R. cathartica* in northeastern Serbia. 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. Gall swelling is not visible on damaged fruits. 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.

On 11 July 2005, over 3000 fruits of *R. catharica* apparently attacked by *W. krumbholzi* were collected in Serbia and sent to Switzerland. 280 larvae of presumably *W. krumbholzi* from *R. cathartica* were transferred into Petri dishes with a mixture of sieved soil and vermiculite. The soil was sieved in early September. Seven larvae alive and 123 larval cocoons were recovered (i.e. 44% of the larvae successfully built a larval cocoon for overwintwering). 60% of this material was kept in a 3°C incubator for overwintering. The remaining 40% was kept continuously in an outdoor shelter.

Emergence of gall midge adults kept in outdoor shelter started on 19 May 2006, i.e. at the same time as in 2005 (Gassmann et al. 2006), and was completed on May 28 (Fig 10). Percent of emergence was low (14%) as in the previous year. On 20 June 2006, gall midge cocoons kept in the 3°C incubator were moved into a 20 °C incubator. Adult emergence started two weeks later and was completed within six days. 10 males and 27 females were obtained (i.e. 46%) and were put in 80% alcohol for confirmation of their identification.

Eight parasitoids emerged from the same batches of larval cocoons. Keeping larval cocoons at 3°C and moving them to 20°C seem to be a reliable method to synchronize adult emergence with plant phenology. The high larval mortality in Petri dishes in the outdoor shelter seems to be due partly to drought. In 2006, larvae of *W. krumbholzi* are being stored in 9x9x9 cm ventilated plastic boxes half filled with a mixture of sterilized sieved soil and vermiculite.

In early July 2006, over 5000 fruits of *R. cathartica* apparently attacked by *W. krumbholzi* were collected in Serbia and sent to Switzerland on 14 July. Some 800 larvae have been reared from this material and transferred to Petri dishes filled with a mixture of sterilized sieved soil and vermiculite. In late August, the soil was sieved and 685 larval cocoons recovered (86%). Batches of 50 larval cocoons have been placed in 9x9x9 cm ventilated plastic boxes half filled with a mixture of sterilized sieved soil and vermiculite. 400 larval cocoons are being at in a 3°C incubator for overwintering and the remaining 285 ones in an outdoor shelter.

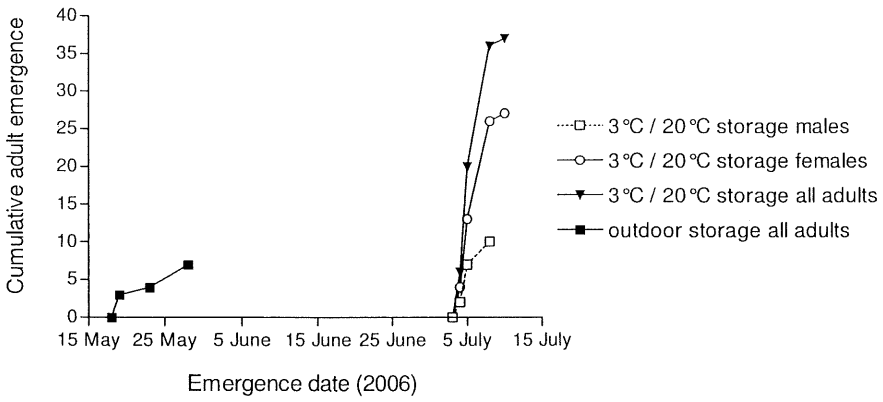

Fig 10 Emergence of *W. krumbholzi* adults under different conditions (2005-2006)

2.3.2 Others

Five larvae of an unknown lepidoptera species have been found in the fruits of *R. cathartica* in Switzerland in 2005. These larvae have been kept in an outdoor shelter for overwintering. No adult but one parasitoid emerged in 2006. Two adults of an unyet identified Lepidoptera species have emerged form the fruits collected in Serbia in early July 2006.

3 General discussion

Work carried out in 2006 has highlighted the difficulty to rear or to test some of the potential biological control agents for buckthorns. In spite of this, additional data with the shoot-tip boring moth *S. janiszewskae* confirm that both *R. cathartica* and *F. alnus* are suitable hosts for this species. 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 we suggest giving *S. janizewskae* a low priority.

Work in 2006 has also highlighted the difficulty to demonstrate with certainty the absence of consistence oviposition by *T. walkeri* on non target plants in no-choice conditions. However, the probability for high oviposition rate and gall and larval development on non target *Rhamnus* species is extremely low. Oviposition choice tests should confirm the high specificity of *T. walkeri*.

Work carried out in 2006 has also highlighted progress with the rearing and testing of another potential biological control agent. Transfer of newly hatched larvae of *P. vetulata* on potted plants at the time of leaf bud expansion has produced remarkable results. Species in genus *Rhamnus* appear to be suitable hosts for larval development of this moth although variability in food quality may result in lower pupal weight or longer time for development to pupal stage. *Philereme vetulata* is restricted to *R. cathartica* in its European native range. The feasibility of oviposition choice tests will be studied in 2007.

Finally progress has been made in mass collection, and rearing to the adult stage of the seed feeding midge *W. krumbholzi*. Oviposition and larval development will be studied in 2007. Growing plants to fruiting stage and the synchronisation between adult emergence and plant phenology will be the main challenges in future tests.

Acknowledgments

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