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# Report 2008-2009

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## Summary

Following a reassessment of the potential for biological control of *Rhamnus cathartica* and *Frangula alnus*, work carried out in 2008–09 concentrated on the biological control of the former species using the leaf-feeding moth *Philereme vetulata*, the leaf-margin gall psyllid *Trichochermes walkeri* and the seed-feeding midge *Wachtiella krumbholzi*.

Fecundity of *P. vetulata* in 2008–09 was much lower than in previous years. A test to see whether the fecundity of adults reared on *R. alnifolia* from North America was lower than that of adults reared on *R. cathartica* therefore yielded inconclusive results. Larval feeding and development tests were conducted with eight plant species, six of which are native to North America. Complete larval development was recorded on *R. cathartica, R. alnifolia* and *R. erythroxylon*, a species native from China. As in previous years, we were unable to obtain oviposition under field cage conditions. This method therefore appears unsuitable for testing the egg-laying behaviour of *P. vetulata.*

In a no-choice test, *T. walkeri* laid the same number of eggs on *R. alaternus* from Europe and on *R. cathartica*. In contrast, only a few eggs were laid on *R. prinoides* from South Africa in a similar test. A single-choice test confirmed that *R. alnifolia* may be used for oviposition. In summary, a few species in the genus *Rhamnus* (*R. alnifolia*, *R. alaternus* and to a lesser extent *R. prinoides*) appear suitable hosts for oviposition by *T. walkeri* in no-choice and/or choice conditions but neither gall nor larval development were recorded on any of the non-target *Rhamnus* species. The North American species *R. ilicifolia* does not seem to support adult survival.

The phytoplasma '*Candidatus* Phytoplasma rhamni' (16SrX-E group) has been detected in two populations of *T. walkeri* in Switzerland. This is the first record of '*Candidatus* Phytoplasma rhamni' in Switzerland and *T. walkeri* is also the first insect host record for this phytoplasma.

The cecidomyiid *W. krumbholzi* is much more common in Europe than previously indicated in the literature since it has been recorded on *R. cathartica* at all sites sampled. Successful oviposition was obtained in the very young developing fruits of *R. cathartica*. In contrast, no oviposition occurred in the well-developed, one-month-older fruits. No midge larvae were found in the fruits of *F. alnus* at two sites where *R. cathartica* and *F. alnus* co-occur and no oviposition was recorded on the latter species in confinement.
A review of successes and failures in biological control of trees and shrubs included in this report shows that beetles, sap suckers, gall wasps and rust fungi are the most successful taxonomic groups for these target plants. In addition, any agents directly or indirectly reducing seed output are expected to facilitate management of the target tree or shrub. Based on this review, further recommendations are made for biological control of *R. cathartica*.

# 1 Introduction

*Rhamnus cathartica* (common buckthorn) and *Frangula alnus* (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 a landscape plant and used as a shelter-belt tree because of its winter hardiness and its ability to grow in multiple soil types and habitats (Archibold et al. 1997). It has spread extensively and is currently found in most Canadian provinces (Nova Scotia to Saskatchewan) and 27 US states predominantly in the north-central and north-eastern portion of the country (Gale 2001; USDA/NRCS 2001). It is one of the most invasive woody perennials in natural ecosystems and has negative impacts on agriculture. Common buckthorn is a suitable overwintering host for soybean aphid, *Aphis glycines*, and the spring host for oat crown rust, *Puccinia coronata* (see Yoder et al. 2008 for references).

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

Research to develop biological control for buckthorns was initiated in 1964 and preliminary screening tests were conducted in 1966–1967 (Malicky et al. 1970). A new programme was started in 2001 and has taken into consideration increasing concerns over potential non-target impacts of biological control agents and greater demands for high levels of specificity (Louda et al. 1997; Pemberton 2000).

In 2008, we presented a reassessment of the potential for biological control of *R. cathartica* and *F. alnus* by target species and by the arthropod-feeding guilds (Gassmann et al. 2008a). It was based on work conducted in Europe in 2002–2007 on selected potential biological control agents (Gassmann et al. 2006, 2007). The assumption was that candidate biological control agents should be monospecific to *R. cathartica* or *F. alnus* or their host ranges should be restricted to a few species in either the genus *Rhamnus* or the genus *Frangula*. Following discussions with our counterparts in the USA, it was decided to focus on the biological control of *R. cathartica* and on the leaf-feeding moth *Philereme vetulata*, the leaf-margin gall psyllid *Trichochermes walkeri*, and the seed-feeding midge *Wachtiella krumbholzi*. This report presents work carried out in 2008–09.

The project is presenting a range of difficulties and its feasibility needs to be readdressed. We have reviewed 25 past or current programmes on biological control of invasive trees and shrubs in order (1) to assess the feasibility and likelihood of success of such programmes, and (2) to assess which groups of agents appear to work better than others. This review is presented in section 6.

# 2 *Philereme vetulata* (Lep., Geometridae)

The leaf-feeding moth *P. vetulata* is exclusively associated with *R. cathartica* in Europe with the exception of one record on *R. alpina* (Malicky et al. 1965). *Philereme vetulata* has one generation per year and overwinters in the egg stage on the bark of its host plant. Larvae feed within folded leaves.

Larval feeding and development tests on potted plants carried out in the past few years indicated that survival to pupal and adult stages was similar on *R. cathartica* EU (= of European origin), *R. alpina* EU and the native North American species *R. alnifolia* (= NA). However, *R. alpina* and *R. alnifolia* NA seem to be slightly less optimal food sources for *P. vetulata* (Gassmann et al. 2006). The pupae reared on *R. alnifolia* NA weighed significantly less than those reared on *R. cathartica* and *R. alpina*, and the time to pupation was significantly shorter on *R. cathartica* than on *R. alnifolia* NA and *R. alpina*. No larval establishment or damage was observed on *Frangula alnus* EU and *F. caroliniana* NA. No oviposition on the field host plant was obtained in confinement.

In 2008–09, larval development and oviposition tests were carried out on a few *Rhamnus* species, three additional species in the family Rhamnaceae, and species in the families Elaeagnaceae and Vitaceae. Tests concentrated on species native to North America.

## 2.1 Biology and rearing

### 2.1.1 Collections and adult emergence

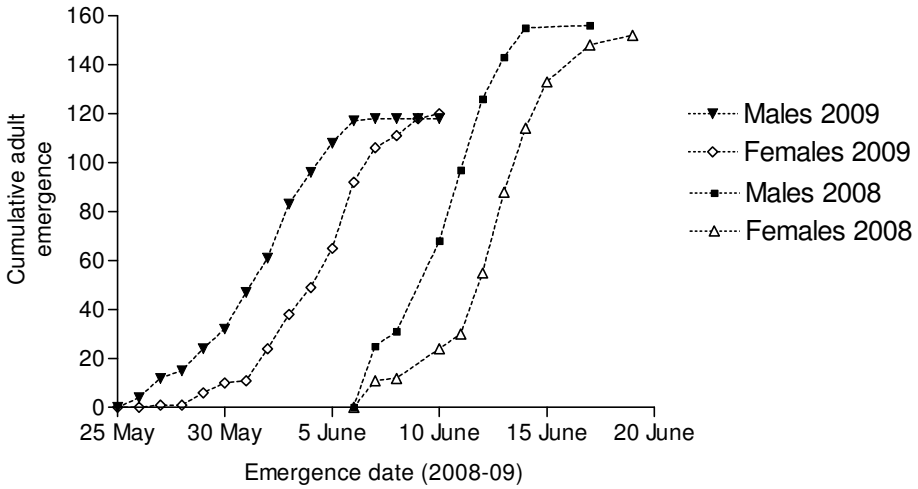


**Figure 1** Emergence of *Philereme vetulata* adults reared from field-collected larvae in 2008–09

Following field collection, larvae were reared on leaves of *R. cathartica* in ventilated plastic boxes lined with moist paper to keep leaves fresh. Boxes were stored in an outdoor shelter. Pupae were kept in ventilated plastic cups half-filled with vermiculite to allow adults to emerge. In 2008, a total of 156 males and 152 females (84%) emerged from the 368 larvae collected on 8 and 9 May 2008 in Germany and Switzerland (Figure 1). In 2009, a total of

118 males and 120 females (74%) were obtained from 320 larvae collected at the same sites on 23 April and 5 May.

### 2.1.2 Rearing and fecundity tests

We tried several rearing methods, varying the number of pairs released:

1. Between 11 May and early July 2008, 64 pairs of *P. vetulata* were kept in groups of two to six pairs (mostly five pairs) in 15 cardboard cylinders (11 × 25 cm) in an outdoor shelter. A total of only 582 eggs were obtained of which 63.9% (372) were fertile.

2. Between 8 May and early July 2008, 12 pairs reared from larvae collected in Germany in spring 2008 were kept individually in cardboard cylinders (11 × 25 cm) in an outdoor shelter. A total of only 91 eggs were obtained of which 81% (74) were fertile, and of these, 59 were obtained from the same female.

3. Between 26 May and early July 2009, 92 pairs were kept in groups of two pairs in ventilated plastic cylinders (11 x 15 cm) in an outdoor shelter. A total of 1,256 eggs were obtained of which about 78% (980) were fertile.

### 2.1.3 Maternal impact

We initiated this trial to test whether the fecundity of adults reared on *R. alnifolia* NA was lower than that of adults reared on *R. cathartica*.

In 2007, five pairs of *P. vetulata* adults which had been reared throughout the larval stages on *R. alnifolia* NA laid a total of 220 eggs of which 68% (150 eggs) were fertile. Eggs were kept in an outdoor shelter and then transferred to a 1°C incubator on 4 January 2008 to help synchronize egg hatch with plant availability. On 5 May, eggs were transferred to a 20°C incubator. Larvae hatched within three days. Successful hatch for eggs laid by *P. vetulata* reared on *R. alnifolia* was 73%, compared to 95% for eggs from *P. vetulata* reared on *R. cathartica*.

Thirty larvae each were reared on potted *R. alnifolia* NA and *R. cathartica* (Table 1)**.** In total, 12 males and five females were obtained from *R. alnifolia* (56.7% successful development) and 13 males and ten females were reared from *R. cathartica* (76.7%) (Table 1). Only four infertile eggs were obtained from five pairs of the '*R. alnifolia* strain'. Seven fertile eggs and 49 infertile eggs were obtained from ten pairs of the '*R. cathartica* strain'. Dead females were in too poor a condition to be dissected for eggs in ovaries to be counted.

### 2.1.4 Discussion

Mass and individual rearing of *P. vetulata* was not successful in 2008 and only slightly improved in 2009. Such low fecundity and fertility had never been observed in previous years. At this stage, it is difficult to explain why so few females mated and laid eggs over the past two seasons. Regarding mass rearing, it is possible that disturbance arose because too many adults were put in the same container. But this suggestion does not explain the low fecundity recorded from individual rearing in 2008–09; average fecundity in previous years was 62–88 eggs when one or two pairs were reared in similar conditions. Because of the very low fecundity recorded in 2008–09, no conclusions could be drawn from the maternal impact experiment.

A total of 412 eggs were kept in an outdoor shelter in preparation for larval feeding and development tests in 2009. In 2009, 980 eggs are being kept for potential work in 2010.

## 2.2 Host specificity

### 2.2.1 Larval feeding and development tests

**Methods.** In early spring, *P. vetulata* larvae hatching from eggs obtained in the previous year were transferred onto potted plants of *Rhamnus cathartica* and eight test species, including six native to North America.

**Results.** Complete larval development to the pupal stage was recorded on *R. cathartica* (Table 1). Percent successful development was as high on *R. alnifolia* NA and *R. erythroxylon* from China as on the target plant. Larval feeding damage was negligible on *R. alaternus* EU. No feeding and no larval development were recorded on any of the other species.

**Table 1** Results of no-choice larval survival and development tests with *Philereme vetulata* in 2008–09

| Test plant[a] | No. of L1 transferred (no. of potted plants) | Percent successful development (pupae, adults) |
|---|---|---|
| **Rhamnaceae** | | |
| *Rhamnus cathartica* | 232 (24) | 40.5 |
| *R. cathartica* | 30 (3)[b] | 76.7 |
| *R. alnifolia* NA | 30 (3)[b] | 56.7 |
| *R. erythroxylon* | 88 (9) | 48.9 |
| *R. alaternus* | 178 (15) | 0 |
| *Frangula caroliniana* NA | 40 (3) | 0 |
| *Hovenia dulcis* NA | 32 (4) | 0 |
| **Elaeagnaceae** | | |
| *Elaeagnus commutata* NA | 40 (4) | 0 |
| *Hippophae rhamnoides* NA | 66 (5) | 0 |
| **Vitaceae** | | |
| *Parthenocissus quinquefolia* NA | 31 (2) | 0 |

[a], NA: plant species native to North America; [b], first instar (L1) larvae from an F1 generation reared on *R. alnifolia* in 2007 (see section 2.1.3).

### 2.2.2 Multiple-choice field cage oviposition tests

**Methods.** In 2007, no oviposition was recorded in 2 × 2 × 1.6 m field cages in which twelve pairs of *P. vetulata* had been released. In 2008 we reassessed the feasibility of conducting cage oviposition tests in three similar field cages, releasing 15 pairs plus five females into each cage (Plate 1). Each cage contained two potted *R. cathartica*, one potted *R. alpina* and one potted *R. alnifolia* NA (about 50–80 cm high) embedded in sawdust. All cages were protected from excess rain and sun by green gauze covers. Each cage was provided with branches from *Fagus*, *Quercus* or *Corylus* trees as well as with

cardboard plates to allow the moths to hide. In 2009, we conducted one last trial exposing two potted *R. cathartica* and three potted *R. alnifolia* NA of about the same size (30–50 cm high) in a cage containing two bushes (*Salix* and *Corylus*) and several herbaceous species, thus creating a more natural environment (Plate 1). Twenty pairs of six- to eight-day-old moths and 12 newly emerged pairs were released into the cage. The tests were established in early June and all plants were removed from cages two months later and searched for eggs.

**Results.** No eggs were found on any part of the plants. It is concluded that oviposition tests in confinement can definitively be discarded as a method of evaluating the oviposition behaviour of *P. vetulata*.

**Plate 1** Field cage oviposition test in 2008 (left) and inside the 'natural' field cage in 2009 (right)

### 2.2.3 No-choice open-field oviposition test

On 19 June 2008, 25 female and 20 male *P. vetulata* were released on the margin of an orchard in which five large, potted *R. cathartica* had been placed in order to try and assess the oviposition behaviour of the moth in open-field conditions. No naturally growing *R. cathartica* was visible for a distance of at least 300 m from the release point. All plants were removed from the field plot on 18 July and searched for eggs.

No eggs were found on any part of the *R. cathartica* plants although one mating pair was observed on a trunk base just after release (Plate 2).

**Plate 2** Potted *Rhamnus cathartica* plants at the margin of an orchard (left) and a mating pair of *Philereme vetulata* just after release (right)

### 2.3 Conclusions and outlook

The larval feeding and development tests with *P. vetulata* indicated that larval development to the adult stage is restricted to a few *Rhamnus* species. Oviposition tests carried out in 2008–09 confirm the previous finding that egg laying does not occur in confined conditions. Eggs were also not found on *R. cathartica* in the open-field oviposition test established in the vicinity of the CABI Europe – Switzerland (E-CH) Centre. This was probably influenced by the small size of our potted *R. cathartica* and test plant species, making results from any open-field test unreliable. At this point it appears impossible to study the oviposition behaviour of *P. vetulata*.

Currently, host-specificity studies with *P. vetulata* rely on larval feeding and development tests. It is likely that specific requirements for larval establishment related to plant phenology, stage of the developing leaf bud, and leaf shape and toughness, as well as habitat requirements, will restrict host acceptance and host suitability to a few species in the genus *Rhamnus*. Results obtained so far suggest that larvae will not complete development on small tough or thick evergreen leaves such as those of *R. alaternus*. Therefore, the native North American *Rhamnus* species *R. crocea*, *R. ilicifolia*, *R. serrata* and *R. smithii* are unlikely to be suitable for development of *P. vetulata* larvae through to the adult stage. Critical native North American non-target species are *R. alnifolia* and *R. lanceolata* because of their leaf shapes and smoothness and their geographical distributions which partially overlap that of *R. carthartica*.

## 3 *Trichochermes walkeri* (Hem., Triozidae)

The leaf-margin curl galler *T. walkeri* is known only from *R. cathartica* in Europe. It is also one of the most common insect species on *R. cathartica* and certainly one of the most conspicuous. The galls of *T. walkeri* seem to be aggregated on certain trees, while within a tree they appear to have a more random distribution. The species has one generation per year and overwinters in the egg stage. Females lay small orange eggs during late summer on leaf buds. The nymphs hatch in spring from overwintered eggs. First-instar nymphs migrate to the leaves, feed, and induce rolling of the leaf margin. Egg laying by *T. walkeri* begins about 3–4 weeks after adult emergence and lasts from late August until mid October. Oviposition tests were continued in 2008–09.

### 3.1 Collections and rearing

Between 28 July and 6 August 2008, 3,600 leaf galls of *T. walkeri* were collected at three sites in western Switzerland. Between 31 July and 18 August, 60 females and 84 males emerged from this material. The last adult emerged on 3 September 2008. A late collection of 550 galls carried out on 19 August 2008 provided only three additional females and ten males. These adults were used in oviposition tests.

On 7 July 2009, a first small collection of leaf galls of *T. walkeri* was made in western Switzerland to assess larval development and larval size. Between 27

July and 6 August 2009, 1,700 leaf galls were collected at the same three sites in western Switzerland as in 2008. Between 28 July and 26 August, 106 females and 150 males emerged from this material. No adults emerged from a late collection of 100 galls carried out on 19 August 2009.

Inspection of the 7 July collection indicated that 23% of the larvae had reached the third larval stage and 77% the fourth larval stage (Figure 2). One month later, one-third of the larvae were in the fourth larval stage and two-thirds in the fifth and last larval stage.

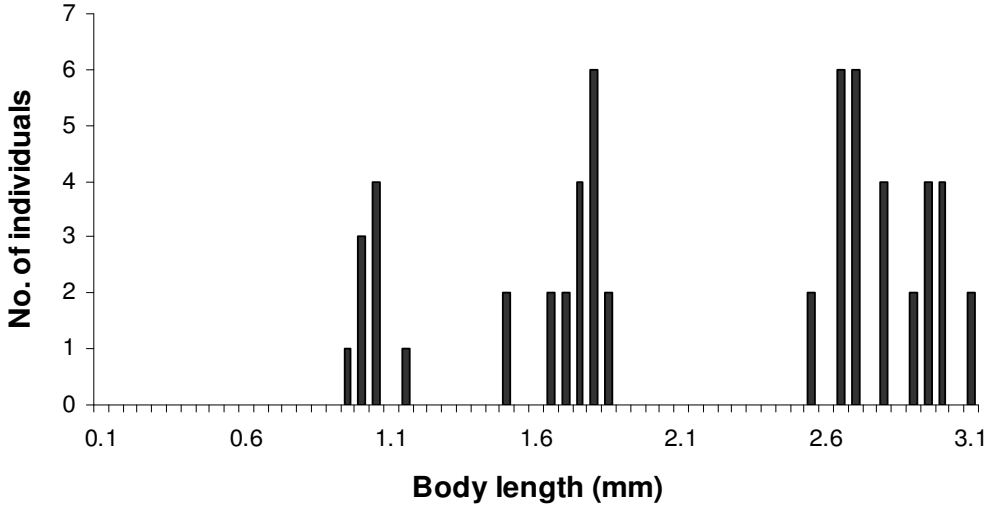


**Figure 2** Body lengths of the third, fourth and fifth larval stages of *Trichochermes walkeri*

## 3.2 Host specificity

### 3.2.1 Sequential no-choice tests in 2008–09

In 2004, no eggs were laid in no-choice oviposition tests carried out with *R. alnifolia* NA, *R. alpina*, *F. alnus* and *F. caroliniana* NA. These preliminary results also indicated that none of the test plants was suitable for adult feeding and that adults did not survive until oviposition. Therefore, in 2005, no-choice oviposition tests were carried out with females which had previously been exposed to *R. cathartica* for three weeks. Even under these conditions, female longevity was much reduced on all test plants compared to the field host, *R. cathartica*. In 2006, we tested *R. alnifolia* NA and *R. alpina* in sequential no-choice conditions (Gassmann et al. 2006), in 2008 *R. alaternus* and the South African species *R. prinoides*, and in 2009 *R. ilicifolia* NA.

**Methods.** Females and males were first exposed to *R. cathartica* in pre-oviposition boxes for three weeks in groups of 2–5 pairs in 44 (21 in 2008 and 23 in 2009) ventilated plastic cylinders (diameter 11.0 cm, height 15.0 cm) fixed on branches of potted *R. cathartica.* After this period, i.e. at the start of the oviposition period, each pair of *T. walkeri* was transferred into a small, ventilated plastic cup (diameter 7.0 cm, height 8.5 cm), which was fixed on a branch of a potted test or control plant. Plants were kept outside underneath a suspended tarpaulin to protect them from rain and sun.

Insects were sequentially alternated between the test plant and the target plant, *R. cathartica,* in 'test series'. The assumption was that females would be able to survive the period on the test plants, and would then 'recover' on *R. cathartica*, thus allowing them to oviposit on perhaps less preferred but acceptable plant species. The test series were established in late August – early September and lasted for 3–4 weeks. Because no-choice adult feeding and survival tests carried out during previous years showed that *T. walkeri* usually survives at least 3–4 days on non-target hosts, adult survival and oviposition were recorded every four days.

Branches with eggs were marked with coloured threads. All plants used in tests were protected from natural infestation by *T. walkeri* and other herbivores under a large gauze tent in a greenhouse from July until late October. All attacked plants were overwintered in the same greenhouse and gall and larval development assessed the following spring.

**Results.** In 2008, 60% of males and females survived the three-week pre-oviposition period during which two late-emerged females laid 101 eggs. In the sequential no-choice oviposition tests, *T. walkeri* laid a similar number of eggs on *R. alaternus* and *R. cathartica* (Table 2) although the leaf buds of *R. alaternus* are smaller and tougher than those of *R. cathartica*. The average fecundity and female longevity were similar to those observed for *R. cathartica* in no-choice tests in previous years. Only a few eggs were laid by *T. walkeri* in the five *R. cathartica – R. prinoides* test series suggesting that *R. prinoides* is less suitable than *R. alaternus* for supporting normal adult longevity and egg laying.

In 2009, 90 females and 121 males were put into pre-oviposition boxes. Female mortality was high (87%) and only 12 females could be used in the oviposition trial. Ten sequential no-choice oviposition tests were carried with *R. ilicifolia* NA, starting with six replicates of the control *R. cathartica* and four of *R. ilicifolia* NA. Only 38 eggs were laid on *R. cathartica* and female longevity was much reduced. All eggs were laid by the female with highest longevity (16 days). Only three females survived a four-day period on *R. ilicifolia* NA which appears to be a lethal plant for this insect.

### 3.2.2 Single-choice tests in 2008–09

In 2005 and 2006, little oviposition occurred on *R. alnifolia* NA in no-choice tests, and no gall and larval development was recorded the following year. To check whether this test species was attacked in the presence of the target weed, single-choice tests were conducted. In 2007, single-choice oviposition tests were evaluated in three 2 × 2 × 1.6 m field cages, but no eggs were found in any of these tests.

**Methods.** In 2008, single-choice oviposition tests were carried out in five 40 × 40 × 70 cm cages (= replicates) which were kept outdoors underneath a suspended tarpaulin to give protection from rain and sun. Each cage contained one potted *R. cathartica* and one potted *R. alnifolia* NA. Between 21 August and 9 September, three newly emerged pairs of *T. walkeri* were released into each cage. On 3 November, all plants were checked for eggs.

In 2009, one *T. walkeri* pair was added to each of two small cages, each of which contained one potted *R. cathartica* and one potted *R. ilicifolia* NA. The test was set up on 15 September and completed on 6 October.

**Results.** In 2008, a total of 557 eggs were recorded from four replicates with *R. cathartica* (mean = 111.4 ± 102.9; *n*=5) and 24 eggs from three replicates with *R. alnifolia* NA (mean = 4.8 ± 5.2; *n*=5). Thus egg laying on *R. alnifolia* NA represented 4.1% of the total number of eggs laid in the test. Over 90% of the eggs were laid on the bark of branches and the trunk of *R. alnifolia* NA. In contrast, on *R. cathartica*, over 60% of the eggs were laid on leaf bud axils, thus facilitating gall development in spring.

In 2009, two eggs only were recorded from one replicate with *R. cathartica* and 49 eggs from the second. No eggs were recorded on *R. ilicifolia* NA.

**Table 2** Sequential no-choice oviposition tests with *Trichochermes walkeri* in 2008–09 (after a three-week feeding and pre-oviposition period on *Rhamnus cathartica*)

| | **TEST SERIES (2008)** | | | |
|---|---|---|---|---|
| | ***R. cathartica – R. alaternus* (*n=5*)** | | ***R. cathartica – R. prinoides* (*n=5*)** | |
| | ***R. cathartica*** | ***R. alaternus*** | ***R. cathartica*** | ***R. prinoides*** |
| Total no. of ♀ days | 107 | 85 | 67 | 41 |
| Total no. of eggs laid | 245 | 302 | 53 | 7 |
| Mean no. of eggs/♀ (SD) | 49.0 (28.6) | 60.4 (40.1) | 10.6 (22.6) | 1.4 (2.6) |
| No. of ovipositing females (% of total no. of females) | 4 (80) | 4 (80) | 2 (40) | 2 (40) |
| Mean female longevity in the test series (SD) | 21.4 (8.3) | | 13.4 (3.6) | |
| Mean total female longevity (SD) | 43.4 (8.3) | | 35.4 (3.6) | |
| | **TEST SERIES (2009)** | | | |
| | ***R. cathartica – R. ilicifolia (n=6)*** | | ***R. ilicifolia – R. cathartica (n=4)*** | |
| | ***R. cathartica*** | ***R. ilicifolia*** | ***R. ilicifolia*** | ***R. cathartica*** |
| Total no. of ♀ days | 30 | 16 | 10 | 2 |
| Total no. of eggs laid[a] | 38 | 0 | 0 | 0 |
| Mean female longevity in the test series (SD) | 7.7 ± 4.8 | | 3.0 ± 2.0 | |
| Mean total female longevity (SD) | 27.7 ± 4.8 | | 23.0 ± 2.0 | |

[a], all eggs laid by one female.

### 3.2.3 Leaf gall development 2008–09

Potted plants, onto which eggs of *T. walkeri* had been laid in autumn 2008 in no-choice and single-choice oviposition tests, were protected from natural oviposition under a large gauze tent in a greenhouse until the end of November 2008, and then kept outdoors until late May 2009. A total of 179 galls and 261 larvae (mostly second and third larval stages) were obtained from 855 eggs laid on *R. cathartica* in 2008 (Table 3). Thus, 30.5% of the

eggs developed successfully to the early larval stages. Sixty-five percent of attacked leaves had one gall and 34% had two galls. One leaf carried three galls.

About 54% of the galls contained one larva, 37% two larvae and 9% three or four larvae. About 70% of all potted plants and branches with eggs developed leaf galls. Ten percent of all branches did not develop leaf galls because they were heavily infested by aphids.

No galls and larval development occurred on *R. alaternus*, *R. alnifolia* NA and *R. prinoides*.

**Table 3** Results of gall and larval development with *Trichochermes walkeri* in 2008–09

| Test plant[a] | No. of eggs (2008) | No. of galled leaves (2009) | No. of galls (2009) | No. of larvae (2009) |
|---|---|---|---|---|
| ***Rhamnus cathartica*** | 855 | 133 | 179 | 261 |
| ***R. alaternus*** | 302 | 0 | 0 | 0 |
| ***R. alnifolia* NA** | 24 | 0 | 0 | 0 |
| ***R. prinoides*** | 7 | 0 | 0 | 0 |

[a], NA: plant species native to North America.

## 3.3 Detection of '*Candidatus* Phytoplasma rhamni'

### 3.3.1 Background

Plant-pathogenic phytoplasma are non-culturable, insect-transmitted wall-less prokaryotes of the class *Mollicutes* that are associated with diseases in several hundred plant species, including many woody shrubs or small trees (Marcone et al. 2004; Weintraub and Beanland 2006). Based on 16S rRNA gene sequences, Lee et al. (1998) describe the 12 main groups of phytoplasmas (designated 16Sr I-XII); their subgroups are designated with a letter suffix.

A lethal witches'-broom disease of *R. cathartica* was observed for the first time in south-western Germany in the 1990's (Mäurer and Seemüller 1996). This disease, known as buckthorn witches'-broom (BWB) phytoplasma, belongs to the 16SrX-E group (i.e, 16Sr ten group, E subgroup). The BWB phytoplasma is phylogenetically more closely related to phytoplasmas of the apple proliferation (AP) group (16SrX) than to other phytoplasma subclades (see Marcone et al. 2004 for references). The 16SrX group of phytoplasmas includes for example the apple proliferation phytoplasma (16SrX-A) and the pear decline phytoplasma (16SrX-C).

For uncultured phytoplasmas, a novel putative species may be described when its 16S rRNA gene sequence (>1200 bp) has ≤ 97.5% similarity to any previously described '*Candidatus* Phytoplasma' species (IRPCM, 2004). The BWB phytoplasma share < 97.5% 16S rDNA sequence similarity with other known phytoplasmas, including the AP group phytoplasmas. This is the reason why Marcone et al. (2004) proposed the BWB phytoplasma as a novel '*Candidatus* Phytoplasma' species, i.e. '*Candidatus* Phytoplasma rhamni'. According to these authors, the BWB phytoplasma has clearly distinct

molecular and biological properties, especially a different and unique field host plant, *R. cathartica*.

According to Mäurer and Seemüller (1996), symptoms are brush-like witches' brooms which arise from the stems or major branches. These witches' brooms develop from young, premature shoots that start to grow in January. The leaves of diseased plants were often distorted and the vigour of such plants steadily decreased. Severely affected trees and shrubs did not bear fruits and declined.

The single most successful insect vectors of phytoplasma are the Hemiptera. Phytoplasmas are phloem-limited; therefore, only phloem-feeding insects can potentially acquire and transmit the pathogen. However, within the groups of phloem-feeding insects only a small number, primarily in a very few taxonomic groups, have been confirmed as vectors of phytoplasmas (Weintraub and Beanland 2006). The main group of known vectors is the Cicadeliidae. Another seven families including 15 species are also known as vectors of phytoplasmas (Weintraub and Beanland 2006).

Two genera of Psyllidae are vectors. Six species of *Cacopsylla* transmit AP group (16SrX) phytoplasmas on apple, stonefruit and pear trees. Another genus, *Bactericera*, has one vector species, *B. trigonica*, which transmits a stolbur (16SrXII) phytoplasma to carrots. *Trichochermes walkeri* was not recorded as a potential vector of phytoplasma.

### 3.3.2 Material and Methods

Six pairs and four pairs of *T. walkeri* were reared from galls collected in early August 2009 at two well separated sites in Switzerland, respectively, and kept in 95% ethanol for further studies. Phytoplasma detection and characterization was carried out by PCR amplification of 16S ribosomal RNA gene with universal and group specific primers. Amplification was performed in nested PCR with P1/P7 (Deng and Hiruki, 1991; Smart *et al*., 1996) followed by F2n/R2 universal primer pair (Gundersen and Lee, 1996) or R16(X)F1/R1 primers specific for amplification of 16SrX group phytoplasmas (Lee et al., 1995). To obtain longer fragments for sequencing, 16S rRNA amplicons were obtained in nested PCR assay with the universal primers P1A/P7A with reaction conditions according to Lee *et al*. (2004).

### 3.3.3 Results

The presence of the p*hytoplasma* named '*Candidatus* Phytoplasma rhamni' (16SrX-E group) was detected in all four insect pulls analyzed from the locality along lake Neuchatel while from the second locality, in the Jura hills, only one out of four analyzed pulls was positive. One isolate from each locality was sequenced and an approximately 1500bp long sequence was obtained. Sequences were identical among themselves. Comparison with available sequences from the NCBI database (using BLAST analyses) confirmed the presence of '*Candidatus* Phytoplasma rhamni' in *T. walkeri* samples.

This finding is a first record of '*Candidatus* Phytoplasma rhamni' in Switzerland, and the first record of a phytoplasma detected in *T. walkeri*.

### 3.4 Conclusions and outlook

Unlike results with other non-target *Rhamnus* species (i.e. *R. alnifolia* NA, *R. alpina* and *R. prinoides*), consistent egg laying occurred in 2008 on *R. alaternus* under sequential no-choice conditions. However, no gall development was recorded on this species in 2009 and there are no records of *T. walkeri* galls on *R. alaternus* in Europe, confirming that this plant is not a suitable host for larval development of *T. walkeri*. The single-choice tests confirmed that *R. alnifolia* NA is a much less suitable host than the target weed for oviposition. As in previous years, no gall development was recorded on *R. alnifolia* NA the following spring. Female longevity was about 20 days in the tests with *R. alnifolia* NA, *R. alaternus* and *R. alpina*, and much reduced in those with *R. prinoides* and *R. ilicifolia* NA (see also Gassmann et al. 2007). Adult longevity was also much reduced on *Frangula* spp.

*Trichochermes walkeri* overwinters as eggs, which are usually laid on the leaf buds. The difficulties encountered in the manipulation and overwintering of eggs on cut material and the transfer of first-instar or older larvae from young galls onto the leaves of potted plants means this is not a suitable method for assessing the physiological host range of *T. walkeri*. Therefore, host-specificity tests need to rely on oviposition tests and subsequent larval and gall development. Oviposition tests carried out so far indicate that usually only limited oviposition occurs on non-target *Rhamnus* species under no-choice and choice conditions. No gall development was recorded the following spring on any non-target species indicating that *T. walkeri* has a very narrow host range. Because oviposition usually starts 3–4 weeks after adult emergence, it will not occur on non-target hosts in field situations where *R. cathartica* is not present since the longevity of *T. walkeri* females is much reduced on those plants.

*Trichochermes walkeri* has been recorded exclusively on *R. cathartica* in Europe and no larval and gall development has been observed so far on any other *Rhamnus* species.

The detection of a phytoplasma in *T. walkeri* adults raises several important questions: 1) is the phytoplasma '*Candidatus* Phytoplasma rhamni' common on *R. cathartica* in Europe?; 2) does '*Candidatus* Phytoplasma rhamni' already occur in North America, and if yes, which insect is the vector?; 3) does the phytoplasma occur on other *Rhamnus* species in Europe?; 4) does *T. walkeri* transmit the phytoplasma, and if not, which other insect is the vector, and 5) is '*Candidatus* Phytoplasma rhamni' specific to *R. cathartica* as it is suggested in the literature?

## 4 *Wachtiella krumbholzi* (Dipt., Cecidomyiidae)

Little is known about this insect, which was identified by Dr M. Skuhrava (Czech Republic). Interestingly, with the exception of a few specimens reared from *R. cathartica* in the Czech Republic, Skuhrava has not found this species during 50 years of investigations of cecidomyiids in 1800 European localities (Simova-Tosic et al. 2000, 2004; Skuhrava et al. 2005). The main characteristics of fruits attacked by *W. krumbholzi* are premature fruit

maturation with changes in colour, with the fruits also larger in size than normal and irregularly shaped. Attacked fruits become dark-red/black while healthy fruits remain green (see Plate 3). Casual observations revealed up to nine midge larvae per fruit and three larvae in one seed. The midge larva leaves the fruit and enters the soil to prepare a larval cocoon made of silk and debris.

Work on *W. krumbholzi* started in 2007.

### 4.1 Collections and rearing in 2008–09

Fruits of *R. cathartica* were collected in Austria (six sites), southern Germany (two sites) and Switzerland (two sites) during late June – early August 2008 (Plate 3). Midge larvae were reared from all sites. In total, about a thousand larvae emerged and were transferred to Petri dishes filled with a mixture of sterilized sifted soil and vermiculite. In late August, the soil was checked and 850 larval cocoons recovered. Batches of larval cocoons were overwintered in an outdoor shelter in a mixture of sifted soil and vermiculite.

**Plate 3** Larvae of *Wachtiella krumbholzi* feeding in the fruit and seeds of *Rhamnus cathartica* (left) and a sample of fruits, including many attacked ones with exit holes (right)

Several hundred fruits of *R. cathartica* were collected in southern Germany on 2 July and 20 July 2009. Fruits were kept on a wire grille, allowing the larvae to drop into a container beneath filled with a mixture of vermiculite and sifted soil. In late August, the soil was checked and 213 larval cocoons were recovered. Batches of larval cocoons are being overwintered in an outdoor shelter in a mixture of sifted soil and vermiculite for potential work in 2010.

No midge larvae were reared from the fruits of *Frangula alnus* collected in 2008 at one site in Austria and at one site in Switzerland, where *R. cathartica* and *F. alnus* co-occur.

### 4.2 Adult emergence in 2009

**Methods.** In early March 2009, 600 larval cocoons reared in 2008 were transferred from outdoor storage into a 1°C incubator to delay adult emergence according to experimental needs. On 18 May 2009, as the first adults emerged from the outdoor storage boxes, about 50% of this material was transferred into a 10°C incubator to investigate whether adult emergence

could be delayed by this means. On 2 June, all cocoons from cold storage were returned to outdoor conditions.

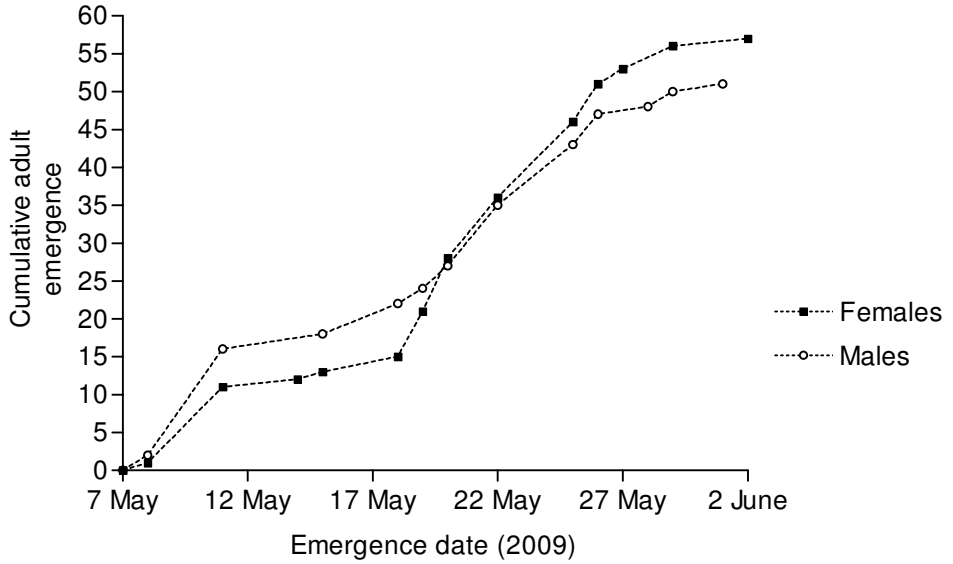


**Figure 3** Emergence of *Wachtiella krumbholzi* adults reared from field-collected larvae in 2008

**Results.** A total of 57 females and 51 males emerged between 8 May and 1 June 2009 from 120 cocoons held permanently in outdoor storage (Figure 3). Larval mortality was quite high for the material which had been kept at 1°C until 2 June, since only 96 females and 90 males emerged between 24 June and 13 July. No adults emerged from the cocoons held in the 10°C incubator indicating that the cold treatment was lethal for adults ready to emerge.

### 4.3 No-choice oviposition tests

#### 4.3.1 Methods

Only a few potted *R. cathartica* yielded flowers and developing fruits, thus limiting the number of oviposition trials. One individual branch of two potted *R. cathartica* and one potted *F. alnus* were each exposed to four pairs of *W. krumbholzi***.** Branches were covered with a gauze bag and the plants kept outdoors. All tests were set up on 22–25 May and the fruits collected on 6 July for dissection. Fruits of an *R. cathartica* tree growing naturally in the vicinity of the Centre were dissected on 24 June to confirm the presence of *W. krumbholzi* in the area.

On 24 June, one branch of potted *R. cathartica* and two branches of potted *F. alnus* with well-developed fruits were each exposed to five pairs of *W. krumbholzi*. Fruits of potted *R. cathartica* and potted *F. alnus* were dissected on 6 July to check whether *W. krumbholzi* oviposited naturally on the test plants in the Centre's garden.

#### 4.3.2 Results

Our preliminary tests indicate successful oviposition by *W. krumbholzi* in the very young developing fruits of *R. cathartica* (Table 4). In contrast, no

oviposition occurred in fruits that were one month older and well developed. No midge larvae were found in the unexposed fruits of potted *R. cathartica* used as a control for this experiment. Attack by *W. krumbholzi* on a *R. cathartica* tree growing naturally in the vicinity of the Centre was lower than in the oviposition tests. No midge larvae were found in the fruits of *F. alnus*.

**Table 4** Results of no-choice oviposition tests with *Wachtiella krumbholzi* in 2009

| | Set-up date | No. of fruits dissected | No. of fruits attacked (%) | Mean no. of larvae/fruit (± SD) | Max. no. of larvae/fruit |
|---|---|---|---|---|---|
| ***Rhamnus cathartica*** | | | | | |
| **Test 1** on potted plant with very young developing fruits | 22 May | 25 | 15 (60) | 2.4 ± 4.2 | 18 |
| **Test 2** on potted plant with very young developing fruits | 22 May | 20 | 19 (95) | 5.6 ± 4.1 | 14 |
| **Test 3** on potted plant with well-developed fruits | 24 June | 4 | 0 | - | - |
| **Test 4** on potted plant with well-developed fruits | 24 June | 11 | 0 | - | - |
| **Control 1** dissection of unexposed fruits from potted plants | - | 16 | 0 | - | - |
| **Control 2** dissection of fruits from a nearby tree | - | 30 | 10 (33) | 1.0 ± 2.0 | 8 |
| ***Frangula alnus*** | | | | | |
| **Test 1** on potted plant with very young fruits | 22 May | 4 | 0 | - | - |
| **Test 2** on potted plant with well-developed fruits | 24 June | 9 | 0 | - | - |
| **Control** dissection of unexposed fruits from potted plants | - | 21 | 0 | - | - |

## 4.4 Conclusions and outlook

Host-range tests with this fruit-attacking gall midge species will rely entirely on oviposition tests. The main difficulty will be to get test plants at the right

phenological stage, i.e. probably in a very early stage of fruit development, to coincide with when *W. krumbholzi* lays eggs. The difficulty is enhanced because, to the best of our knowledge, *Rhamnus* species are mostly dioecious (i.e. male and female flowers are on separate plants) and pollination could be a problem.

Batches of cocoons should be kept at below-ambient temperatures in order to delay adult emergence and to match it with plant phenology even though maintaining larval cocoons at low temperatures seems to induce high mortality.

Work conducted on midges in Europe over several decades suggests that *W. krumbholzi* is specific to *R. cathartica* (Simova-Tosic et al. 2000, 2004; Skuhrava et al. 2005). *Contarinia rhamni* and *Dasyneura frangulae,* recorded in the literature on *F. alnus* (Gassmann et al. 2008b), have not been reared from *R. cathartica* fruits. No adult midges have yet been reared from the fruits of *F. alnus*.

# 5 Discussion

Despite the fact that some difficulties have been encountered with rearing *Philereme vetulata*, work in 2008 and 2009 has confirmed that the physiological host range of this leaf-feeding moth is restricted to species in the genus *Rhamnus*, most probably to deciduous species with large smooth leaves. No oviposition was obtained in confinement; hence assessing the host specificity of *P. vetulata* will rely on larval feeding and development in no-choice tests.

In contrast, assessing host specificity of the leaf-margin gall psyllid *Trichochermes walkeri* and the seed-feeding midge *Wachtiella krumbholzi* will rely on oviposition and larval development tests. Adult feeding and oviposition by *T. walkeri* are restricted to species in the genus *Rhamnus* and larval development is likely on *R. cathartica* only. The likelihood of *T. walkeri* accepting a non-target species for oviposition in containment that would not be accepted in the field (a false positive) is considered high.

The detection of a phytoplasma in *T. walkeri* adults raises several questions that will need to be answered in parallel with host range studies (see 3.4).

The challenges in working with *W. krumbholzi* will be obtaining pollination of female buckthorn flowers and synchronizing fruit development with midge oviposition and larval development. More generally, one current constraint in developing biological control of buckthorns is the difficulty of obtaining seeds for a number of test plant species and/or growing plants from seeds.

With one exception (*P. vetulata*), the three candidate agents *P. vetulata*., *T. walkeri* and *W. krumbholzi* have been recorded exclusively on *R. cathartica* in Europe where, however, only a few *Rhamnus* species occur.

Likely specific requirements for host acceptance and suitability will be related to plant phenology, stage of the developing leaf bud, and leaf shape and toughness, as well as habitat requirements. There are indications that larvae of *P. vetulata* and *T. walkeri* will not complete development on small tough or

thick evergreen leaves such as those of *R. alaternus*. Therefore, the native North American *Rhamnus* species *R. crocea*, *R. ilicifolia*, *R. serrata* and *R. smithii* are unlikely to support development of *P. vetulata* and *T. walkeri* larvae to the adult stage. Critical native non-target North American species are *R. alnifolia* and *R. lanceolata* because of their leaf shapes and smoothness and their geographical distributions which partially overlap that of *R. carthartica*.

A range of difficulties has not been solved over the past two years. Collecting and growing a couple of critical test plant species are still difficult. The success of this programme is complicated by two other factors: the need to work with genus- or species-specific species which considerably limits the number of potential biological control agents and the difficulty of rearing and testing some candidate agents. At this point, and after several years of research, the feasibility of biological control of *R. cathartica* needs to be addressed and considered from another standpoint.

The following review of successes and failures in biological control programmes for trees and shrubs has been carried out in order (1) to assess the feasibility and likelihood of success of such programmes, and (2) to further help prioritize potential biological control agents for buckthorn based on the most successful groups of biological control agents for invasive trees and shrubs.

# 6 Successes and failures in the biological control of invasive trees and shrubs and implications for the buckthorn project (A. Gassmann)

## 6.1 Introduction

This review is based on a paper by Moran et al. (2004) and updated from most-recent information extracted from CAB Direct (www.cabdirect.org/) and a search on the internet. It focusses on programmes against invasive trees and shrubs for which biological control agents have already been released.

Since the paper by Moran et al. (2004) was written, insect releases have been made against one other tree genus, *Tamarix* spp., in North America. Of the list of plants analysed by Moran et al. (2004), *Mimosa pigra* is the only species which can be considered as a shrub exclusively and not a tree/shrub. Unlike these authors, I have included in this review other 'obvious' shrub species such as *Ulex europaeus*, *Cytisus scoparius*, *Clidemia hirta* and *Mimosa invisa* but have, like them, excluded vines.

Of the 25 tree/shrub species which have been targeted for biological control, seven are invasive in North America (including three exclusively in Hawaii), seven in Australia, New Zealand and the Pacific islands and 14 in South Africa (of which nine are *Acacia* spp.). With the exception of *Tamarix*, *U. europaeus* and *C. scoparius*, the species targeted occur mostly in the dry or humid tropical or subtropical ecoregions according to the definitions proposed by Bailey (1996). With the exception of the Hawaiian programmes of the 1960s, and a few early insect introductions against *U. europaeus* and *C. scoparius*, releases for biological control of trees and shrubs started in the late 1970s, mainly in South Africa.

## 6.2 Successes and failures in biological control of trees and shrubs

Demonstrating that an agent introduced to a new geographical area is effective against a target weed across its range and over the long term is a difficult task. Almost without exception, early publications on biological control provide definitions of success that refer to reductions in either 'density' or 'abundance' of the target plant (see Hoffmann and Moran 2008).

The initiation of biological control of trees in the late 1970s in South Africa revealed another aspect of what is meant by success (see Moran et al. 2004) and pleas have been made to develop other performance criteria for the role of biological control in weed management. First, the economic importance of some invasive *Acacia* species in South Africa has limited the choice of biocontrol agents to those that reduce flower and seed production and thus have the potential to limit the spread of cultivated exotic acacias (Dennill et al. 1999). Second, the apparent failure of biological control agents to reduce the distribution or density of, e.g., *Acacia pycnantha*, *A. cyclops* and *Sesbania punicea* in South Africa is hiding the fact that management of the weeds was much faster and therefore cheaper after biological control agents had reduced the levels of seeding, and hence seedling recruitment (Moran et al. 2004). The conclusion is that there is increasing evidence from the studies of biological control of invasive trees in South Africa that any reduction in seed output aids management and that after seed-destroying agents are deployed, agents that attack other parts of the plant should be considered.

A rather similar innovative goal-based approach was used for the *Melaleuca quinquenervia* programme in Florida, USA, showing that this programme could be considered as a success even though vast stands of *M. quinquenervia* still exist that overtly appear unchanged (Center et al. 2008). The hypothesis was that biological control cannot eliminate the huge amounts of woody biomass present in large infestations. The role of biological control is instead to neutralize the reproductive potential of those populations which are reduced to maintenance level by other control methods, or to reduce it in other small isolated stands such as those on private lands.

From the 25 species targeted for biological control, the programmes against *M. quinquenervia* (Center et al. 2008), *Tamarix* spp. (DeLoach et al. 2008), *S. punicea* (Hoffmann and Moran 1999), *Acacia longifolia*, *A. saligna*, *A. pycnantha*, *A. cyclops* (Dennill et al. 1999) and *Mimosa invisa* (Kuniata and Korowi 2004) are considered as successes either in a classical sense of stand and density reduction or in terms of improved management of the weed (see also Julien and Griffiths 1998). Biological control was also effective in preventing the spread of *Clidemia hirta* into open pastures and cultivated land but failed in shaded areas (Nakahara et al. 1992). In summary, biological control programmes against seven of 20 targeted trees (35%) and two of five targeted shrubs (40%), i.e. *C. hirta* and *M. invisa*, have resulted in some level of satisfactory control so far. These numbers are at least as encouraging as those proposed two decades ago for biological control of weeds in general (Crawley 1989; Waage 1992; Bruzzese 1993). In terms of the amount of work done and the number of biological control agents released, the most obvious recalcitrant trees are *Schinus terebinthifolius* in Hawaii in the 1960s, *Prosopis* spp. in South Africa and Australia in the 1990s, *Parkinsonia aculeata* in

northern Australia in the 1990s and *Acacia nilotica* ssp. *indica* also in Australia. With regard to shrubs, no control has been achieved so far for *M. pigra*, *Ulex europaeus* and *Cytisus scoparius* in spite of a number of introductions.

### 6.3 Prioritization of agents for shrub and tree control

Weed biological control projects are not often undertaken on the basis of the likelihood of a successful outcome and biological control of many weed problems would likely never be attempted if target choice was based primarily on maximizing the probability of success. For example, in the recent *Melaleuca quinquenervia* programme, no pre-release studies were carried out to prioritize the potentially most efficient agents and all species cleared for release so far have been selected according to their host specificity exclusively (Center et al. 2008).

There is a vast literature on pre-release modelling and experimental studies to evaluate the effectiveness of biological control agents that could thus has assisted agent selection and prioritization and helped to fine-tune a biological control programme's strategy. These approaches, which have been recently evaluated by Morin et al. (2009), include, e.g., setting performance targets, evaluating agent effectiveness, and performing laboratory and field studies, plant demographic modelling and benefit-cost analyses. However, the ultimate efficacy of an agent will only be demonstrated in the area of release, entailing many more years of research, and a priori predictions of agent efficacy have seldom been explicitly tested by quantifying effectiveness in the field after release (Morin et al. 2009). There are therefore great disincentives to undertaking in-depth pre-release evaluation of agent effectiveness because of the additional time and resources required and the potential likelihood of rejecting agents that could turn out to be effective in the introduced range. Quite obviously, invasive trees and shrubs present even more difficulties in terms of predicting effectiveness of classical biological control. Regardless of the costs of such studies it may take many generations of attack before quantifiable impacts are observed on the target plant. In addition, for seed-feeders, population-level impact is directly related to amount of seed destroyed and the importance of recruitment from seed in the area of introduction.

Given the constraints, only a few authors have tried to analyse which taxonomic groups make the best biological control agents (Crawley 1989; Gassmann 1995; Syrret et al., 1996). These reviews showed that beetles, in particular Chrysomelidae and Curculionidae, are the most effective weed biological control agents in the temperate world. In subtropical and tropical areas, beetles are not of such dominant importance, perhaps because the impact of the prolonged combined feeding period of adult beetles and larvae is counterbalanced by continuous and overlapping breeding of species belonging to other taxa (Gassmann 1995).

In this review I attempt to carry out a similar analysis, predicting which taxonomic group(s) make(s) the best biological control agents of invasive trees and shrubs. Of 72 arthropod species released against invasive trees and shrubs, there are only 16 (22%) that did not become established or whose

establishment status is unknown (Table 5). Over 50% of the species that failed to establish were released under two Australian programmes which turned out to be very problematic, i.e. *Acacia nilotica* ssp. *indica* and *Mimosa pigra*. Thus, in general, the establishment rate of arthropod agents in the biological control of woody perennials has been very successful. The figures are quite similar when trees and shrubs are considered separately.

Of the 56 arthropod species that have established, 28 species are Coleoptera (i.e. 82% of all beetles released, including 20 seed feeders), 16 Lepidoptera (67% of all moths released, including three seed feeders), six Hemiptera (86%), two Thysanoptera, two gall-forming Hymenoptera, one Diptera and one Acari. Approximately 40% of these agents directly reduce seed production.

**Table 5** Successful biological control agents of invasive trees and shrubs by taxonomic groups

| Taxonomic group | No. of species released | No. of species established (%) | No. of species having a substantial impact (% of those established) |
|---|---|---|---|
| Coleoptera | 34 | 28 (82.4) | 8 (28.6) |
| Lepidoptera | 24 | 16 (66.7) | 0 |
| Hemiptera | 7 | 6 (85.7) | 2 (33.3) |
| Diptera | 2 | 1 (50.0) | 0 |
| Hymenoptera | 2 | 2 (100) | 2 (100) |
| Thysanoptera | 2 | 2 (100) | 1 (50.0) |
| Acari | 1 | 1 (100) | 0 |
| Pathogens | 4 | 1 (25.0) | 1 (100) |
| **Total** | **72** | **56 (78%)** | **14 (25%)** |

Of the agents established, 14 species are reported to impact on the target plant and almost two-thirds of these successful agents belong to the Coleoptera; i.e. nearly one-third of all beetles which have become established are contributing to successful control (Table 6). In addition to the four beetle species which directly attack the reproductive parts of the target, four other species have had a major impact on their target weed, e.g. the leaf-feeding chrysomelid *Diorhabda* spp. on *Tamarix*, or the flush feeder *Oxyops vitiosa* on *Melaleuca quinquenervia*.

Gall-forming wasps are also a very successful group, as are the sap-sucking species in the families Phlaeothripidae (Thysanoptera)) and Psyllidae (Hem.). Of four pathogens released, the gall-forming rust fungus, *Uromycladium tepperianum*, turned out to be a very effective agent on *Acacia saligna* in South Africa, two species failed in the *Mimosa pigra* programme in Australia and one did not become established on *Ulex europaeus* in the USA.

The success rate of the beetles drops slightly, from 28.6% to 22.7%, when the nine *Acacia* species targeted for biological control are excluded from the analysis (data not shown) and the pathogens disappear from the list of successful agents.

Interestingly, none of the 16 Lepidoptera species established is considered as having a substantial impact, including three seed-feeding species and two

stem borers. It is perhaps surprising that no Lepidoptera seem to impact on invasive trees or shrubs as a number of Lepidoptera are recorded as major forest pests. One explanation could be that these species never reached population densities capable of defoliating plants to a level resulting in a long-term decrease in the fitness of their host plants. It should also be noted that defoliation is generally more detrimental to coniferous trees than to deciduous ones because the regrowth of foliage of coniferous trees takes longer than for deciduous trees and the plants are not able to overcome complete defoliation (Dajoz 1980).

**Table 6** Taxonomy and food niche of most effective agents in the biological control of trees and shrubs (for references see Annex 1)

| Plant species | Biological control agent | Taxonomy | Food niche | Country of introduction |
|---|---|---|---|---|
| *Melaleuca quinquenervia* | *Oxyops vitiosa* | Col., Curculionidae | Flush feeder, shoot tip, foliage | USA (Florida) |
| | *Boreioglycaspis melaleucae* | Hem., Psyllidae | Sap sucker, foliage | USA (Florida) |
| *Tamarix* spp. | *Diorhabda* spp. | Col., Chrysomelidae | Foliage feeder | Southern USA |
| *Sesbania punicea* | *Trichapion lativentre* | Col., Curculionidae | Bud feeder | South Africa |
| | *Rhyssomatus marginatus* | Col., Curculionidae | Seed feeder | South Africa |
| | *Neodiplogrammus quadrivittatus* | Col., Curculionidae | Stem borer | South Africa |
| *Acacia cyclops* | *Melanterius servulus* | Col., Curculionidae | Seed feeder | South Africa |
| *Acacia pycnantha* | *Trichilogaster* sp. | Hym., Pteromalidae | Stem galler | South Africa |
| *Acacia longifolia* | *Melanterius ventralis* | Col., Curculionidae | Seed feeder | South Africa |
| | *Trichilogaster acaciaelongifoliae* | Hym., Pteromalidae | Stem galler | South Africa |
| *Acacia saligna* | *Melanterius compactus* | Col., Curculionidae | Seed feeder | South Africa |
| | *Uromycladium tepperianum* | Uredinales | Gall former | South Africa |
| *Clidemia hirta* | *Liothrips urichi* | Thysanoptera, Phlaeothripidae | Sap sucker, shoot tips | USA (Hawaii), Fiji |
| *Mimosa invisa* | *Heteropsylla spinulosa* | Hem., Psyllidae | Sap sucker, young leaves | Australia, Papua New Guinea |

In conclusion, the success rate of biological control of invasive trees and shrubs appears to be quite similar to what has been observed in biological control of weeds in general, but how success is defined may considerably differ from that of herbaceous plants. Based on the taxonomic groups of the

most efficient agents used in 25 programmes to date against invasive trees or shrubs, beetles, sap suckers, gall wasps and rust fungi should be prioritized. In addition, any agents reducing regeneration, either through reduced seed output or attack of seedlings, are expected to facilitate management of the target tree or shrub. Although these recommendations may be simplistic, in the absence of any pre-release impact efficacy assessment or other models, they could be used as additional criteria for agent prioritization.

# 7 Recommendations for biological control of *Rhamnus cathartica*

In total, 39 specialized arthropods were recorded from *R. cathartica* and *F. alnus* in Europe (Gassmann et al. 2008b). Lepidoptera (22 species) largely dominate, followed by Hemiptera (eight species), Diptera, (four species) and Acari (four species). There is only one specialized beetle on buckthorn in Europe, the stem-boring longhorned beetle, *Oberea pedemontana* (Col., Cerambycidae), but this species is not specific at the genus level.

Based on the above review, the next best group to consider is the sap suckers. A dozen species have been recorded on buckthorn in Europe including three psyllids, *Trichochermes walkeri* (Hem., Triozidae), *Cacopsylla rhamnicola* (Hem., Psyllidae) and *Trioza rhamni* (Hem., Triozidae). One of them, *T. walkeri*, is currently being studied. One Miridae (Hem.) and three Eriophydidae (Acari) have also been recorded on *R. cathartica* in Europe. With the exception of one species inducing leaf erinea on *R. cathartica*, none of the eriophyid species has been observed on buckthorn in past surveys. We therefore suggest not focussing on these species in the immediate future.

The coccinellid beetle *Harmonia axyridis* was recently found to be abundant on *R. cathartica* in Minnesota (Yoder et al. 2008) and it is possible that predation by *H. axyridis* could pose a particular threat to introduced biocontrol agents. The risk for each species is discussed below:

*Trichochermes walkeri*: Larval development occurs in leaf galls and thus larvae should be safe from predation. The species overwinters in the egg stage and eggs are laid on leaf bud axils. In Switzerland, *H. axyridis* adults start to look for overwintering sites in early October (M. Kenis, pers. comm.). The presence of *T. walkeri* eggs (October–April) may thus not fully coincide with maximum predation activity in *H. axyridis*. It has also been observed that *H. axyridis* does not feed on all insect species. It is planned to study the predation of *T. walkeri* by *H. axyridis* in collaboration with Marc Kenis who is studying the multitrophic interactions of this coccinellid beetle at CABI E-CH (see www.cabi.org/default.aspx?site=170&page=1017&pid=2319).

*Cacopsylla rhamnicola*: this species overwinters in the adult stage (Ossiannilsson 1992). The eggs are found in the inflorescences and young folded leaves, in which we have also observed young larvae. In this case, too, the threat from predation should be minimal.

*Trioza rhamni*: This species overwinters as an adult on conifers (Ossiannilson 1992). Females lay eggs singly on the underside of young leaves of the host plant and before long a pit-gall develops around each egg. The first-instar

larvae remain in the gall, but after each moult the larvae move to another site on the leaf. Of the three psyllids associated with buckthorn, *T. rhamni* seems to be the most susceptible to predation and perhaps also the potentially least efficient species.

The detection of '*Candidatus* Phytoplasma rhamni' in *T. walkeri* adults raises several questions that will need to be addressed before further considering sap-suckers for biological control of *R. cathartica*. In addition to the questions raised about *T. walkeri* in section 3.4, it will be necessary to determine whether *C. rhamnicola* is also host of the phytoplasma and able to transmit it.

Based on the preceding review, Lepidoptera were one of the least successful taxonomic groups for the biological control of shrubs or trees. In addition, the Lepidoptera we have investigated so far were either not sufficiently specific or are very difficult to test. They will therefore be given low priority as potential agents.

As far as insects that directly reduce seed output of buckthorn are concerned, two midge species and two Lepidoptera are known from the fruits of *R. cathartica* in Europe. One midge, *Wachtiella krumbholzi* is under evaluation. We have not found the second midge species, *Lasioptera kosarzewskella* or the two Lepidoptera species, *Sorhagenia rhamniella* (Cosmopterigidae) and *Hysterosia sodaliana* (Tortricidae), which also do not appear to be genus specific according to literature.

*Wachtiella krumbholzi* is the only available potential seed feeder for biological control of *R. cathartica* but the feasibility of host-range testing still needs to be addressed.

# 8 Proposed work programme 2010–2011

Based on the above, we propose the following work programme for 2010 and 2011:

***Trichochermes walkeri* and *Cacopsylla rhamnicolla* (Hem., Psylloidae)**

- Establish a protocol to determine whether the leaf gall psyllid *Trichochermes walkeri* transmits '*Candidatus* Phytoplasma rhamni';
- Sample additional *T. walkeri* populations for the detection of the phytoplasma;
- Collect samples of *R. cathartica* and other *Rhamnus* spp. from Europe and North America for the detection of the phytoplasma;
- Sample populations of the psyllid *Cacopsylla rhamnicolla* for the detection of '*Candidatus* Phytoplasma rhamni';
- Elaborate a protocol to determine the specificity of '*Candidatus* Phytoplasma rhamni';
- Continue host range studies with *T. walkeri* (no-choice and single-choice oviposition and larval development tests);
- Conduct preliminary studies of the predatory behaviour of *Harmonia axyridis* on *T. walkeri.*

***Wachtiella krumbholzi* (Dipt., Cecidomyiidae)**

- Further assess the feasibility of host-range testing of this seed-feeding midge.

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## Annex 1

Deliberate introductions of biological control agents against invasive trees and shrubs (the data are extracted from Julien and Griffiths (1998) and updated by more recent information)[a]

| | Plant growth habit / Agent taxonomic group | Introduced range / date | Native range | Food niche | Success status | References |
|---|---|---|---|---|---|---|
| *Melaleuca quinquenervia* (Myrtaceae) | Tree | Florida, USA | Eastern Australia; New Caledonia | | | |
| *Oxyops vitiosa* (Curculionidae) | Coleoptera | First released in 1997 | | Foliage, flush feeder | Success | Rayamajhi et al. 2002; Center et al. 2008 |
| *Boreioglycaspis melaleucae* (Psyllidae) | Hemiptera | First released in 2002 | | Sap sucker | Success | Rayamajhi et al. 2002; Center et al. 2008 |
| *Fergusonina turneri* (Fergusoninidae) | Diptera | First released in 2005 | | Foliage, flush feeder | Not established | Rayamajhi et al. 2002; Center et al. 2008 |
| *Schinus terebinthifolius* (Anacardiaceae) | Tree | Hawaii, Florida, USA | Argentina, Brazil | | | |
| *Episimus unguiculus* (=*utilis*) (Tortricidae) | Lepidoptera | First released in 1954 in Hawaii | | Foliage feeder | Failure | Hight et al. 2002 |
| *Lithraeus atronotatus* (Bruchidae) | Coleoptera | First released in 1960 in Hawaii | | Seed feeder | Failure | Hight et al. 2002 |
| *Crasimorpha infuscata* (Gelechiidae) | Lepidoptera | First released in 1961 in Hawaii | | Stem galler | Not established | Hight et al. 2002 |
| *Tamarix* spp. (Tamaricaceae)[a] | Tree | Western USA | Western Asia | | | |
| *Diorhabda* spp. (Chrysomelidae) | Coleoptera | First releases of populations from China/Kazakhstan in 2001 and Greece in 2003 | | Foliage feeder | Success | Hudgeons et al. 2007; DeLoach et al. 2008 |
| *Solanum mauritianum* (Solanaceae) | Tree | South Africa, New Zealand, India, Pacific islands | South America | | | |
| *Gargaphia decoris* (Tingidae) | Hemiptera | First released in South Africa in 1999 | | Sap sucker | Failure | Olckers and Borea 2009 |

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|---|---|---|---|---|---|---|
| *Clidemia hirta* (Melastomataceae) | Shrub | Hawaii, USA | Central America, Trinidad and Tobago | | | |
| *Liothrips urichi* (Phlaeothripidae) | Thysanoptera | First released in Fiji in the 1930s and in Hawaii in 1953 | | Sap sucker | Success | Simmonds 1937; Reimer and Beardsley 1989; Conant 2002 |
| *Ategumia matutinalis* (syn. *ebulealis* ?) (Pyralidae) | Lepidoptera | First released in Hawaii in 1969 | | Foliage feeder | Failure | Nakahara et al. 1992; Julien and Griffiths 1996; Conant 2002 |
| *Antiblemma acclinalis* (Noctuidae) | Lepidoptera | First released in Hawaii in 1995 | | Foliage feeder | Failure | Julien and Griffiths 1998; Conant 2002; Culliney et al. 2003 |
| *Carposina bullata* (Carposinidae) | Lepidoptera | First released in Hawaii in 1995 | | Flowerbud feeder | Not established | Julien and Griffiths 1998; Conan 2002; Culliney et al. 2003 |
| *Lius poseidon* (Buprestidae) | Coleoptera | First released in Hawaii in 1988 | | Foliage feeder | Failure | Julien and Griffiths 1998; Conant 2002 |
| *Mompha trithalama* (Momphidae) | Lepidoptera | First released in Hawaii in 1995 | | Seed feeder | Failure | Julien and Griffiths 1998; Conant 2002; Culliney et al. 2003 |
| *Sesbania punicea* (Fabaceae) | Tree | South Africa | South America | | | |
| *Trichapion lativentre* (Curculionidae) | Coleoptera | Accidental introduction in South Africa in the 1970s | | Foliage, flush feeder | Success | Julien and Griffiths 1998; Hoffmann and Moran 1999 |
| *Rhyssomatus marginatus* (Curculionidae) | Coleoptera | First released in South Africa in 1984 | | Seed feeder | Success | Julien and Griffiths 1998; Hoffmann and Moran 1999 |
| *Neodiplogrammus quadrivittatus* (Curculionidae) | Coleoptera | First released in South Africa in 1984 | | Stem borer | Success | Julien and Griffiths 1998; Hoffmann and Moran 1999 |
| *Hakea sericea* (Proteaceae) | Tree | South Africa | Australia | | | |
| *Erytenna consputa* (Curculionidae) | Coleoptera | First released in South Africa in 1972 | | Seed feeder | Failure | Gordon 1999 |
| *Cydmaea binotata* (Curculionidae) | Coleoptera | First released in South Africa in 1979 | | Stem borer | Failure | Gordon 1999 |
| *Carposina autologa* (Carposinidae) | Lepidoptera | First released in South Africa in 1991 | | Seed feeder | Failure | Gordon 1999 |
| *Prosopis* spp. (Mimosaceae)[a] | Tree | South Africa, Australia | North and South America | | | |
| *Algarobius prosopis* (Bruchidae) | Coleoptera | First released in South Africa in 1987 and in Australia in 1996 | | Seed feeder | Failure | Impson et al. 1999 |

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|---|---|---|---|---|---|---|
| *Algarobius bottimeri* (Bruchidae) | Coleoptera | First released in South Africa in 1990 | | Seed feeder | Failure | Impson et al. 1999 |
| *Neltumius arizonensis* (Bruchidae) | Coleoptera | First released in South Africa in 1993 | | Seed feeder | Failure | Impson et al. 1999 |
| *Evippe* sp. (Gelechiidae) | Lepidoptera | First released in Australia in 1998 | | Foliage feeder | Failure | van Klinken et al. 2003 |
| *Prosopidopsylla flava* (Psyllidae) | Hemiptera | First released in Australia in 1998 | | Sap sucker | Failure | van Klinken et al. 2003 |
| *Parkinsonia aculeata* (Caesalpiniaceae) | Tree | Northern Australia | America | | | |
| *Mimosetes ulkei* (Bruchidae) | Coleoptera | First released in Australia in 1993 | | Seed feeder | Failure | Lockett et al. 1999; Grace et al. 2006 |
| *Penthobruchus germaini* (Bruchidae) | Coleoptera | First released in Australia in 1995 | | Seed feeder | Failure | Lockett et al. 1999; Grace et al. 2006 |
| *Rhinacloa callicrates* (Miridae) | Hemiptera | First released in Australia in 1989 | | Seed feeder | Failure | Lockett et al. 1999; Grace et al. 2006 |
| *Leptospermum laevigatum* (Myrtaceae) | Tree | South Africa | Australia | | | |
| *Parectopa thalassias* (Gracillariidae) | Lepidoptera | First released in South Africa in 1996 | | Foliage feeder | Failure | Gordon 1999 |
| *Myrica faya* (Myricaceae) | Tree | Hawaii | Azores, Canary Islands, Madeira | | | |
| *Caloptilia* nr *schinell* (Gracillariidae) | Lepidoptera | First released in Hawaii in 1991 | | Foliage feeder | Failure | Leen and Markin 1996; Markin 2001 |
| *Acacia nilotica* ssp. *indica* (Fabaceae) | Tree | Australia | Pakistan, Kenya and southern Africa | | | |
| *Bruchidius sahlbergi* (Bruchidae) | Coleoptera | First released in Australia in 1982 | | Seed feeder | Failure | Dhileepan 2009 |
| *Cuphodes profluens* (Gracillariidae) | Lepidoptera | First released in Australia in 1983 | | Flush feeder, shoot-tip borer | Not established | Dhileepan 2009 |
| *Homicloda barkeri* (Chrysomelidae) | Coleoptera | First released in Australia in 1996 | | Foliage feeder | Establishment unknown | Lockett and Palmer 2003; Dhileepan 2009 |

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|---|---|---|---|---|---|---|
| *Cometaster pyruoctuidae* (Noctuidae) | Lepidoptera | First released in Australia in 2004 | | Foliage feeder | Not established | Dhileepan 2009 |
| *Chiasmia inconspicua* (Geometridae) | Lepidoptera | First released in Australia in early 2000s | | Foliage | Not established | Palmer et al. 2007 |
| *Chiasma assimilis* (Geometridae) | Lepidoptera | First released in Australia in early 2000s | | Foliage | Failure | Palmer et al. 2007 |
| *Acacia cyclops* (Fabaceae) | Tree | South Africa | Australia | | | |
| *Dasyneura dielsi* (Cecidomyiidae) | Diptera | First released in South Africa in 2001 | | Seed feeder | Failure | Adair 2005; Dennill et al. 1999; Moseley et al. 2009 |
| *Melanterius servulus* (Curculionidae) | Coleoptera | First released in South Africa in 1991 | | Seed feeder | Success | Dennill et al. 1999 |
| *Acacia dealbata* (Fabaceae) | Tree | South Africa | Australia | | | |
| *Melanterius maculatus* (Curculionidae) | Coleoptera | First released in South Africa in 1991 | | Seed feeder | Not established | Dennill et al. 1999 |
| *Acacia decurrens* (Fabaceae) | | South Africa | Australia | | | |
| *Melanterius maculatus* (Curculionidae) | Coleoptera | First released in South Africa in 2001 | | Seed feeder | Not established | Moran et al. 2004 |
| *Acacia longifolia* (Fabaceae) | Tree | South Africa | Australia | | | |
| *Melanterius ventralis* (Curculionidae) | Coleoptera | First released in South Africa in 1985 | | Seed feeder | Success | Dennill et al. 1999 |
| *Trichilogaster acaciaelongifoliae* (Pteromalidae) | Hymenoptera | First released in South Africa in 1982 | | Stem galler | Success | Dennill 1985; Dennill et al. 1999 |
| *Acacia mearnsii* | Tree | South Africa | Australia | | | |
| *Melanterius maculatus* (Curculionidae) | Coleoptera | First released in South Africa in 1994 | | Seed feeder | Failure | Dennill et al. 1999 |
| *Acacia melanoxylon* | Tree | South Africa | Australia | | | |
| *Melanterius acaciae* (Curculionidae) | Coleoptera | First released in South Africa in 1986 | | Seed feeder | Failure | Dennill et al. 1999 |

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|---|---|---|---|---|---|---|
| *Acacia pycnantha* | Tree | South Africa | Australia | | | |
| *Trichilogaster* sp. (Pteromalidae) | Hymenoptera | First released in South Africa in 1987 | | Stem galler | Success | Dennill et al. 1999; Hoffmann et al. 2002 |
| *Acacia saligna* | Tree | South Africa | Australia | | | |
| *Melanterius compactus* (Curculionidae) | Coleoptera | First released in South Africa in 2001 | | Seed feeder | Success | Impson and Moran 2004 |
| *Uromycladium tepperianum* (Uredinales) | Pathogen | First released in South Africa in 1987 | | Galler of any young tissue | Success | Morris 1999; Wood and Morris 2007 |
| *Paraserianthes lophanta* | Tree | South Africa | Australia | | | |
| *Melanterius servulus* (Curculionidae) | Coleoptera | First released in South Africa in 1989 | | Seed feeder | Failure | Dennill et al. 1999 |
| *Mimosa pigra* | Shrub | Northern Australia | Tropical America | | | |
| *Acanthoscelides puniceus* (Bruchidae) | Coleoptera | First released in Australia in 1983 | | Seed feeder | Failure | Ostermeyer and Grace 2007 |
| *Acanthoscelides quadridentatus* (Bruchidae) | Coleoptera | First released in Australia in 1983 | | Seed feeder | Not established | Ostermeyer and Grace 2007 |
| *Chlamisus mimosae* (Chrysomelidae) | Coleoptera | First released in Australia in 1985 | | Foliage | Failure | Ostermeyer and Grace 2007 |
| *Neurostrota gunniella* (Gracillariidae) | Lepidoptera | First released in Australia in 1989 | | Foliage | Failure | Ostermeyer and Grace 2007 |
| *Carmentosa mimosa* (Sesiidae) | Lepidoptera | First released in Australia in 1989 | | Stem borer | Failure | Ostermeyer and Grace 2007 |
| *Coelocephalapion aculeatum* (Curculionidae) | Coleoptera | First released in Australia in 1992 | | Seed feeder | Not established | Ostermeyer and Grace 2007 |
| *Coelocephalapion pigrae* (Curculionidae) | Coleoptera | First released in Australia in 1994 | | Seed feeder | Failure | Ostermeyer and Grace 2007 |
| *Phloeospora mimosae-pigrae* (Coelomycetes) | Pathogen | First released in Australia in 1995 | | Leaves and stems | Not established | Ostermeyer and Grace 2007 |
| *Chalcodermus serripes* (Curculionidae) | Coleoptera | First released in Australia in 1996 | | Seed feeder, flush feeder | Not established | Ostermeyer and Grace 2007 |

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|---|---|---|---|---|---|---|
| *Diabole cubensis* (Uredinales) | Pathogen | First released in Australia in 1996 | | Leaves and stems | Not established | Ostermeyer and Grace 2007 |
| *Sibina fastigiata* (Curculionidae) | Coleoptera | First released in Australia in 1997 | | Seed feeder, flush feeder | Not established | Ostermeyer and Grace 2007 |
| *Malacorhinus irregularis* (Chrysomelidae) | Coleoptera | First released in Australia in 2000 | | Root feeder | Failure | Ostermeyer and Grace 2007 |
| *Macaria pallidata* (Geometridae) | Lepidoptera | First released in Australia in 2002 | | Foliage | Failure | Ostermeyer and Grace 2007 |
| *Leuciris fimbriaria* (Geometridae) | Lepidoptera | First released in Australia in 2005 | | Foliage | Establishment unknown | Ostermeyer and Grace 2007 |
| *Mimosa invisa* | Vine shrub | Australia, Pacific islands | Tropical America | | | |
| *Heteropsylla spinulosa* (Psyllidae) | Hemiptera | First released in Australia in 1988 | | Sap sucker | Success | Kuniata and Korowi 2004 |
| *Psigida walkeri* (Citheroniidae) | Lepidoptera | First released in Cook Islands in 1994 | | Flush feeder | Not established | Waterhouse 1994 |
| *Scamurius* sp. (Coreidae) | Hemiptera | First released in Australia in 1987 | | Sap sucker | Not established | Waterhouse 1994 |
| *Ulex europaeus* | Shrub | USA, New Zealand, Australia | Temperate Europe | | | |
| *Exapion ulicis* (Brentidae) | Coleoptera | First released in Hawaii, USA, in 1926 | | Seed feeder | Failure | Hill et al. 2008 |
| *Cydia succedana* (Tortricidae) | Lepidoptera | First released in New Zealand in 1992 | | Seed feeder | Failure | Hill et al. 2008 |
| *Tetranychus lintearius* (Tetranychidae) | Acari | First released in New Zealand in 1989 | | Sap sucker | Failure | Hill et al. 2008 |
| *Sericothrips staphylinus* (Thripidae) | Thysanoptera | First released in New Zealand in 1991 | | Sap sucker | Failure | Hill et al. 2008 |
| *Agonopterix ulicetella* (Oecophoridae) | Lepidoptera | First released in Hawaii, USA, in 1988 | | Foliage | Failure | Hill et al. 2008 |
| *Pempelia genistella* (Pyralidae) | Lepidoptera | First released in New Zealand and the USA in 1996 | | Foliage | Failure | Hill et al. 2008 |
| *Scytheris grandipennis* (Scythrididae) | Lepidoptera | First released in New Zealand in 1990 | | Foliage | Not established | Hill et al. 2008 |

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|---|---|---|---|---|---|---|
| *Eutrichapion scutellare* (Curculionidae) | Coleoptera | First released in Hawaii, USA, in 1961 | | Stem galler | Not established | Markin et al. 1996 |
| *Uromyces pisi* (Uredinales) | Pathogen | First released in the USA in 2000 | | Foliage | Not established | Hill et al. 2008 |
| *Cytisus scoparius* | Shrub | USA, New Zealand, Australia | Temperate Europe | | | |
| *Leucoptera spartifoliella* (Lyonetiidae) | Lepidoptera | First released in the USA in 1960 | | Stem borer | Failure | Sheppard et al. 2006 |
| *Arytainilla spartiophila* (Psyllidae) | Hemiptera | First released in New Zealand in 1993 | | Sap sucker | Failure | Sheppard et al. 2006 |
| *Bruchidius villosus* (Bruchidae) | Coleoptera | First released in New Zealand in 1987 | | Seed feeder | Failure | Sheppard et al. 2006 |
| *Exapion fuscirostre* (Curculionidae) | Coleoptera | First released in the USA in 1964 | | Seed feeder | Failure | Coombs et al. 2008 |

[a], the exact number of *Prosopis* species is not given because the genus has formed hybrid communities in the invaded ranges; the number of *Tamarix* species is not given either since most papers refers to the biological control of *Tamarix* spp.

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