

ORIGINAL CONTRIBUTION

Biological control of *Rhamnus cathartica*: is it feasible? A review of work done in 2002–2012A. Gassmann¹ & I. Tosevski^{1,2}¹ CABI, Delémont, Switzerland² Department of Plant Pests, Institute for Plant Protection and Environment, Zemun, Serbia**Keywords**

buckthorn, defoliators, host range, internal feeders, sap suckers

Correspondence

André Gassmann (corresponding author), CABI, Rue des Grillons 1, 2800 Delémont, Switzerland. E-mail: a.gassmann@cabi.org

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Abstract

Rhamnus cathartica (common buckthorn) is a shrub (or small tree) of Eurasian origin, which has become invasive in North America. Internal feeders and sap suckers were prioritized for biological control from over 30 specialized insects identified from the target plant in its native European range. Five leaf-feeding moths were also considered for further investigations. Field observations and preliminary host range tests with the stem-boring beetle *Oberea pedemontana*, the root-boring moth *Synanthedon stomoxiformis*, the shoot-tip-boring moth *Sorhagenia janiszewskae* and the leaf-feeding moths *Ancylis apicella*, *A. unculana*, *Triphosa dubitata*, *Philereme transversata* and *P. vetulata* confirmed that all of these species were lacking host specificity in no-choice conditions. Choice oviposition tests carried out with most of the prioritized species to assess their ecological host range yielded unreliable results. Three psyllids, *Trichoermes walkeri*, *Cacopsylla rhamnifolia* and *Trioza rhamni* are promising in terms of host specificity, but are infected with the plant disease 'Candidatus Phytoplasma rhamni'. Fruit- or seed-feeding insects may present the best potential for biological control of buckthorn in directly reducing seed set and thus seedling establishment. However, it was not possible to obtain adult fruiting trees of native North American *Rhamnus* species for testing. It is concluded that there are no promising arthropod agents based on what is known to date. Pathogens could offer new opportunities for biological control of *R. cathartica* in North America.

Introduction

Rhamnus cathartica L. (common buckthorn) (Rhamnaceae) is a shrub or small tree of Eurasian origin that has become invasive in North America. The species was deliberately introduced in the late 1800s into north-eastern North America primarily as an ornamental hedge plant and shelterbelt tree and was then brought to Saskatchewan for the same purposes in the 1930s (Gourley 1985; Randall and Marnelli 1996; Archibold et al. 1997). It has escaped cultivation and has spread into most Canadian provinces and 34 states predominantly in the North-eastern and Midwestern United States (Zouhar 2011; USDA, NRCS 2013). *R. cathartica* is

declared as noxious in six U.S. states and two Canadian provinces (USDA, NRCS 2013; http://www.omafra.gov.on.ca/english/crops/facts/info_buckthorn.htm).

The most effective management strategies of common buckthorn involved a combination of cutting or girdling with applications of glyphosate or picloram/2,4-D (Qaderi et al. 2009). However, cutting trees near the base provides temporary control only because the plant is able to regrow from the stump (Maw 1984). *R. cathartica* can be controlled by annual or biennial prescribed burns for 5 or more years, but this may be inappropriate because of damage to native species (Heidorn 1991), and burning may also enhance populations (Zouhar 2011).

A project was initiated in 1964 to investigate the possibilities for biological control of *R. cathartica* in Canada (Malicky et al. 1970). The project was halted due to lack of funding 2 years later despite the fact that host-specific and effective herbivores were found (Malicky et al. 1970). In 2001, a new project was started to continue the work initiated by Malicky et al. (1970) and to reassess the potential for biological control of common buckthorn, especially considering the new paradigm shift towards recognizing the value of 'non-useful' native plants from a conservation and ecological perspective. This paper reports field observations and host specificity work done in 2002–2012 on selected biological control arthropod agents. Factors that limit the feasibility of biological control of common buckthorn in North America are discussed.

The target plant

Rhamnus cathartica is found throughout most of Europe, absent only in the extreme south, the area north of southern Sweden and also from most parts of the Iberian Peninsula (Tutin 1968; Anderberg 1998). Common buckthorn is a dioecious shrub or small tree 4–8 m tall, grey to black, 0.5- to 2.2-cm-long thorns grow at the tips of branches or in the forks of two branches. Leaves are toothed and may be arranged both alternately and oppositely on the same branch. Winter buds have dark scales. *R. cathartica* reproduces by seeds and regenerates by sprouting from cut or damaged stems or from the root crown following complete or partial stem removal (Zouhar 2011).

Rhamnus cathartica is adapted to a wide range of climatic and habitat conditions. In Western Europe, the species prefers mesophile to meso-xerophile open or half-shaded habitats on calcareous alkaline or neutral soils, but it can also be found in swampy areas (Rameau et al. 1989). In North America, *R. cathartica* seems to have an affinity for disturbed, fertile, calcium-rich, moist areas, open woods and woodland edges, but it can tolerate both dry and partially flooded conditions. It avoids extreme shading and drought (Qaderi et al. 2009). In North America, common buckthorn can become the dominant understory vegetation, displacing native vegetation through the formation of a dense canopy, thus creating a major threat to native biodiversity (Heidorn 1991; Catling 1997; Moffatt and McLachlan 2004). One of the most important impacts of *R. cathartica* is the alteration of ecosystem processes. Heneghan et al. (2006) found that soil in woodland areas where buckthorn dominates has higher percentage of nitrogen (N) and

carbon (C), modified nitrogen mineralization rates, elevated pH and higher soil moisture than those areas where buckthorn was not present. Indirect economic damage results from *R. cathartica* being an alternate host of the pathogenic fungus causing crown rust and leaf rust of oats, *Puccinia coronata* Corda. f. sp. *avenae* Eriks. & Henn. (Maw 1984), and the primary overwintering host plant for the soybean aphid *Aphis glycines* Matsumura (Zhu et al. 2006).

Natural enemies

In its native European range, the feeding guild of the 36 specialized arthropods reported by Gassmann et al. (2008) on *R. cathartica* is dominated by leaf feeders (17 spp), followed by sap suckers (12 spp), fruit or seed feeders (4 spp) and shoot/root borers (3 spp). Most of the 150 host associations between *R. cathartica* and fungal species are reported from Europe (Farr and Rossman 2012). Also, the cucumber mosaic virus was detected in *R. cathartica* in Germany (Kegler et al. 1994), and the occurrence of '*Candidatus* phytoplasma rhamni' in *R. cathartica* in Europe was confirmed by Jović et al. (2011).

In North America, the soybean aphid and other Hemiptera, such as the green stink bug, *Acrosternum hilare* (Say), or the Say's stink bug, *Chlorochroa sayi* Stal., have been commonly observed feeding on *R. cathartica* in Ontario (Qaderi et al. 2009). In a 2-year study, Yoder et al. (2008) recorded a total of 32 herbivorous arthropod species representing 20 families and six orders from common buckthorn in Minnesota. Only generalists were found and more Hemipterans were encountered than Lepidopterans. There are therefore much fewer insect and fungal species associated with *R. cathartica* in the introduced range in North America than in the native European range, and none appear to be at minimum genus specific. This is another possible reason for the invasiveness of common buckthorn in North America (Knight et al. 2007).

Prioritization of biological control agents for *Rhamnus cathartica*

A meta-analysis of results published after 2000 confirmed previous analyses that Chrysomelidae and Curculionidae families are the most effective weed biological control agents (Clewley et al. 2012). Reviews of successes and failures in 25 programmes against invasive trees and shrubs as of 2010 concluded that Curculionidae are the most effective agents against woody perennials followed by sap-sucking

species in the Phlaeothripidae (Thysanoptera) and Psyllidae (Hemiptera) families (Moran et al. 2004; Gassmann et al. 2010). Internal feeders and sap suckers were therefore prioritized for biological control of buckthorn. From 17 leaf-feeding moths known from buckthorn in Europe, five species were considered in an early stage of the project to confirm their field host records. Fruit- or seed-feeding insects may also present a good potential for biological control of buckthorn through directly reducing seed set and thus seedling establishment. In contrast to the lepidopterous species, seed-feeding midges seem to be potentially host specific enough for biological control of buckthorn.

Test plants are selected using criteria based on phylogenetic relatedness, biogeographic overlap and ecological similarity (Briese 2003). Although much disputed historically, the separation of *Frangula* from *Rhamnus* is now widely accepted, being supported by recent genetic data (Bolmgren and Oxelman 2004) with *Rhamnus* and *Frangula* being predominant in the Old World and New World, respectively (Grubov 1949; Johnston and Johnston 1978). Ten native *Rhamnus* taxa and 20 native *Frangula* taxa are known in North America (USDA, NRCS 2013). The native North American species *R. alnifolia* L'Hér., *R. lanceolata* Pursh and *F. caroliniana* (Walt.) Gray have a broad habitat range, and their geographical distribution overlaps most with common buckthorn. These are therefore key species in preliminary host range studies of potential biological control agents for *R. cathartica*.

Studied biological control candidates

Stem and root borers (3 species)

Three internal root/shoot borers are known on *R. cathartica* in Europe:

The stem-boring beetle *Oberea pedemontana* Chevrolat (Cerambycidae) is the only specialized beetle known from buckthorn in Europe. Although Conatarini and Garagnani (1980) observed beetles in Italy to infest *F. alnus* and to avoid adjacent *R. cathartica* bushes, we found larvae in the branches of both buckthorn species in two neighbouring sympatric sites in northern Italy. In Serbia, we sampled *O. pedemontana* larvae in seven *R. cathartica* sites and one *F. alnus* site, but no adults could be collected on the host trees in five collection trips made in early summer perhaps due to cryptic or nocturnal adult behaviour. Field records confirmed that the beetle lacks specificity at the genus level, and it

was rejected as a potential biological control agent of buckthorn in North America.

The root-boring moth *Synanthedon stomoxiformis* (Hübner) (Lep., Sesiidae) is widely distributed in the Palearctic region (Doczkal and Rennwald 1992). There are three subspecies which are all associated with *Rhamnus* and *Frangula* species in different geographical areas in Europe and Asia Minor (Spatenka et al. 1999). *Synanthedon stomoxiformis* ssp. *stomoxiformis*, which was observed from *R. cathartica* and *F. alnus* between central-southern Europe to the Urals, has been found relatively commonly at several *R. cathartica* sites in Serbia, where its presence has been confirmed by the use of the pheromone lure SYMY *Synanthedon myopaeformis* (PHEROBANK®). *S. stomoxiformis* ssp. *stomoxiformis* has a biennial life cycle (Spatenka et al. 1999) and oviposits on the trunk and branches of buckthorn. Newly hatched larvae crawl down or fall from the oviposition site and start mining in the stem base or root. During the second year, larvae move further down, boring into the roots. In the autumn of that year, the larva builds a long and visible reddish exit tube aboveground, made out of scraps of organic material, sawdust and silk, in which pupation occurs and from which the adult emerges the following spring.

Larval development tests

Methods

Because mating and oviposition could not be achieved in confinement, we used eggs laid by two females mated under field conditions for no-choice larval development tests. The tests were carried out on potted plants in 3–10 replicates of 6 or 12 larvae on 15 plant species and six plant families.

Results and conclusion

The moth completed development in 1 year on all buckthorn species (table 1). Optimal larval development was observed on the European species, *F. alnus* and *R. alpina*. Larval survival was lower on the target plant *R. cathartica*, and similar to that recorded on the native North American species *R. alnifolia* and *F. caroliniana*. No larvae were found on any of the other 10 species tested outside the genera *Rhamnus* and *Frangula*. Larval development tests confirmed that *S. stomoxiformis* ssp. *stomoxiformis* lacks specificity at the genus level. The difficulty of achieving mating

Table 1 Larval survival and development of *Synanthedon stomoxiformis* in no-choice conditions

	2004			2005			
	No. of larvae/ replicate	No. of replicates	Total no. of larvae	Total no. of pupae	No. of larvae	Total% survival	Per cent of plants attacked
Rhamnaceae							
<i>Rhamnus cathartica</i> L.	6	15	90	0	15	16.7	60
<i>R. alpina</i> L.	6	10	60	17	6	38.3	90
<i>R. alnifolia</i> L'Hér.*	6	10	60	6	6	20.0	70
<i>Frangula alnus</i> P. Mill.	6	10	60	8	19	45.0	100
<i>F. caroliniana</i> (Walt.) Gray*	6	10	60	3	9	20.0	30
<i>Hovenia dulcis</i> Thunb.	6	10	60	0	0	0	0
<i>Ziziphus ziziphus</i> (L.) Karst	6	4	24	0	0	0	0
Elaeagnaceae							
<i>Hippophae rhamnoides</i> L.	12	5	60	0	0	0	0
<i>Elaeagnus commutata</i> Bernh. ex Rydb.	12	5	60	0	0	0	0
<i>E. angustifolia</i> L.	6	3	18	0	0	0	0
Vitaceae							
<i>Parthenocissus tricuspidata</i> (Sieb. & Zucc.) Planch.	12	5	60	0	0	0	0
<i>Ampelopsis aconitifolia</i> Bunge	6	5	30	0	0	0	0
Grossulariaceae							
<i>Ribes rubrum</i> L.	15	4	60	0	0	0	0
Rosaceae							
<i>Sorbus aucuparia</i> L.	12	5	60	0	0	0	0
Caprifoliaceae							
<i>Lonicera xylosteum</i> L.	12	5	60	0	0	0	0

*Native North America species.

and oviposition in confinement makes it difficult to determine the ecological host range of this species.

The stem-boring moth *Sorhagenia janiszewskae* Riedl (Lep., Cosmopterigidae) is found in most parts of Europe, except south of the Alps (Malicky and Sobhian 1971). The larvae mine the current year's growing shoots of *F. alnus* and more rarely those of *R. cathartica* and *R. alpina* (Malicky and Sobhian 1971; Gassmann et al. 2008). The species lacks therefore specificity at the genus level. Attempts of oviposition tests failed to provide reliable results for determining the ecological host range of this species.

Sap suckers (3 species)

The leaf-margin curl galler *Trichohermes walkeri* (Foerster) (Hom., Triozidae) is known only from *R. cathartica* in Europe (Ossiannilsson 1992). It is one of the most common insect species on *R. cathartica* and certainly one of the most conspicuous. Adults emerged in August. In a biology study, females started ovipositing 3–4 weeks after emergence. Eggs were laid on leaf bud axils. The nymphs hatched in spring from

overwintered eggs and migrated to developing leaves, fed and induced rolling of the leaf margin. Host specificity was assessed using oviposition tests and subsequent larval and gall development.

No-choice adult survival and oviposition tests with newly emerged adults

Methods

Five buckthorn species and one no-plant control were individually tested with one newly emerged pair of *T. walkeri* in 12–20 replicates in small ventilated plastic cups (Ø 7.0 cm, height 8.5 cm) fixed on branches of potted plants. Adult mortality and oviposition were checked every 3–5 days, and males replaced. All plants were kept outdoors beneath a suspended tarpaulin, protected from rain and sun.

Results

Oviposition occurred only on the target plant, *R. cathartica* (table 2). First, eggs were recorded on *R. cathartica* about 30 days after set-up. Nearly 50% of the

Table 2 No-choice adult survival and oviposition tests with newly emerged *Trichoermes walkeri* adults

Test plants	No. of replicates (female)	No. of replicates with eggs	Total no. of eggs	Mean \pm SD of eggs/replicate (female)	Mean adult longevity \pm SD (days)
<i>Rhamnus cathartica</i>	20	11	1493	74.7 \pm 90.3	♂: 32.0 \pm 28.1 ♀: 43.2 \pm 27.0
<i>R. alnifolia</i> *	15	0	0	0	♂: 5.6 \pm 1.8 ♀: 8.2 \pm 2.7
<i>R. alpina</i>	15	0	0	0	♂: 5.1 \pm 2.2 ♀: 6.7 \pm 3.2
<i>Frangula alnus</i>	15	0	0	0	♂: 6.7 \pm 1.5 ♀: 9.9 \pm 3.6
<i>F. caroliniana</i> *	15	0	0	0	♂: 5.2 \pm 1.4 ♀: 8.4 \pm 3.4
No plant	12	0	0	0	♂: 4.5 \pm 1.2 ♀: 6.4 \pm 2.2

*Native North America species.

females on *R. cathartica* died before starting to oviposit. Females lived longer than males (overall means 14.0 ± 1.37 and 9.9 ± 1.37 days, respectively; $F_{1,174} = 4.56$, $P = 0.034$). Adult longevity was significantly higher on *R. cathartica* than on the other plant species ($F_{5,162} = 32.12$, $P < 0.001$). There was no significant difference in adult longevity between non-target plants and the no-plant control (Tukey HSD, $P > 0.45$) suggesting that little feeding occurred on these plants.

No-choice adult survival and oviposition tests with a 3-week feeding and pre-oviposition period on *R. cathartica*

Methods

Newly emerged adults were kept for 3 weeks on *R. cathartica* until they were ready to oviposit, as described above. Plant species were then individually tested with one pair of *T. walkeri* in 9–10 replicates as described above.

Results

Very little oviposition was recorded on non-target *Rhamnus* species with 3-week-old females (table 3). Females lived longer on *R. cathartica* than on the other plant species ($F_{4,43} = 31.46$, $P < 0.001$). Occasionally, the native North American species *R. alnifolia* sustained prolonged adult feeding: at the most, one female lived up to 26 days but did not lay any eggs. There was no significant difference in female longevity among the non-target plants.

Sequential no-choice oviposition tests

Because very little oviposition occurred on non-target *Rhamnus* species in no-choice tests, we tested oviposition in sequential no-choice tests. This was under the assumption that females would survive on native North American *Rhamnus* species long enough in a post-release environment (before having the possibility to feed again on *R. cathartica*), to oviposit on perhaps less preferred but acceptable plant species.

Table 3 No-choice adult survival and oviposition tests with *Trichoermes walkeri* (after a 3-week feeding and pre-oviposition period on *Rhamnus cathartica*)

Test plants	No. of replicates	No. of replicates with eggs	Total no. of eggs	Mean \pm SD of eggs/replicate (female)	Mean female longevity \pm SD (days)
<i>Rhamnus cathartica</i>	10	9	1164	116.3 \pm 75.4	38.5 \pm 15.6 ^a
<i>R. alnifolia</i> *	9	3	20	2.2 \pm 4.2	9.8 \pm 6.6 ^b
<i>R. alpina</i>	9	1	1	0.1 \pm 0.3	7.1 \pm 2.0 ^b
<i>Frangula alnus</i>	10	0	0	0	7.2 \pm 2.2 ^b
<i>F. caroliniana</i> *	10	0	0	0	6.4 \pm 1.0 ^b

*Native North America species.

Letters indicate significant differences between test plants (Tukey HSD, $P < 0.05$).

Methods

Females and males were first exposed to *R. cathartica* for 4 weeks in groups of three pairs in ventilated plastic cylinders as described above. After this period, pairs of *T. walkeri* were transferred individually onto potted test or target plants as described above. Because previous no-choice adult feeding and survival tests 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 plants were sequentially altered between the test plant and the target plant, *R. cathartica*. For each test plant, about 50% of the replicates started with the test plant and 50% with the target plant.

Results

Oviposition was high on the target plant, *R. cathartica* and the European species *R. alaternus* L. Oviposition was negligible on *R. alpina* and the native North American species *R. alnifolia* (table 4). Compared to the no-choice tests, oviposition on *R. cathartica* and female longevity were also reduced in the sequential no-choice oviposition tests, suggesting that the adults need to feed continuously on their field host to allow normal survival and reproductive output. In the *R. cathartica*–*F. alnus* series, all females died during the first exposure to *F. alnus*.

Single-choice tests

Because some oviposition occurred on *R. alnifolia* in sequential no-choice tests, single-choice tests were

Table 4 Sequential no-choice oviposition tests with *Trichoermes walkeri* (after a 3-week feeding and pre-oviposition period on *Rhamnus cathartica*)

	Mean no. of eggs/♀ (SD)	No. of ♀ × days of exposure	Mean female longevity in the series + SD (days)
Series 1 (N = 29)			
<i>Rhamnus cathartica</i>	11.7 ± 13.1	325	20.1 ± 12.7
<i>R. alnifolia</i> *	0.6 ± 1.4	339	
Series 2 (N = 16)			
<i>R. cathartica</i>	10.2 ± 22.4	161	19.8 ± 13.5
<i>R. alpina</i>	1.3 ± 2.7	143	
Series 3 (N = 5)			
<i>R. cathartica</i>	49.0 ± 28.6	107	21.4 ± 8.3
<i>R. alaternus</i>	60.4 ± 40.1	85	
Series 4 (N = 11)			
<i>R. cathartica</i>	0.5 ± 1.2	40	9.0 ± 4.4
<i>Frangula alnus</i>	0	59	

*Native North America species.

conducted to check whether this non-target North American species was attacked in the presence of the target weed.

Methods

Single-choice oviposition tests were carried out in five replicate 40 × 40 × 70 cm (l × w × h) cages each containing one potted *R. cathartica*, one potted *R. alnifolia* and three newly emerged *T. walkeri* pairs. All cages were kept outdoors beneath a suspended tarpaulin, protected from rain and sun.

Results

A total of 557 eggs were recorded on *R. cathartica* (mean = 111.4 ± 102.9; n = 5) and 24 eggs on *R. alnifolia* (mean = 4.8 ± 5.2; n = 5). On *R. alnifolia*, over 90% of the eggs were laid atypically on the trunk and branches. In contrast, on *R. cathartica*, over 60% of the eggs were laid on leaf bud axils, thus facilitating gall development in spring.

Larval and gall development

Methods

Branches with eggs of *T. walkeri* were marked with colour threads, and the pots were protected from contamination under a large gauze tent in a greenhouse until the end of November. All pots were then kept outdoors until late spring when the number of galled leaves, galls and larvae was counted.

Results

On *R. cathartica*, 13.7% of 2527 eggs developed into larvae in 2005–2006 and 30.5% of 855 eggs in 2008–2009. No galls and larvae were recorded from 24 eggs laid on *R. alnifolia* and from 302 eggs laid on *R. alaternus*.

Conclusions

Trichoermes walkeri is likely to be monophagous on *R. cathartica*. Some atypical oviposition without gall and larval development has been recorded on non-target hosts in the presence of the target weed. Because oviposition usually starts 3–4 weeks after adult emergence, oviposition on non-target hosts can be excluded in field situations where *R. cathartica* does not occur as *T. walkeri* females will die long before oviposition starts. *Frangula alnus* is not a suitable host for adult feeding and survival even in the alternate

presence of the target host. If feeding attempts occur, it is possible that *F. alnus* is lethal to the adults. By contrast, *R. alaternus* might provide a suitable food source for *T. walkeri*. In the sequential no-choice oviposition tests, the females laid a similar number of eggs on *R. cathartica* and *R. alaternus* although the leaf buds of the later species are smaller and tougher than those of *R. cathartica*. However, the leaf structure of *R. alaternus* is not suitable to allow gall and larval development of *T. walkeri*. More eggs laid on the native North American species *R. alnifolia* would be needed to ascertain that this species is not suitable for gall and larval development.

The discovery of a phytoplasma infection in *T. walkeri* adults and larvae (J. Jović unpublished results), however, renders this species problematic for biological control. 'Candidatus Phytoplasma rhamni' was also detected in 25% of all *R. cathartica* samples at 12 sites in Switzerland, Germany, Austria and Serbia, but not in samples of *R. alpina*, *R. saxatilis* Jacq., *R. rupestris* and *F. alnus* (Jović et al. 2011). In contrast, the phytoplasma was not detected in a composite sample of several trees from 75 *R. cathartica* sites in Minnesota, USA (Becker and Mollov, unpubl. results). The presence of the phytoplasma could not be associated with any particular symptoms although a lethal witches' broom disease of *R. cathartica* was observed for the first time in the Rhine Valley in south-western Germany in the 1990s (Mäurer and Seemüller 1996). Non-destructive phytoplasma detection and clean mass rear of *T. walkeri* would be theoretically possible by feeding the adults with artificial media for at least 48 h to insure that infection rate is zero (Landi et al. 2013). However, adult mortality would likely be high and fitness of survivors much reduced because of inadequate food source. Another option would be to expose adults to healthy *R. cathartica* plants. However, plants could not be screened with 100% confidence. For example, if the defence mechanisms disabled propagation of the phytoplasma in the plant sieve elements, then the psyllid could be a vector, but the plant would show a negative reading of the phytoplasma. Additionally, single adult psyllids inject a low amount of phytoplasma bodies, which require time to multiply to a level which is detectable.

'Candidatus Phytoplasma rhamni' was also detected in *Cacopsylla rhamnifolia* (Scott) (Hom., Psyllidae) and *Trioza rhamni* (Schrank) (Hom., Triozidae), two other Psyllid species associated with *R. cathartica* in Europe (J. Jović unpublished results), making these species problematic for biological control of common buckthorn.

Defoliators (5 species)

The leaf-feeding moth *Ancylis apicella* (Denis & Schiffermüller) (Lep., Tortricidae) is widely distributed in Europe from the British Islands to Scandinavia and Asia Minor (Razowski 2003). Malicky et al. (1970) found *A. apicella* on *F. alnus*, *R. cathartica*, *R. saxatilis*, *R. alaternus* and *R. alpina*. Early larval instars develop singly within a folded leaf, later spinning two leaves flatly together, eating parenchyma and blanching the leaves in irregular patches. *A. apicella* is bivoltine and overwinters as a larva in a silk web in the soil.

Larval development tests

Methods

Adults reared from field-collected larvae bred easily in captivity. Eggs were usually laid on the lower leaf surface close to the veins. Preliminary no-choice larval development tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Five plant species were included in the test in 15–25 replicates.

Results and conclusions

Ancylis apicella larvae completed development on *F. alnus*, *R. cathartica*, *R. alpina* and the native North American species *R. alnifolia* and *F. caroliniana* (table 5). The pupae produced on *R. alnifolia* weighed significantly less than those reared on *R. cathartica* ($F_{4,44}=12.11$, $P = 0.036$). Field observations and preliminary host range tests indicate that this species lacks specificity at the genus level. The difficulty of carrying out reliable oviposition tests in confinement makes it difficult to determine the ecological host range of *A. apicella*.

The geographical distribution of the congeneric leaf-feeding moth *Ancylis unculana* (Haworth) (Lep., Tortricidae) is similar to that of *A. apicella* (Razowski 2003). Unlike *A. apicella*, the species is found more commonly on *R. cathartica* than on *F. alnus* (Gassmann et al. 2008). No other field host is known in Europe. The biology of *A. unculana* and *A. apicella* are similar.

Larval development tests

Methods

Adults reared from field-collected larvae bred easily in captivity. Preliminary no-choice larval development

Table 5 Larval survival and development of *Ancylis apicella* and *A. unculana* on cut leaves in no-choice conditions

Test plant	No. replicates (L1)	Per cent larval development to the pupal stage	Pupal weight (mg) (mean \pm SD) (N)
<i>Ancylis apicella</i>			
<i>Rhamnus cathartica</i>	25	72	11.1 \pm 1.7 ^a (13)
<i>R. alpina</i>	10	40	9.1 \pm 2.2 ^{ab} (3)
<i>R. alnifolia</i> *	25	76	8.5 \pm 1.5 ^b (15)
<i>Frangula alnus</i>	15	53	9.6 \pm 1.3 ^{ab} (8)
<i>F. caroliniana</i> *	25	40	9.6 \pm 3.3 ^{ab} (10)
<i>Ancylis unculana</i>			
<i>Rhamnus cathartica</i>	30	37	9.6 \pm 1.6 (11)
<i>R. alpina</i>	20	10	8.9 \pm 3.4 (2)
<i>R. alnifolia</i> *	30	40	9.3 \pm 1.8 (12)
<i>Frangula alnus</i>	30	3	7.2 (1)
<i>F. caroliniana</i> *	30	7	4.8 (1)

*Native North America species.

Letters indicate significant differences between test plants (Tukey HSD, $P < 0.05$).

tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Five plant species were included in the test in 20–30 replicates.

Results and conclusion

Rhamnus cathartica and *Frangula* species are less suitable hosts than *R. cathartica* and the native North American species *R. alnifolia* (table 5). Similar to *A. apicella*, this species lacks specificity at the genus level, and the difficulty of carrying out reliable oviposition tests in confinement makes it difficult to determine its ecological host range.

The leaf-feeding moth *Triphosa dubitata* L. (Lep., Geometridae) is widely distributed in Europe, but it is rare in most Northern Europe (Forster and Wohlfahrt 1981). *T. dubitata* larvae were found in small numbers on *R. cathartica* and *R. alpina* in nearly all surveyed areas in Austria, Germany, Switzerland and the Czech Republic (Gassmann et al. 2008). There is one record of *T. dubitata* on *F. alnus* (Malicky et al. 1970). The species overwinters as an adult in natural caves (Jacobi and Menne 1991), and females mate prior to hibernation (Malicky et al. 1970). Eggs and first-instar larvae can be found in late April. The species is univoltine.

Larval development tests

Methods

Preliminary no-choice larval development tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Neonate larvae were reared from eggs collected from *R. cathartica* and *R. alpina* and tested separately. Three and five plant species were included in the test, respectively, each with 15–35 replicates.

Results and conclusion

Larval survival to the pupal stage was higher on the native North American species *R. alnifolia* than on *R. cathartica* and *R. alpina* in both populations (table 6). No larvae developed to the pupal stage on *F. alnus* and the native North American species *F. caroliniana*. Time to pupation was significantly higher on *R. alpina* than on *R. cathartica* and the native North American species *R. alnifolia* for both populations. Pupal weight was significantly affected by both the

Table 6 Larval survival and development of *Triphosa dubitata* on cut leaves in no-choice conditions

Test plant	No. of replicates (L1)	Per cent larval development to the pupal stage	Time to pupation (days) (mean \pm SD) (N)	Pupal weight (mg) (mean \pm SD) (N)
From <i>R. cathartica</i>				
<i>R. cathartica</i>	25	48	40.2 \pm 2.6 (12) ^a	140.0 \pm 19.7 (12) ^a
<i>R. alpina</i>	29	38	46.5 \pm 6.7 (11) ^b	116.5 \pm 18.7 (11) ^b
<i>R. alnifolia</i> *	21	81	40.6 \pm 4.7 (17) ^a	135.3 \pm 12.5 (17) ^{ab}
From <i>R. alpina</i>				
<i>R. cathartica</i>	35	49	40.5 \pm 4.4 (17) ^a	127.7 \pm 22.7 (17) ^a
<i>R. alpina</i>	35	37	48.9 \pm 6.4 (13) ^b	100.0 \pm 25.1 (13) ^b
<i>R. alnifolia</i> *	30	70	41.3 \pm 3.8 (21) ^a	127.4 \pm 23.3 (21) ^a
<i>F. caroliniana</i> *	19	0	0	0
<i>F. alnus</i>	15	0	0	0

*Native North America species.

Letters indicate significant differences between test plants (Tukey HSD, $P < 0.05$).

test plant and the field host plant (ANOVA: $F_{2,85} = 9.84^{***}$ and $F_{1,85} = 8.02^{**}$ for test plant and field host, respectively). *T. dubitata* is likely to be specific to the genus *Rhamnus*, but the native North American species *R. alnifolia* is a more suitable host for *T. dubitata* from either field host (*R. cathartica* and *R. alpina*) in no-choice larval development tests. Oviposition preference tests would be needed to assess the potential ecological host range of *T. dubitata*. However, this is not practical, given the adult biology of the species. These tests do not confirm species in genus *Frangula* as suitable host plants for larval development of *T. dubitata*.

The leaf-feeding moth *Philereme transversata* Hufnagel (Lep., Geometridae) is reported to be common across Europe (Carter 1987). *P. transversata* larvae were found in small numbers on *R. cathartica* and also very occasionally on *R. saxatilis*, *R. orbiculata* Bornm. and *F. alnus* (Malicky et al. 1970; Gassmann et al. 2008). The species is univoltine and hibernates in the egg stage.

Larval development tests

Methods

Adults reared from field-collected larvae did not breed easily in captivity. Preliminary no-choice larval development tests consisted of one neonate larva offered one test plant leaf in individual Petri dishes. Four plant species were included in the test, each with 20–55 replicates.

Results and conclusions

The native North American species *R. alnifolia* was a less suitable host than *R. cathartica* (table 7). *F. alnus*

and the native North American species *F. caroliniana* were not suitable host plants for larval development of this species. *P. transversata* is likely to be specific to the genus *Rhamnus*. In confinement, eggs of *P. transversata* were laid mostly on the cage frame making it difficult to determine the ecological host range of this species.

The congeneric leaf-feeding moth *Philereme vetulata* Denis & Schiffermüller (Lep., Geometridae) is widely distributed in Europe where it can be locally abundant (Forster and Wohlfahrt 1981). *P. vetulata* is associated exclusively with *R. cathartica* in Europe with the exception of one record on *R. alpina* (Malicky et al. 1965). Larvae feed within young folded leaves. *P. vetulata* is univoltine and overwinters in the egg stage on the bark of its host plant.

Larval development tests

Methods

Adults reared from field-collected larvae bred easily in cardboard cylinders (Ø 10 cm, height 27 cm). Preliminary no-choice larval development tests consisted of (i) one neonate larva offered cut shoots of four plant species with young folded leaves in individual Petri dishes and (ii) 5–10 neonate larvae transferred each onto potted plants of five species with newly developed leaf buds.

Results and conclusion

Drying of cut plant material resulted in a much higher larval mortality in Petri dishes than on potted plants (tables 7, 8). On cut shoots, larval development to the pupal stage was much higher on *R. cathartica* than on

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

Test plant	No. of replicates (L1)	Per cent larval development to the pupal stage	Time to pupation (days) (mean ± SD) (N)	Pupal weight (mg) (mean ± SD) (N)
<i>Philereme transversata</i>				
<i>Rhamnus cathartica</i>	40	23	35.7 ± 3.4 (9)	83.7 ± 24.5 (9)
<i>R. alnifolia</i> *	20	5	36	47
<i>Frangula alnus</i>	55	0	0	0
<i>F. caroliniana</i> *	40	0	0	0
<i>Philereme vetulata</i>				
<i>Rhamnus cathartica</i>	55	24	35.5 ± 2.1 (13)	53.9 ± 6.2 (13)
<i>R. alnifolia</i> *	50	6	40.7 ± 5.1 (3)	39.5 ± 23.2 (3)
<i>Frangula alnus</i>	60	0	0	0
<i>F. caroliniana</i> *	60	0	0	0

*Native North America species.

Table 8 Larval survival and development of *Philereme vetulata* on potted plants in no-choice conditions

Test Plant	No. of L1 transferred (No. of potted plants)	Per cent larval development to the pupal stage	Time to pupation (days) (mean \pm SD) (N)	Pupal weight (mg) (mean \pm SD) (N)
<i>Rhamnus cathartica</i>	119 (25)	72	32.8 \pm 3.7 (86) ^a	0.055 \pm 0.012 (86) ^a
<i>R. alpina</i>	80 (9)	60	37.3 \pm 3.9 (40) ^c	0.051 \pm 0.010 (48) ^{ab}
<i>R. alnifolia</i> *	58 (5)	69	34.5 \pm 2.4 (48) ^b	0.046 \pm 0.001 (40) ^b
<i>F. alnus</i>	80 (12)	0	0	0
<i>F. caroliniana</i> *	75 (10)	0	0	0

*Native North American species.

Letters indicate significant differences (Tukey HSD, $P < 0.05$).

the native North American species *R. alnifolia*. On potted plants, larval development to the pupal stage was similar on *R. cathartica*, *R. alpina* and *R. alnifolia*. However, pupae reared on *R. alnifolia* weighed significantly less than those reared on *R. cathartica*. The time to pupation was shortest on the field host *R. cathartica*. No larval establishment or damage was observed on *F. alnus* and *F. caroliniana*. *P. vetulata* appears to be specific to the genus *Rhamnus*. No oviposition was recorded on *R. cathartica* in confinement, thus making it difficult to determine the ecological host range of this species.

Fruit and seed feeders (1 species)

From the two midge species known on *R. cathartica* (*Wachtliella krumbholzi* Stelter and *Lasioptera kozarzewskella* Mar.), only the former has been collected during surveys for potential biological control agents of buckthorn (Gassmann et al. 2008). No midges were reared from the fruits of *F. alnus* collected at two sites in Austria and in Switzerland where *R. cathartica* and *F. alnus* co-occur. The main characteristics of fruits attacked by *W. krumbholzi* are a change in colour resembling premature fruit maturation, fruits larger in size and irregular shape. Once mature, the midge larva leaves the fruits and enters the soil to prepare a larval cocoon made of silk and debris. Field records suggest that *W. krumbholzi* is specific to *R. cathartica*. Due to the inability to obtain fruits on potted target and non-target *Rhamnus* species, no host specificity testing could be carried out with *W. krumbholzi*.

Discussion

In 2001, a new research programme to develop biological control for common buckthorn was initiated, taking into account increasing concerns about the safety of native plants in the potential release areas of

biological control agents. Candidate biological control agents would need to be monospecific to *R. cathartica* or their host ranges restricted to a few non-native species in the genus *Rhamnus*. Over 30 specialized insects were identified from *R. cathartica*, most of them with a likely lack of specificity at the species or genus level (Gassmann et al. 2008). Field observations and preliminary host range tests confirmed that the three internal feeders associated with buckthorn in Europe lack specificity at the genus level. Literature records of 12 leaf-feeding Lepidoptera known from buckthorn in Europe (Gassmann et al. 2008) combined with our work on another five species suggest that specificity requirements will not be met with those species as well.

Three psyllids, *T. walkeri*, *C. rhamnicolla* and *T. rhamni* are promising in terms of host specificity, but are infected with the plant disease 'Candidatus Phytoplasma rhamni'. Transmission trials with *R. cathartica* were negative twelve months after exposure to phytoplasma-infected *T. walkeri* adults (J. Jović unpublished results). Due to the limited research on this disease and as it is not known to be present in the United States, there is low potential that the psyllids would be approved for release in the United States.

There is increasing evidence from studies of biological control of invasive trees in South Africa that reduction in the levels of seeding and hence of seedling recruitment by biological control agents greatly facilitate the management of invasive woody plants (Moran et al. 2004). Seed-feeding midges seem to be potentially host specific enough for further research on biological control of buckthorn. However, attempts to work with *W. krumbholzi* proved to be difficult in a research setting as it was not possible to obtain adult fruiting trees of native North American *Rhamnus* species for testing in Switzerland.

All arthropods considered for biological control of buckthorns so far have been discarded from further

consideration because of either a lack of specificity at the species or genus level, the occurrence of a phytoplasma disease or the lack of feasibility of host range testing. For example, there are a few potentially genus-specific Lepidoptera, that is *P. vetulata*, *P. transversata* and *T. dubitata*, however, it was not possible to achieve oviposition in confinement and to assess their ecological host range. Given that Lepidoptera have not shown to contribute significantly to the successful control of invasive trees and shrubs (Moran et al. 2004; Gassmann et al. 2010), it is questionable to pursue host range testing with lepidopteran species to demonstrate a hypothetical specific host range. Other species known from buckthorns such as the mirids, *Heterocordylus erythrophthalmus* Hb and *Lygocoris rhamnicola* Reuter and the free-living or erineum gall mites, *Aceria rhamni* Roiv., *Tetra rhamni* Roiv., *Eriophyes rhamni* (Pgst) and *Phyllocoptes annulatus* (Nal.) were not considered in this project because of either their lack of visible impact on the target plant or the lack of feedback in using such organisms in biological control of weeds. Also, species in the genus *Rhamnus* are dioecious making plant breeding to the reproductive stage difficult for testing fruit- or seed-feeding candidate agents.

After 11 years of searching for biological control arthropods that are host specific and damaging to buckthorn, we conclude that we do not have any promising agents based on what is known to date. It is rarely the case in weed biological control that a project is terminated without field releasing any agents. One further recent example, however, includes biological control of *Potentilla recta* L. (sulphur cinquefoil) (Cortat et al. 2013).

Pathogens have not yet been considered for biological control of buckthorn. Based on literature and herbarium records from the Royal Botanical Gardens, Kew, UK, a few pathogens show potential as biological control agents, for example *Coniothyrium rhamnigenum* (Sacc.) Bubák (leaf spot damage), *Septoria rhamni-cathartica* Ces (leaf spot damage), *Mycosphaerella vogelii* (Syd.) Tomilin (leaf spot damage, host record on *R. alnifolia* needs to be confirmed) and *Phyllosticta rhamnicola* Desm. (leaf spot damage). We believe that pathogens could therefore potentially offer new opportunities for biological control of *R. cathartica* in North America.

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