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Published online: 11 Oct 2019.

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The host range and impact of *Aceria angustifoliae* (Eriophyidae), a potential biological control agent against Russian olive, *Elaeagnus angustifoliae* (Elaeagnaceae) in North America

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ABSTRACT

Russian Olive, *Elaeagnus angustifolia* was introduced into North America primarily as a wind break and shade tree. Today it is listed as a noxious weed in the U.S. and Canada. During field surveys in the native range, the eriophyid mite, *Aceria angustifoliae* was identified as a promising biological control agent. Results from no-choice and open-field tests suggest that this is a highly specialized herbivore and that the risk to non-target plants in North America is negligible. The impact study revealed significant reductions in fruit set, which will likely translate to a reduction in long-distance dispersal in the invaded range.

Text

Russian olive, *Elaeagnus angustifolia* L. (Elaeagnaceae), a small deciduous tree or shrub native to south-eastern Europe and Asia. After winter the leaves begin to appear in mid-spring (April) with flowering in late spring usually second half of May into June depending on local climatic conditions. The plant was reportedly first brought to the U.S. in the nineteenth century by Russian Mennonites who used it for windbreaks and as a shade tree. Throughout the twentieth century, it had been planted in numerous U.S. states and Canadian provinces (Katz & Shafroth, 2003). This species is naturalized and spreading from original plantings in the western U.S. and Canada (Katz & Shafroth, 2003). Russian olive has now become the fourth most frequently occurring woody riparian plant in the U.S.A. and the fifth most abundant in the western U.S.A. (Nagler et al., 2008). Russian olive currently occurs throughout the continental U.S.A. except for 10 states, mainly in the southeast. It is designated as a noxious weed in Colorado, Connecticut, New Mexico, Utah and Wyoming, as an invasive weed in California, Nebraska and Wisconsin, and as a regulated species in Montana. In Canada, Russian olive is recorded in all nine provinces.

There is growing evidence that Russian olive causes multiple negative environmental impacts in both terrestrial and aquatic compartments of riparian ecosystems (Fischer et al., 2012; Lesica & Miles, 2001). For example, it increases the nitrogen level in the soil, alters the composition of native plant communities (Lesica & Miles, 2001) and reduces the cavity nesting guild of bird communities (Fischer et al., 2012).

Due to the high cost and poor control with management options to date (Combs, 2010), surveys for biological control were initiated in 2007. However, because of the potential benefits of planting Russian olive, developing a classical biological control programme against it could give rise to a conflict of interest. It was therefore decided to focus initially on biological control agents that reduce the seed output and hence the long distance dispersal of this invader, without killing established trees. Several countries have been surveyed in the native range including Armenia, Georgia, Iran, Kazakhstan, Serbia, Turkey and Uzbekistan. The eriophyid mite, *Aceria angustifoliae* Denizhan, Monfreda, De Lillo, & Cobanoglu, 2008 (Eriophyidae), appears the most promising. In early spring, the mites that have overwintered in the buds begin to feed and develop on the vigorous growth of the buds and shoots, creating galls and deformities of the leaves, flowers and fruits (Figure 1). During the flowering and fruiting period (late May–June), the flower buds and developing fruits are preferred by the mite. The mite is present within the leaf galls throughout the spring and summer and when the tree begins to shed its leaves, the mites retreat into the latent buds to overwinter. The distribution of *A. angustifoliae* has not been published in the literature apart from the type specimen information collected from Kurtuluş Parkı, Ankara, Turkey, 852 m elev.; 39°51'43N, 32°43'58E (Denizhan et al., 2008). During field surveys in the native range of *E. angustifolia,* this mite has been collected from Serbia in the western range, through Turkey, Iran, Uzbekistan and China and does not appear to be restricted to particular climatic conditions or host varieties

Figure 1. Symptoms of *Aceria angustifoliae* feeding on the shoots, flowers and fruits of *Elaeagnus angustifolia.*

(M. Cristofaro, U. Schaffner & P. Weyl, unpublished data). The taxonomic identity of the different collections was confirmed by both morphological and molecular means (Vidović et al., 2014). Molecular identification was based on amplification of the barcode region of the mitochondrial cytochrome oxidase subunit I gene (COI) (genbank accession number KT070217.1). The similarity at the COI region for gall forming mites morphologically identified as *A. angustifoliae* among different populations ranged from 100% to 96.1%, with a 99.6% similarity between the Iranian and Serbian populations.

The aim of this study was to determine the host range and potential impact of this erio-phyid mite, through both laboratory and open field testing in the native range.

Using the centrifugal phylogenetic approach (Wapshere, 1974), a test plant list was developed for biological control candidates of Russian olive. Molecular analyses suggest that, within the Order Rosales, the family Elaeagnaceae belongs to a well-supported clade that includes the Rhamnaceae, Dirachmaceae and Barbeyaceae, of which only the Rhamnaceae contains native species found in North America. The selection of species (outlined in Table 1) within each family followed the risk categories defined in the TAG Reviewers Manual (USDA/APHIS/TAG, 2003).

The specimens of *A. angustifoliae* used for the host-range studies were collected from Russian olive populations near Shirvan, Iran (37°25'02.7"N 57°50'14.8"E). The mites that were used for host-range testing under open-field conditions were collected between May and July each year. While the mites for laboratory testing were collected from Shirvan usually in mid-June each year from 2011 to 2016 after which a colony was established in quarantine for testing in 2017. Since several new collections were made for testing, the mites were regularly screened using both molecular and morphological methods to confirm their identity. The host range of *A. angustifoliae* was assessed between 2011 and 2018, when a total of 33 non-target plant species were tested. Laboratory host specificity tests followed a no-choice design which is the most conservative test to define the funda-mental host range of any herbivore (Schaffner, 2001). For each cohort of no-choice testing under quarantine conditions there was a selection of test plants as well as control plants. The Russian olive used as control plants originated from several locations in Wyoming, Idaho and Montana. The plants correspond to two different genotypes that are present in North America: H1 and H4 (recently identified by Gaskin et al., 2019). The quarantine testing was done from mid-June to July of each year. The test plants that were used during the quarantine host-range tests were grown outdoors in pots (2–10 L pots depending on the size of the plant). Only plants that were in good health and had actively growing fresh shoots were selected for host-range testing. The selected trees were placed in the CABI Switzerland, quarantine approximately two weeks prior to the tests to allow them to adapt to the conditions. On occasion, some plants shed the leaves soon after being placed into quarantine and these were either given a longer period to recover or were discarded from tests. From the collected field material or colony, leaves showing visual symptoms of mite attack either from the colony or from field-collected shoots were checked under the microscope for the presence of living mites. If mites were abundant (over 100 per leaf gall) and active, 3–7 leaves were pinned to shoot tips close to young devel-oping plant material of each test and control plant. All trees were kept in the quarantine facility throughout the experiment (20–26°C; 16 h light: 8 h dark). Using this method, from 2011 to 2017, we were able to test under laboratory conditions between 6 and 68 repli-cates of 29 test plant species as well as 105 replicates of the control (Table 1). Each shoot that

Table 1. Results from no-choice host-specificity tests with *Aceria angustifoliae* conducted in quarantine at CABI Switzerland from 2011 2017 and the open field test in the experimental farm of Ferdowsi University, Mashhad, Iran from 2011 to 2018.

Family and plant species	Common name	Status in North America	No-choice tests			Open-field tests	
			# of shoots inoculated	# of shoots with mites	# of mites/ leaf (Mean ± SE)	# of trees tested	# of trees with mite attack
Category 1: Genetic types of the target weed species.							
Family ELAEAGNACEAE							
Elaeagnus angustifolia	Russian Olive	Introduced	105	82	4.9 ± 1.0	9	9
Category 2: Species in the same (or closely related) genus as the target weed, including environmentally and economically important species.							
Elaeagnus commutata	Silverberry	Native	67	2	0.2 ± 0.2	10	0
Elaeagnus multiflora	Cherry silverberry	Introduced, Cultivated	18	0	0	10	0
Elaeagnus pungens	Spiny Oleaster	Introduced	–	–	–	10	0
Elaeagnus umbellata	Autumn olive	Introduced, Cultivated	43	0	0	10	0
Category 3: Species in other genera of the same family as the target weed including environmentally and economically important species.							
Hippophae rhamnoides	Seabuckthorn	Introduced, Cultivated	41	0	0	7	0
Shepherdia argentea	Silver buffaloberry	Native	68	0	0	8	0
Shepherdia canadensis	Russet Buffaloberry	Native	11	0	0		
Shepherdia rotundifolia	Roundleaf buffaloberry	Native	24	0	0	8	0
Category 4: Threatened and endangered species in the same family as the target weed.			—	—	—		
Category 5: Species in other families in the same order that have some physiological, morphological, or biochemical similarities to the target weed, including environmentally and economically important species.							
Family RHAMNACEAE							
Berchemia scandens	Alabama supplejack	Native	15	0	0		
Ceanothus americanus	New Jersey tea	Native	21	0	0		
Ceanothus pallidus	New Jersey tea	Hybrid, Cultivated	9	0	0		
Condalia hookeri	Brazilian bluewood	Native	45	0	0		
Colubrina texensis	Texas hogplum	Native	44	0	0		
Frangula alnus	Glossy buckthorn	Native	20	0	0	10	0
Hovenia dulcis	Japanese raisintree	Introduced	18	0	0		
Karwinskia humboldtiana	Coyotillo	Native	37	0	0		
Rhamnus alnifolia	Alderleaf buckthorn	Native	19	0	0		
Rhamnus californica		Native	17	0	0		
Rhamnus cathartica	Common buckthorn	Introduced	18	0	0	10	0
Ziziphus jujuba	Common jujube	Introduced, Cultivated	35	0	0		

(Continued)

Table 1. Continued.

			No-choice tests			Open-field tests	
Family and plant species	Common name	Status in North America	# of shoots inoculated	# of shoots with mites	# of mites/leaf (Mean ± SE)	# of trees tested	# of trees with mite attack
Ziziphus spina-christi	Christ's thorn buckthorn	Introduced	–	–	–	10	0
Family ULMACEAE							
Celtis laevigata	Sugarberry	Native, Cultivated	18	0	0		
Celtis australis	Common hackberry	Introduced	15	0	0		
Family CANNABACEAE							
Humulus lupulus	Common hop	Native, Cultivated	15	0	0		
Family MORACEAE							
Maclura pomifera	Osage orange	Native	6	0	0		
Morus alba	White mulberry	Introduced, Cultivated	15	0	0		
Morus nigra	Black mulberry	Introduced, Cultivated	15	0	0	10	0
Family VITACEAE							
Nekemia (=Ampelopsis) arborea	Bogan peppervine	Native	15	0	0		
Parthenocissus quinquefolia	Virginia creeper	Native	27	0	0		
Family ROSACEAE							
Crataegus monogyna	Oneseed hawthorn	Introduced, Cultivated	18	0	0		
Malus pumila	Apple	Introduced, Cultivated	–	–	–	10	0
Prunus armeniacus	Apricot	Introduced, Cultivated	–	–	–	10	0
Pyracantha coccinea	Scarlet firethorn	Introduced, Cultivated	15	0	0		
Pyrus communis	Pear	Introduced, Cultivated	15	0	0		

Category 6: Species in other orders that have some physiological, morphological or biochemical similarities to the target weed, including environmentally and economically important species.

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Category 7: Any plant on which the biological control agent or its close relatives have been previously found or recorded to feed and/or reproduce.

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was inoculated was subsequently sampled after two weeks. The sampled leaf material was checked under a stereomicroscope and any living mites were counted, collected and identified, to be certain they were *A. angustifoliae* and not contamination.

The only plant species that supported a population of the mite, *A. angustifoliae* was Russian olive, with no apparent preference for population used in the tests. Since the separation of the genotypes is recent (Gaskin et al., 2019), it was unknown during the earlier testing to which genotype each individual tree belonged and thus all control plant data were lumped together. Two live individuals of *A. angustifoliae* were recorded in the same year (2014) on 2/67 sampled shoots of *Elaeagnus commutata* (Table 1). The two live mites found on two shoots of *E. commutata* cannot be easily explained, especially since numerous additional tests over three years could not replicate the results. It is possible

that the mites recorded were contamination, especially considering space constraints in quarantine the *E. commutata* could have been touching individuals of Russian olive or air movements within the room moved the mites from one tree to another.

To assess the host range of *A. angustifoliae* under natural open-field conditions, in spring 2011, a common garden plot with between 7 and 10 individual trees from 13 test species (Table 1) were randomly distributed in a plot with 5 m planting distance that was established on the experimental farm of Ferdowsi University, Mashhad, Iran. Each summer from 2011 to 2018, all trees were inoculated with *A. angustifoliae* three times between May and July by attaching three batches of mite-infested leaves to young shoots of all trees growing in the experimental garden using plastic clips or pins. All trees were regularly watered and monitored for symptoms of mite attack. Samples of control trees showing signs of mite attack and any suspicious leaves or shoots from test plants were regularly collected and any mites were identified. The only plant species that supported a population of *A. angustifoliae* and showed symptoms of mite attack (i.e. galling and malformations of the leaves) was the target species Russian olive, *E. angustifolia* (Table 1). No mites or symptoms of mite attack were recorded on any other species tested, despite the multiple inoculations over eight years.

Measuring the impact of this mite in the native range experimentally has been challenging since it takes many years for saplings to finally reach the flowering stage. In addition, the trees are environmentally sensitive during the flowering period, and on several occasions, the trees suddenly dropped all fruits and flowers for no apparent reason. Thus, the impact of *A. angustifoliae* on the reproductive output of Russian olive has been assessed by comparing Russian olive branches naturally infested by *A. angustifoliae* with branches that were free of symptoms of mite presence. On 9 and 10 June 2018, 20 infested and uninfested branches were collected at three sites near Belgrade, Serbia. The diameter and total length of the branches were measured to ensure that similar-sized (and potentially age) branches were compared. On each branch, ten random fruit-bearing stems were measured in length and the number of fruits on each counted. These stems are the current year's growth and the fruits are borne only on them. They are easily recognized since they are white due to a thick layer of hairs. The number of fruit/cm was then calculated by dividing the number of fruits by the length of the stem. The mean of these measurements was calculated per branch and this value compared between treatment, i.e. with or without mites using a 2-tailed *t*-test.

The mean ± SE branch diameter (un-infested 1.6 ± 0.1 cm vs. infested 1.7 ± 0.1 cm) and mean ± SE total length (un-infested 95.1 ± 3.4 cm vs. infested 96.5 ± 2.7 cm) of the branches collected were independent of mite presence ($t_{(38)} = 1.6$; $P = 0.107$ and $t_{(38)} = 0.3$; $P = 0.747$ respectively), suggesting that the branches were comparable. The mean length ± SE of fruit-bearing stems attacked by mites was reduced by about 50%, from 25.4 ± 0.4 cm to 12.7 ± 0.2 cm ($t_{(38)} = -25.6$; $P = 0.000$), while the mean ± SE of number of fruits per stem was reduced three fold, from 2.7 ± 0.2 fruits per stem to 0.9 ± 0.1 fruits per stem ($t_{(38)} = -7.155$; $P = 0.000$). There was also a significant reduction in the number of fruits per centimetre of stem, from 0.11 ± 0.01 fruits/cm where the mites were absent to 0.07 ± 0.01 fruits/cm when mites were present ($t_{(38)} = -3.474$; $P = 0.001$). These results suggest that the mites' impact is not only on the flower and fruit production but also on the development and ultimate length of these fruit-bearing stems, compounding further the reduction in reproductive output.

From the results of the host specificity tests conducted in Iran and Switzerland, in combination with host records of the mite in its native range (Denizhan et al., 2008), we conclude that *A. angustifoliae* is very likely to be restricted to Russian olive, *E. angustifolia* under natural conditions. We do not anticipate negative impacts on any native, non-target plant species. Under field conditions, *A. angustifoliae* negatively impacts the flower and fruit production of Russian olive by at least threefold, however, this may be an underestimate due to the compounding effect of the reduction in length of the fruit-bearing stem. Therefore, negative impacts on the spread of Russian olive can be expected if this mite were to be implemented as a biological control agent, provided that it will reach high densities in the introduced range. Considering the economic, social and ecological costs associated with Russian olive infestations in North America, we consider that these benefits outweigh unforeseen risks to non-target vegetation or other ecological interactions due the introduction of the mite.

Acknowledgements

Disclosure statement

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