



Annual Report 2010

Biological control of Russian olive, *Elaeagnus angustifolia*

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Summary

1. Russian olive, *Elaeagnus angustifolia*, is a shrub or small tree of Eurasian origin. It was originally planted in North America as a horticultural plant but has started escaping from cultivation and invading riparian and other moist habitats in western North America. This report summarizes the results of investigations carried out in 2010 on the biology, host specificity and impact of potential biological control agents of Russian olive. The investigations were conducted in close collaboration with the Biotechnology and Biological Control Agency (BBCA), Rome, Italy, the Uzbek Academy of Sciences, Tashkent, Uzbekistan, and the University of Mashhad, Iran.
2. In 2010, the herbivore assemblages associated with Russian olive were further surveyed in Uzbekistan as well as in north-eastern and north-western Iran. In Uzbekistan, we were able to survey in two pristine habitats of Russian olive and found two yet to be identified curculionid species damaging young shoot tips and flowers. Fruits collected in Iran were sent to CABI Europe – Switzerland to monitor adult emergence during spring 2011.
3. Significant progress was made in 2010 in assessing the host range of the gall mite *Aceria angustifoliae*. Suitable methods were developed to inoculate plant species under both open-field and quarantine conditions. First results obtained from host-range tests indicate that this mite is indeed highly specific, probably even strictly monophagous. Impact studies will need to be repeated in Turkey and continued in Iran. Preliminary data suggest that mite-infested branches produce fewer fruits. In summary, *A. angustifoliae* remains the agent with the highest priority.
4. The moth *Ananarsia eleagnella* attacks and develops inside fruits of the wild and cultivated forms of Russian olive. Life-cycle studies carried out in Iran indicate that this species has at least two generations in summer/autumn. While previous studies indicated that in no-choice feeding bioassays the larvae accept fruits of certain non-target species, no non-targets were attacked in choice feeding bioassays carried out in 2010. We therefore decided to continue working with this species.
5. In 2010, a common garden was established in Shirvan, Iran, consisting of Russian olive and eight woody test plant species. This garden will be used to study the host specificity and impact of *Aceria angustifoliae*, *Ananarsia eleagnella* and other potential agents under open-field conditions. The majority of the Russian olive trees inoculated with *Aceria angustifoliae* in summer 2010 showed signs of mite attack at the end of the season, but none of the test plant species did. Additional woody test species will be planted in the garden in 2011.
6. In 2011, we will continue our investigations on the host range and impact of the mite *Aceria angustifoliae* and the moth *Ananarsia eleagnella* under quarantine and open-field conditions. Depending on the level of funding, we will also consider extending surveys for new biological control candidates into the Central Asian mountain ranges such as the Pamir (Uzbekistan) or Tien Shan (China) ranges.

1. Introduction

Russian olive (*Elaeagnus angustifolia*), a small tree or multi-stemmed shrub native to south-eastern Europe and Asia, was introduced to North America in the late 19th century as a horticultural plant and was used for hedgerows and erosion control, reclamation purposes, and as a shade tree and a nectar source for honey bees. According to Nagler *et al.* (2010), Russian olive escaped cultivation between the 1920s and 1950s and has now become the fourth most frequently occurring woody riparian plant and the fifth most abundant riparian plant in the western USA. In contrast to saltcedar (*Tamarix* spp.), which is limited in its distribution by its sensitivity to hard frosts, Russian olive can survive harsh winter temperatures and is therefore able to colonize higher latitudes. To date, it is a declared noxious weed in four US states (Colorado, Connecticut, New Mexico, Wyoming), a number that is likely to increase in the near future. Habitat suitability maps for Russian olive created by the National Institute of Invasive Species Science (NIISS; www.niiss.org) indicate that Russian olive has not yet colonized its whole potential range in North America.

Because of the potential benefits of planting Russian olive near human settlements, developing a classical biological control programme against Russian olive could give rise to a conflict of interest. It was therefore decided to initially focus on biological control agents that reduce the seed output, and hence the spread, of this invader without killing established trees. However, Russian olive has been spreading to such an extent in recent decades that the economic and environmental damage caused by the woody invader may soon outweigh its horticultural benefits nationally.

In 2007, CABI Europe – Switzerland (CABI E-CH) and the Biotechnology and Biological Control Agency (BBCA), Italy, initiated surveys in Eurasia to determine whether biological control of Russian olive is a feasible option and to come up with a priority list of potential agents. This report summarizes the work conducted by CABI and BBCA in the fourth year of the Russian olive project.

2. Work programme for period under report

The following work programme was proposed for 2010.

Search for biological control candidates

- Continue quantitative surveys in Uzbekistan and Iran, eventually also in eastern Asia;
- Send herbivores and pathogens to taxonomists for identification.

***Aceria angustifoliae* (Acari, Eriophyidae)**

- Establish rearing under quarantine conditions;
- Repeat open-field host-range test in Iran;
- Start host-range testing in quarantine;
- Repeat impact experiments in Iran and Turkey.

***Ananarsia eleagnella* (Lepidoptera, Gelechiidae)**

- Determine field host range by surveying closely related plant species;
- Set up laboratory rearing if a sufficient number of adults emerge from collected fruits;
- Continue with host-range testing.

Rearing of biological control candidates

- Establish rearing colonies and study biology of 1–2 new biological control candidates.

Test plant list:

- Finalize test plant list and submit to TAG.

3. Field surveys

3.1. Surveys in Iran and Uzbekistan

METHODS In 2010, surveys of the herbivore assemblages associated with Russian olive were carried out in Uzbekistan in two pristine Russian olive habitats, i.e. in one of last remnants of the formerly widely distributed togay forest and in the mountain forests in the foothills of the Pamir Mountains (Plate 1). All herbivores were collected, and species not found during previous surveys sent to taxonomists for identification.

Also, surveys of fruit-attacking insects were made in the Mashhad (north-eastern) and Täbriz (north-western) regions of Iran. In both regions, some 1000 fruits each were collected at five different sites. All fruits were sent to CABI E-CH where they are being overwintered in quarantine for adult emergence in 2011.

Field surveys in Uzbekistan were done in collaboration with Prof. A. Khamraev, Uzbek Academy of Sciences, and Prof. H. M. Khaidarov, University of Samarkant, and in Iran with Dr R. Ghorbani and Dr G. Asadi, Mashhad University.



Plate 1. Two of the original habitats of Russian olive in Central Asia: mountain forests (left) and togay forest (right; photos: U. Schaffner).

RESULTS Surveys in the mountain forests in north-eastern Uzbekistan revealed two curculionid species that have not been found during previous surveys in Turkey or Uzbekistan. At least one of the two species significantly damages young shoot tips and also feeds on flowers (Plate 2). Specimens of both weevils have been sent to a taxonomist for identification.

So far, no adult insects have emerged from the fruits collected in the Mashhad and Täbriz regions of Iran in autumn 2010. Monitoring of emergence will be continued in spring 2011. However, larvae of a lepidopteran species have been observed crawling between the fruits; these larvae are considerably larger than those of *Ananarsia eleagnella*, suggesting that a new herbivore species has been found attacking fruits in the Täbriz region.



Plate 2. An as yet unidentified curculionid weevil found during surveys in the mountain forests of north-eastern Uzbekistan (photo: U. Schaffner).

3.2. Summary and outlook

The identification of most insects collected in Iran and Uzbekistan in 2010 is still pending. However, it appears that at least one new potential biological control agent has been found during these surveys. It has been hypothesized that the centre of origin of Russian olive may be somewhere in the mountain ranges of central or eastern Asia (Prof. H. M. Khaidarov, pers. comm.). Depending on the level of funding, it is therefore planned to expand surveys further into the mountain regions of eastern Uzbekistan and/or western China.

4. *Aceria angustifoliae* DENIZHAN (Acari, Eriophyoidae)

This gall mite, which was recently described by Denizhan *et al.* (2008), attacks shoot tips and inflorescences of Russian olive. We therefore believe that this species is a promising biological control candidate that has the potential to reduce the reproductive output of Russian olive trees. During surveys carried out in the first two years of this project, we found the mite at several sites in Turkey and Iran. In 2010, emphasis was put on developing and improving the success rate of artificial inoculation of Russian olive with *A. angustifoliae* under both open-field and quarantine conditions and then using this method to start conducting host-specificity tests.

4.1. Developing methods for host-range testing

METHODS Different methods for experimentally inoculating Russian olive trees with *A. angustifoliae* were tested both under open-field conditions in Iran and under quarantine conditions at CABI E-CH. In early May, 20 Russian olive trees were planted in a common garden on the campus of Shirvan University, Iran. Sites near Shirvan with a natural population of *A. angustifoliae* were visited once a week during spring and summer 2010. Samples of shoots tips were taken to the laboratory and checked for the presence of adult and juvenile mites on leaves and flowers. The first infestation in the area was observed on 27 May 2010. Subsequently, various attempts were made to inoculate Russian olive trees planted in the experimental garden:

- On 29 May, 50-cm-long mite-infested shoot pieces were fixed to shoot tips of Russian olive trees;
- On 4 June, inoculation was repeated by fixing 5- to 10-cm-long infested side-shoots to shoot tips of Russian olive trees;
- On 21 June, all the trees were inoculated for a third time, but this time infested leaves were attached to the leaves of Russian olive trees using plastic clips.

The Russian olive trees were regularly watered and monitored for signs of mite attack throughout the season.

To develop methods for experimental inoculation of potted *E. angustifolia* trees under quarantine conditions, *A. angustifoliae* was collected in central Anatolia, Turkey, and shipped to the quarantine facility at CABI E-CH. The Russian olive trees used in these bioassays were grown from roots collected by John L. Baker in Wyoming, USA. First collections of *A. angustifoliae* were made in May and early June, but by the time the shipments arrived in Delémont all mites were dead. An additional collection was therefore made in mid June, and the mite-infested Russian

olive shoots were hand-carried to Delémont. This time the mites were still active upon arrival. In quarantine, potted Russian olive trees were inoculated using two different methods:

- Some 50 mites were transferred using a fine paintbrush under a stereo microscope onto one or two leaves near a shoot tip;
- 5- to 10-cm-long infested side-shoots were attached to shoot tips of Russian olive trees using string.

All trees were maintained in quarantine throughout the experiment. The shoot tips that were inoculated were marked and repeatedly monitored for the presence of mites during summer and autumn 2010.

RESULTS The first two attempts to inoculate the Russian olive trees in the experimental garden at Shirvan University were unsuccessful. However, on 5 July first infestations were observed on two Russian olive trees to which mite-infested leaves had been attached using plastic clips. Single leaves of the Russian olive trees with signs of mite attack were inspected under the microscope, and the presence of active mites confirmed. On 6 August, no further active mites were observed, suggesting that they had started overwintering by then. By that time six Russian olive trees had shown signs of attack by *A. angustifoliae*.

Inoculation of potted Russian olive trees under quarantine conditions was also successful. Signs of mite attack and active mites were detected in five out of 26 cases where 50 mites were transferred onto shoot tips of Russian olive trees. Moreover, attack by *A. angustifoliae* was confirmed in seven out of 12 attempts where single infested leaves were attached to Russian olive shoot tips. One month after inoculation, mites were found on leaves that were up to 15 cm away from the release site. In many cases, only a few mites were found per inspected leaf, and the leaves showed only weak or moderate signs of mite attack. However, in a few cases up to 50 individuals were found per leaf, and the leaves were significantly damaged. In contrast to the observations made under field conditions, the mites were still active in mid September.



Plate 3. Russian olive leaves infested by *Aceria angustifoliae* (photo: G. Asadi).

4.2. Open-field host-specificity test

METHODS In spring 2010, an common garden was set up on the campus of Shirvan University by planting ten individuals each of Russian olive and seven Eurasian test plant species (Table 1). The trees were planted in a grid of 3 × 4 m in five blocks of two plants of each species.

Given the experience gained in developing methods for host-specificity tests (see section 4.1), all 80 trees were inoculated on 6 July by attaching infested Russian olive leaves to the leaves of exposed trees using plastic clips. All trees were regularly monitored for signs of mite attack.

RESULTS On 25 July, six out of ten Russian olive trees showed signs of mite attack. The presence of *A. angustifoliae* was confirmed by visual inspection under the microscope. None of the test plants revealed any signs of mite attack (Table 1).

Table 1. Results of host-specificity tests with *Aceria angustifoliae* conducted under open-field conditions in Iran in 2010.

Tree family/species	No. of trees exposed	No. of trees with mite attack
ELAEAGNACEAE		
<i>Elaeagnus angustifolia</i>	10	6
RHAMNACEAE		
<i>Ziziphus jujube</i>	10	0
MORACEAE		
<i>Morus alba</i>	10	0
<i>M. rubra</i>	10	0
VITACEAE		
<i>Vitis vinifera</i>	10	0
ROSACEAE		
<i>Malus domestica</i>	10	0
<i>Cerasus avium</i>	10	0
<i>Rosa canina</i>	10	0



Plate 4. Inoculation of the test plant species *Morus alba* with the mite *Aceria angustifoliae* in the open-field host-range test carried out at Shirvan, Iran. Infested Russian olive leaves were attached to the shoots of the test plant using plastic clips (photos: G. Asadi).

4.3. No-choice host-specificity tests

METHODS Since, under quarantine conditions, successful inoculation of Russian olive trees with *A. angustifoliae* was highest where single infested leaves were attached to Russian olive shoot tips (see above), this method was used to start no-choice host-specificity tests at CABI E-CH. Investigations in 2010 focused on test plant species belonging to the same family as Russian olive, i.e. the Elaeagnaceae (Table 2). Test plant species and Russian olive trees were inoculated during the second half of June. Subsequently, leaves next to the inoculation sites were inspected under the microscope for the presence of *A. angustifoliae* at 2-week intervals until September.

RESULTS Seven out of the ten inoculation attempts on Russian olive were successful. However, the average number of mites found per leaf was low (Table 2). None of the test plants revealed signs of mite attack, and no *A. angustifoliae* were detected during inspections of their leaves under the microscope.

Table 2. Results of no-choice host-range tests with *Aceria angustifoliae* under quarantine conditions in 2010. All test plants belong to the family Elaeagnaceae.

Plant species	Status ^a	No. of transfers	No. of attacks	No. of mites/attack
<i>Elaeagnus angustifolia</i>	I	10	7	5.4 ± 2.2
<i>Elaeagnus commutata</i>	N	6	0	0.0
<i>Hippophae rhamnoides</i>	C	10	0	0.0
<i>Shepherdia argentea</i>	N	6	0	0.0

^a I = invasive in North America; N = native to North America; C = cultivated in North America.

4.4. Experimental assessment of impact

METHODS In 2010, two different approaches were used to assess the impact of *A. angustifoliae* on fruit production of Russian olive. Firstly, a long-term experiment was set up in the common garden at Shirvan University to compare the reproductive output of Russian olive trees that were either experimentally inoculated with the mite, or sprayed with an acaricide to keep them free of attack (control plants). As described above, twenty Russian olive trees were planted in 2010, and successful infestation was achieved on six of the ten inoculated Russian olive trees. The other ten Russian olive trees were used as controls.

The second approach to assess the impact of *A. angustifoliae* on the reproductive output of Russian olive was to monitor the probability of transition from flower bud formation to fruit ripening (i) on branches of Russian olive that are attacked by the mite and (ii) on branches that are free of mite attack. Flower bud formation in spring occurs at a time when mites are just about to become active after winter dormancy. In contrast, mite damage is most pronounced during flowering and during early fruit formation. Previous comparative studies indicated that fruit production on mite-attacked branches is significantly lower than on branches without mite attack. Also, fruit production was found to be significantly lower on branch segments with mite

attack than on segments of the same branch that showed no signs of mite attack (Schaffner *et al.*, 2010). However, such comparative studies do not allow us to determine whether the reduced fruit production on attacked branches is due to mite attack, or whether mites prefer young shoots that invest more in vegetative than in reproductive growth. Therefore, a trip was made to central Turkey in early spring 2010 to mark individual shoots on naturally growing Russian olive trees at the time of flower bud formation. On the apical 50 cm of each branch, the number of side-shoots was counted and the number of leaves and flower buds on each side-shoot recorded. Care was taken to only sample branches on the sun-exposed side of each tree, since Russian olive trees tend to produce more fruits on this side of the tree. The site was revisited several times during the summer. On each occasion the number of infested and non-infested leaves and flowers as well as the number of healthy and damaged fruits were recorded on each marked branch.

RESULTS As indicated above, first signs of mite attack on the experimentally inoculated Russian olive trees in the garden at Shirvan University became apparent in early July, and mite damage remained restricted to the neighbourhood of the inoculation site. We expected that only minor mite damage would occur during the first season; we explicitly chose to set up this open-field impact study as a long-term experiment. The fact that we have planted Russian olive trees of similar age and size in a protected common garden will allow us to study mite population build-up over several years and compare growth and reproductive output of infested and non-infested Russian olive trees.

The monitoring study in central Turkey started well; the numbers of flowers, leaves and young fruits were recorded on each side-shoot of each marked branch in April and in mid May. However, when the site was revisited again in late June, all Russian olive trees in the study region had aborted almost all of their young fruits. We suspect that fruit abortion happened because of a hard frost that occurred very late in the season, i.e. during the first half of June. It was therefore decided to stop the experiment in Turkey and repeat it in 2011.

4.5. Summary and outlook

In 2010, we succeeded in developing methods to experimentally inoculate Russian olive trees with *A. angustifoliae* under both field and quarantine conditions. The fact that the best methods resulted in attack rates of more than 50% both in the field and in quarantine allowed us to start meaningful host-range tests. The results obtained so far suggest that this mite has a very narrow host range and may be strictly monophagous. Not even the closely related native North American *Elaeagnus commutata* was attacked.

While the long-term impact study in Iran was successfully established, the monitoring study carried out in Turkey did not provide new information regarding the impact of this mite on the reproductive output of Russian olive. First results obtained in 2010 suggest that branches attacked by the mite produce only very few fruits; depending on the extent of infestation, *A. angustifoliae* might reduce fruit production in infested branches by as much as 50–75% (Schaffner *et al.*, 2010). We therefore think it is justified to continue investigations with *A. angustifoliae* as a biological control agent against Russian olive. However, more detailed information on the actual impact of this mite on Russian olive may only become available in a few years time.

In 2011, we plan to continue with both open-field and quarantine host-range testing, as well as with the open-field impact studies. We will further extend the experimental

garden at Shirvan University by planting and inoculating additional test plant species, mainly from the families Elaeagnaceae and Rhamnaceae.

5. *Ananarsia eleagnella* (KUZNETZOV)

This moth is the most common fruit-feeding herbivore on Russian olive in Uzbekistan and Iran. To study the life cycle of the moth under field conditions, Russian olive fruits were collected in northern Iran every fortnight from early July 2010 onwards. Fruits were taken back to the laboratory of Shirvan University and checked for infestation by *A. eleagnella*. The first infestation was observed on 1 August, suggesting that adults of the first generation were active during the second half of July. Larvae were found to feed on the fruit pericarp, but they also penetrated the seed coat and fed inside the seeds (Plate 5). Larvae were transferred to Petri dishes containing Russian olive fruits and kept in the laboratory under ambient temperature. First pupae were observed on 14 August, and adult emergence started on 19 August. Mating was observed within the first hours after emergence. In insect cages, females oviposited directly onto young Russian olive fruits. The average lifespan of adult males was one to two days, and of the females three to four days. Hence, the life cycle of *A. eleagnella* lasts approximately 25–30 days. These observations also indicate that this moth has at least two generations in the autumn.

Seeds of infested Russian olive fruits were collected in north-eastern (Mashhad) and north-western (Täbriz) Iran, and a first experiment was set up in autumn 2010 to compare the viability of seeds from fruits on branches attacked by *A. eleagnella* and from fruits on non-attacked branches. However, none of the seeds germinated. Therefore, all remaining seeds were transferred to a fridge (temperature 5°C) for cold stratification, and the experiment will be repeated in spring 2011.



Plate 5. Russian olive fruits infested by *Ananarsia eleagnella*. Note the feeding damage on the seed inside the fruit.

5.1. Single-choice host-range testing

METHODS Larvae obtained from laboratory rearing were used for single-choice larval development tests. Ten larvae were transferred into a Petri dish containing

three fruits each of Russian olive and of a test plant species, with three replicates of each test species (see Table 3). The Petri dishes were kept in a laboratory under ambient temperature, and larval feeding and survival was monitored over a period of 14 days.

RESULTS In this single-choice bioassay, all larvae accepted Russian olive fruits, but none of the test plant species were attacked (Table 3).

Table 3. Results from single-choice larval development tests with the fruit-attacking moth *Ananarsia eleagnella* in 2010. Three fruits each of a test plant species and of Russian olive were offered to ten larvae in a Petri dish.

Plant family/species	No. of Petri dishes	No. of larvae feeding on	
		test species	control species
MORACEAE			
<i>Ficus carica</i>	3	0	30
VITACEAE			
<i>Vitis vinifera</i>	3	0	30
ROSACEAE			
<i>Crataegus oxyacantha</i>	3	0	30
<i>Malus domestica</i>	3	0	30
<i>Prunus persica</i>	3	0	30
<i>Rosa canina</i>	3	0	30

5.2. Summary and outlook

According to Ponomarenko (1997), the ecological host range of *A. eleagnella* is restricted to the genera *Elaeagnus* and *Hippophae* (Elaeagnaceae). First investigations on the host specificity under no-choice conditions indicated, however, that larvae of this moth have a relatively broad fundamental host range; under no-choice conditions, larvae fed on fruits of relatively distantly related plant species, including apple and hawthorn (*Crataegus monogyna* Jacq.). However, the preliminary single-choice host-range studies conducted this year suggest that the larvae have a very strong preference for Russian olive. These results are in agreement with the fact that this moth has never been recorded feeding on fruits of cultivated or ornamental species of the family Rosaceae. Moreover, Schaffner *et al.* (2010) found no evidence for attack on sea buckthorn (*Hippophae rhamnoides*) growing at a site with a large *A. eleagnella* population near Samarkant, Uzbekistan. Sea buckthorn, a medicinal plant which is also cultivated in North America, has soft-bodied fruits filled with a reddish juice, which appears to render these fruits unsuitable for larvae that, like *A. eleagnella*, develop internally. Hence, evidence is accumulating that this moth has indeed a very narrow ecological host range.

The host range of *A. eleagnella* will be further investigated in Iran in 2011 by extending the no-choice and single-choice tests in Petri dishes. Because fruits of many of the test plant species degenerate quickly when offered in Petri dishes, these

tests will not allow us to test for complete larval development. Therefore, we will also transfer adults of *A. eleagnella* onto bagged branches of the control and test plant species in the experimental garden at Shirvan University, to assess whether the plants are accepted for oviposition and whether they support complete larval development. As indicated above, the list of test plant species (cf. Table 1) will be extended by planting additional trees, particularly of the families Elaeagnaceae and Rhamnaceae, in the garden.

Provided that we are able to send infested Russian olive fruits from Uzbekistan to CABI E-CH, we will also repeat our efforts at establishing a quarantine rearing colony that will allow us to conduct host-range tests with North American test plant species under quarantine conditions.

6. Test plant list

Using the centrifugal phylogenetic approach proposed by Wapshere (1974) and revised by Briese (2003), we developed a list of proposed plant species for testing the host specificity of biological control candidates for Russian olive (Schaffner *et al.*, 2009). The test plant list of the Russian olive biological control project was presented and discussed at the Annual Meeting of the Technical Advisory Group (TAG) in Mission, Texas, in early October 2009.

The test plant list with an accompanying description was subsequently revised and sent to the project partners in the USA. Once the general information on the target weed and the rationale for conducting a biological control project are completed, the list will be formally submitted to TAG.

7. Work programme proposed for 2011

In line with the results obtained during the field season 2010, it is proposed that work in 2011 should focus on the following elements.

Search for biological control candidates

- Continue quantitative surveys in Uzbekistan and Iran, eventually also in China;
- Send herbivores and pathogens to taxonomists for identification.

***Aceria angustifoliae* (Acari, Eriophyidae)**

- Establish rearing under quarantine conditions;
- Repeat open-field host-range test in Iran;
- Continue host-range testing in quarantine;
- Repeat/continue impact experiments in Iran and Turkey.

***Ananarsia eleagnella* (Lepidoptera, Gelechiidae)**

- Conduct laboratory no-choice and choice tests;
- Determine the field host range by surveying closely related plant species in the native range;
- Carry out a no-choice oviposition and larval development test in the field in Iran;

- Set up laboratory rearing if a sufficient number of adults emerge from collected fruits;
- Continue with host-range testing under both field and quarantine conditions.

Rearing of biological control candidates

- Establish rearing colonies and study biology of 1–2 new biological control candidates.

Test plant list

- Finalize test plant list and submit to TAG.

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