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Article

The formation of the consortia relations of *Molipteryx fuliginosa* (Uhler, 1860) (Hemiptera, Coreidae) with *Ambrosia artemisiifolia* in the Primorskii Krai of Russia

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Abstract
Currently, invasive species spreading is becoming a key problem on the global scale. Scope of invasive species control get solved at governmental level in many countries; hundreds of billions dollars a year are allocated as financial support; global programs are created. For the first time, biological control of invasive species *Ambrosia artemisiifolia* using an introduced phytophage insect was applied in Russia (former USSR) in the 60-70s years of the 20th century and it remains a unique large-scale example until to nowadays. Ambrosia leaf beetle (*Zygogramma suturalis* (Fabricius, 1775) was brought to the Russian Far East for biological control of *Ambrosia artemisiifolia* in the 80-90s years of the 20th century for the first time, however the targeted result was not achieved. At present, *Zygogramma suturalis* were adapted at the Primorskii Krai in the south of the Far East, but due to climatic conditions its numbers are low and, as a result, the impact on ambrosia is minimal. The search for native insect species with capable of effectively suppressing the spread of aggressive quarantine weed in local conditions remains in the area of focus. At the moment, two species of native leaf beetles have been identified, using *Ambrosia* as an additional element in the diet. Climate changes and the shift of biogeographic boundaries cause the natural expansion of the areals of some East Asian insect species to the north within the Russian Far East. Bug-edge *Molipteryx fuliginosa* (Uhler, 1860) is one of such species. Expanding the area to the north and east, the species also corrects trophic preferences. As a result of our research in the south of Primorskii Krai, the trophic relations of *Molipteryx fuliginosa* were studied in the field conditions and in rearing cages. The same work was carried out in places where *Ambrosia artemisiifolia* were growing in a great number. For insects observing were used standard methods. As a result, firstly was established and confirmed that the bug-edge uses *Ambrosia artemisiifolia* as a feed plant only in select developmental stages, including imago. The feeding of larvae of IV – V ages was noted in natural conditions. The long-time *Molipteryx fuliginosa* development cycle on ambrosia leads to break the course of normal plant ontogenesis and causes wilting of individual organs.

Key words: trophic relations, bug-edge, invasion organisms, the Russian Far East.

Introduction

The problem of introduction and spread of invasive species is the key component of global changes taking place in the existing ecosystems and their functioning. Many invasive plants are highly allergenic. It has been estimated that annual medical costs for treatment of allergies inflicted by invasive plants amount to 290 million euro in Austria and Germany (Katza *et al*., 2014), 110 million euro in Hungary (Oude Lansink *et al.*, 2018), and 120 million USD in the USA (Susaeta *et al*., 2016). Yield loss caused by common ragweed (*Ambrosia artemisiifolia* L.) can be as high as millions euro: for example, in Hungary this figure was 130 million euro per year (Oude Lansink *et al.*, 2018).

Today, funds ranging from several dozens to several hundreds billion USD are annually spent to combat invasive species (Culliney, 2005). SMARTER, HALT-AMBROSIA, and NOVARA are the European government-supported projects launched to control the hotspots of aggressive invasive species, and many countries are currently participating in these projects (Oude Lansink *et al*., 2018). Their objective is to share experience with biological control over spread of invasive species. In 2013, 121 species of specialized insects were used for biological control of 34 weed species in the USA, and 94 insect species were used for 22 weed species in Canada (Zimdahl, 2018). However, the introduced biological agents are not always successful in new environments. Thus, the hawk moth (*Hyles euphorbiae* (L., 1758) (Lepidoptera, Sphingidae) imported from Austria, Hungary, and India had a negligible effect when used for weed control in the USA (Zimdahl, 2018).

In the USSR, the biological control method using introduced phytophagous insects was first employed in the 1960–70ss for combatting the invasive species *A. artemisiifolia*. Today, it is the only example of large-scale application of a biological method to control this weed species. For this purpose, the moth species *Tarachidia candefacta* Hübner, [1831] (Lepidoptera, Noctuidae), fruit fly *Euaresta bella* (Loew, 1862) (Diptera, Tephritidae), ragweed leaf beetles *Brachytarsus tomentosus* (Say, 1827) (Coleoptera, Anthribidae) and *Zygogramma suturalis* (Fabricius, 1775) (Coleoptera, Chrysomelidae) (Esipenko, 2012; Kovalev *et al*., 2013) were introduced to the European part of the USSR. Among the four candidates, only two were efficient for combatting common ragweed: *Tarachidia candefacta* and *Zygogramma suturalis*, which managed to acclimatize under conditions of Southern Russia (Esipenko, 2013).

In the Russian Far East, an attempt to biologically control the *Ambrosia artemisiifolia* population was made in the Primorskii Krai in the 1980–90ss (Kovalev & Medvedev, 1983; Kuznetsov & Esipenko, 1991; Esipenko, 1998). Application of ragweed leaf beetle *Zygogramma suturalis* has not yielded the anticipated results. Today, this species has established in several districts of the Primorskii Krai, but its population is rather small (Esipenko, 2013; Kuznetsov & Storozhenko, 2010; Aistova & Bezborodov, 2015).

Application of the classical biocontrol method can be an important and even the only option for managing aggressive invasive species in the localities where chemical or mechanical control is infeasible (Culliney, 2005; Lommen *et al*., 2017). Therefore, searching for native insects to be used for biocontrol is less threatening for ecosystems compared to the alien species that can run out of control and become invasive, and sometimes in a totally unpredictable way. Furthermore, application of native species is less cost demanding.

The climate change taking place over the past decades has naturally expanded the habitats of many East Asian insect species in the Russian Far East (Koshkin *et al*., 2015). Leaf-footed bug *Molipteryx fuliginosa* (Uhler, 1860) (Hemiptera, Coreidae) is one of such species. The genus *Molipteryx* Kiritshenko, 1916 comprises three species commonly occurring in the southeastern Palearctic region (Vinokurov *et al*., 2010). It was reported in literature that this species is noxious (Wang *et al*., 2002; Chen *et al*., 2014; Luo *et al*., 2014). In China, *M. fuliginosa* was included in the list of economically important insects (Zhang, 1985). In Russia, this species was first encountered in the late 1980s in the Khabarovskii Territory (Kerzhner & Kanyukova, 1998). Later, the species was gradually spreading to the Primorskii Krai (Kanyukova & Vinokurov, 2009; Kanyukova, 2012), where it can be found today.

Application of *A. artemisiifolia* as a feeding plant for M. fuliginosa has been reported in neither foreign (Zhang, 1985; Wang *et al*., 2002; Chen *et al*., 2014; Luo *et al*., 2014; Zhou *et al*., 2017) nor Russian literature (Kerzhner & Kanyukova, 1998; Vinokurov, 2009; Kanyukova, 2012; Markova *et al*., 2016a). In most studies discussing the measures for reducing the common ragweed population, the primary focus was placed on its natural enemies, the insects (Esipenko, 1998; Kovalev & Medvedev, 1983; Kuznetsov & Esipenko, 1991; Kovalev *et al*., 2013; Zhou *et al*., 2017; Lommen *et al*., 2018). However, the climatic conditions of the region or country where *A. artemisiifolia* is currently found are not always favorable for the insects recommended for introduction. Therefore, it is extremely important to study the native insect species that can be used for biocontrol of invasive plants. Among all the phytophagous insects found in the Russian Far East (Kuznetsov & Storozhenko, 2010; Esipenko, 2014; Aistova *et al*., 2014), *M. fuliginosa* has the longest life cycle on *A. artemisiifolia*, which disrupts the natural ontogenesis of *A. artemisiifolia*.

This paper presents the results of the studies demonstrating the features of *M. fuliginosa* development on *A. artemisiifolia* in natural plant communities and stationary cages in the southern Primorskii Krai.

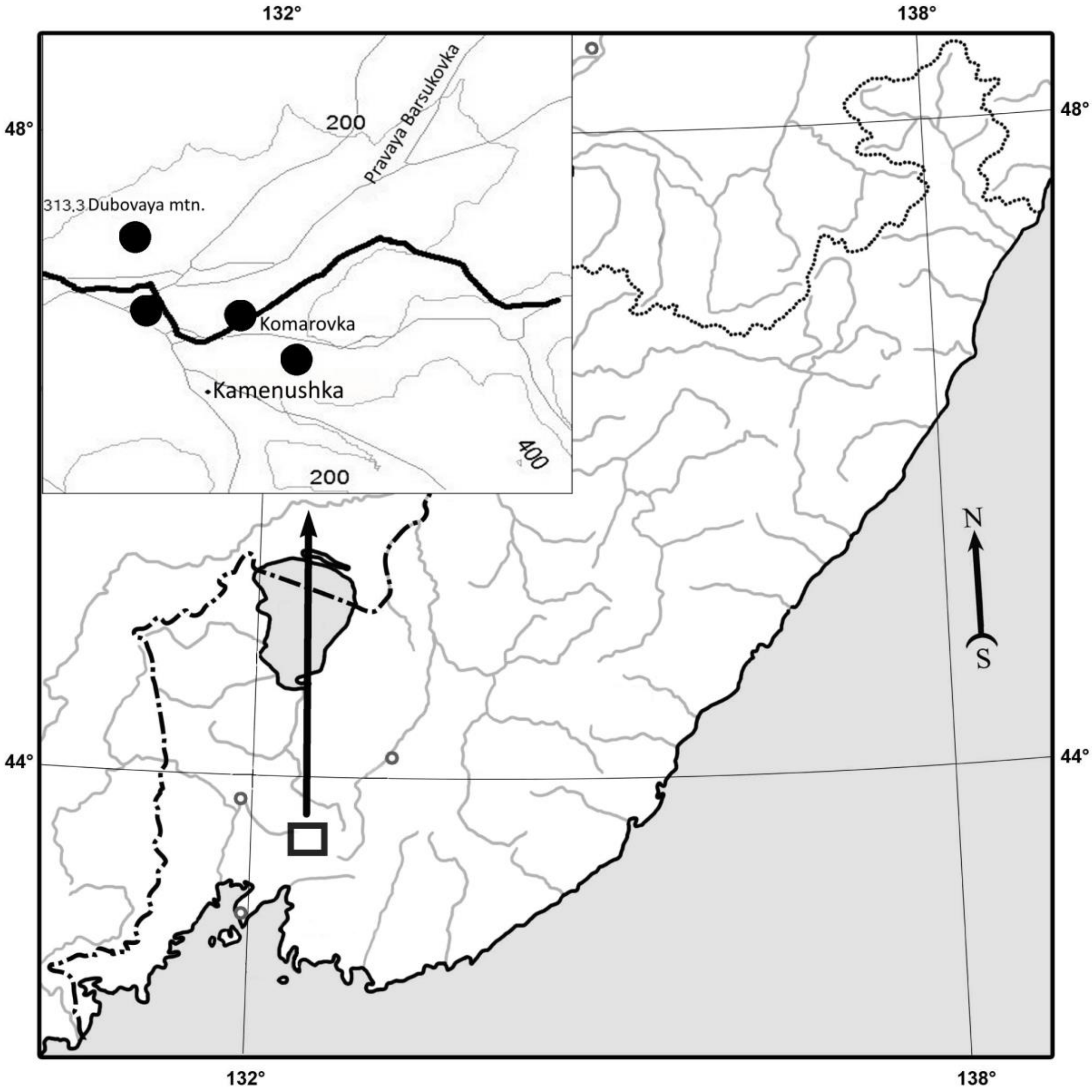

Figure 1. Points in the *Molipteryx fuliginosa* (Uhler) research sites on *Ambrosia artemisiifolia* L. (circles indicate the location of the bug on an ambrosia).

Materials and methods

The studies were carried out in the southern Primorskii Krai near the villages of Kaimanovka and Kamenushka (forest tree nursery), in the specially protected natural area of the natural landmark of regional importance "Barsukovskaya groove" (the Ussuriysk urban district), in the Ussuri State Nature Reserve named after V.L. Komarov (nature reserve cordons: Staraya baza, Peishula, and Anikin in the Ussuriysk urban district and Shkotovskii district) in May through October, 2014–2018. All the field studies in the Primorskii Krai were conducted by T.O. Markova and M.V. Maslov.

Preferential localities of *A. artemisiifolia* (verges of dirt and forest roads, anthropogenically transformed and waste areas, gardens, and woodland areas) were inspected every ten days. The standard methods (visual inspection and hand picking) were used to collect insects. Photos were taken. A GPS navigation unit was utilized for the route examination of plant communities; the data were plotted on a GPS map using the MapSours Trip Waypoint Manager software (Fig. 1). All the photos presented were made by the authors of this paper.

The trophic associations were studied under field conditions and in stationary cages (Fig. 2).

Figure 2. Stationary cages.

The stationary cages allow one to simulate near-natural housing conditions for insects (with feeding quality of plants retained) and to observe the insects for a long period, between the oviposition stage and adult emergence (Markova *et al.*, 2017 a, b; 2018).

We conducted three variants of studies using different food plants to prove that the leaf-footed bug uses *A. artemisiifolia* during its life cycle. In the control variant, we used only the food plants *Cirsium pendulum* Fischer (Asteraceae)and *Agrimonia striata* Michaux (Rosaceae); in the first variant, *Ambrosia artemisiifolia*, *C. pendulum* and *A. striata*; and *A. artemisiifolia* only was used in the second variant.

Ten *M. fuliginosa* adult specimens were placed into each stationary cage containing undamaged plants pre-planted into the soil. Since *M. fuliginosa* nymphs feed on a more constant and narrow range of plants than adults do (Puchkov, 1956), similar experiments were also conducted using II–V instar nymphs.

Results and Discussion

The features of the biology of *Molipteryx fuliginosa* in the Primorskii Krai

The leaf-footed bug *Molipteryx fuliginosa* is a species native to East Asia and is widespread in the northeastern China, the Korean Peninsula, and Japan (Kyushu and Hokkaido islands). In Russia, this species has been found in the southern parts of the Khabarovskii and Primorskii Krai (Park, 1996; Kerzhner & Kanyukova, 1998; Kanyukova & Vinokurov, 2009; Vinokurov *et al.*, 2010; Kanyukova, 2012; Markova *et al.*, 2016b).

The experiments conducted in the Primorskii Krai have demonstrated that overwintered *M. fuliginosa* imagines were found in nature starting with the late second ten-day interval of May; mating and oviposition were observed between the last ten-day interval of May until the first ten-day interval of August (Markova *et al.*, 2017b). According to foreign literature sources, there is only one generation of the leaf-footed bug per year in Korea; this species is found there from early May until late September, and overwinters as adults (Park, 1996). In the stationary experiments in cages conducted in the Primorskii Krai, four to eighteen repeated copulations lasting between two and 48 hours were reported for one female bug. A total of five bug pairs were kept in the stationary cages. The observations were carried out continuously, both visually and using a photo camera. The average number of eggs laid between the copulations was 3.8; the average number of oviposition events was 7.8; and female fertility was 28.4 (n = 5) (Markova *et al.*, 2017b). Copulation of *M. fuliginosa* was also observed under natural conditions. After mating, the females were leaving the plants in search of a suitabble place for oviposition. Single batches of eggs laid by *M. fuliginosa* were found on plants *Solanum lycopersicum* L., *Carex* sp., *Elytrigia repens* (L.) Nevski, and T*araxacum officinale* Wigg. It seems that female leaf-footed bugs are not selective to oviposition spots; i.e., it is not always associated with food plants even if they are abundant and easily accessible (Markova *et al.*, 2017b).

Trophic associations between *Molipteryx fuliginosa* and plants

Whether an individual insect species is a good candidate for being used as a biological agent or not depends on ability of instar nymphs to complete the cycle of development to the adult stage or at least spend the individual stages of their life cycle on an invasive plant, as well as on the rate of ontogenesis (Lommen *et al.*, 2017).

Figure 3. Larvae of IV – V instar *Molipteryx fuliginosa* (Uhler, 1860) on the inflorescence of *Ambrosia artemisiifolia* in natural conditions (env. Kamenushka; road to the nursery)

Different species of phytophagous insects (the ones naturally feeding on the common ragweed) are used in different countries to eradicate *A. artemisiifolia* hotspots. Leaf beetles belonging to the genus *Ophraella* Wilcox, 1965 (Coleoptera, Chrysomelidae Latreille) have been used most successfully in Europe. Species within this genus have consortive ties with plants in the family Asteraceae (Lommen *et al.*, 2018). The studies conducted in Switzerland and focused on simulation of climatic suitability of *O. slobodkini* (Futuyma, 1991) and *O. communa* LeSage, 1985 have demonstrated that the former species can be used only in the subtropical climate, while the latter one has a broader habitat and can be utilized as a biological agent for biocontrol in Europe (Lommen *et al.*, 2017). Today, *O. communa* has established in northern Italy (Lommen *et al.*, 2018). The moth *Epiblema strenuana* (Walker, 1863) (Lepidoptera, Tortricidae Latreille, 1803) and leaf beetle *Zygogramma bicolorata* Pallister, 1953 (Coleoptera, Chrysomelidae Latreille, 1802) (Lommen *et al.*, 2017, 2018) are widely used to control *A. artemisiifolia* in Australia. *Epiblema strenuana* (Walker) and *Ophraella communa* LeSage are the species efficiently utilized to control *A. artemisiifolia* in

South China. As demonstrated by the Chinese researchers (Zhou *et al.*, 2017), cold-tolerant populations of *O. communa* have been revealed there. Their succeeding generations might be able to establish in North China, where the common ragweed is also spreading rapidly. Today, chemical control is the only method used in North China to control *A. artemisiifolia* (Zhou *et al.*, 2017). In Russia, *O. communa* is planned to be introduced in the southern part of the country as a potential agent for controlling common ragweed (Esipenko, 2013).

Foreign researchers reported that the leaf-footed bug feeds on different taxonomic groups of plants. Hence, the leaf-footed bug feeds on *Liquidambar formosana* Hance (Altingiaceae), *Rhus chinensis* Mill. (Anacardiaceae), *Petasites* sp. (Asteraceae) and *Potentilla fragarioides* L. (Rosaceae) in China and *Zelkowa serrata* Makino, *Ulmus davidiana* Planchon var. *japonica* Nakai (Ulmaceae) (Zhang, 1985; Park, 1996) in South Korea. Some authors (Wang *et al.*, 2002; Luo *et al.*, 2014; Chen *et al.*, 2014) mentioned that this species can also damage cultivars, such as *Camellia oleifera* Abel (Theaceae), *Bambusa* sp. (Poaceae, Bambusoideae), and *Oryza* sp. (Poaceae).

Preliminary studies have demonstrated that, along with *Rubus idaeus*, *R. caesius* and *R. crataegifolius* (Rosaceae), the leaf-footed bug feeds on *Sanguisorba officinalis*, *Agrimonia striata* (Rosaceae), *Cirsium pendulum*, *C. setosum* (Willd.) Bess. (Asteraceae) (Kanyukova, 2012; Markova *et al.*, 2016a, b).

Our studies aimed to confirm the trophic associations between the leaf-footed bug and common ragweed. Detailed observations of the leaf-footed bug living on *A. artemisiifolia* have been conducted since 2014. In June 2016, we first found a V instar nymph of *M. fuliginosa* feeding on the base of *A. artemisiifolia* inflorescences (Fig. 3). In July–August 2018, the evidence supporting the fact that *M. fuliginosa* at the nymph and adult stages feeds on common ragweed under natural conditions was obtained. The studies carried out in natural biocenoses demonstrated that in wood habitats, the insects choose moderately insolated ecotonic plant associations (preferring the mesophyticones ones), as well as anthropogenically disturbed areas (forest roads, fringes, overgrowing wood glades, etc.). It was observed that the *M. fuliginosa* species was feeding on *A. artemisiifolia* in these biotopes. No leaf-footed bugs were found on *A. artemisiifolia* in xerophytic biotopes and monodominant communities.

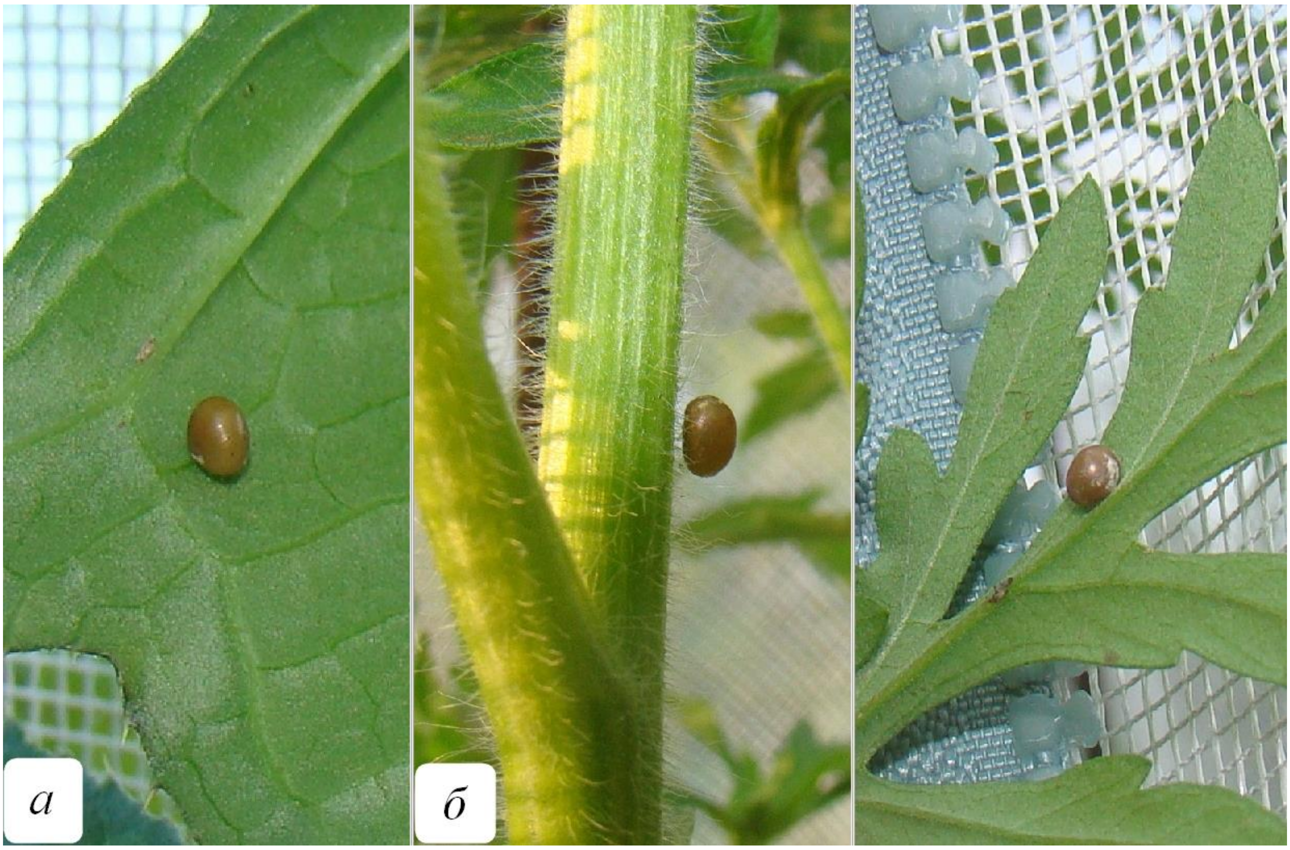

Figure 4. Eggs laid by the bug-riddling on the leaf plate *Cirsium pendulum* (a) and the stem and leaf plate of *Ambrosia artemisiifolia* (b).

After the leaf-footed bugs were feeding on *A. artemisiifolia* during one week, inflorescences of this plant started to slowly dry out, without passing through the full development cycle. Although the number of damaged *A. artemisiifolia* plants was insignificant yet, it is fair to say that this bug species gradually establishes trophic associations with *A. artemisiifolia*. In July–August 2018, we conducted an experiment to confirm the existence of trophic association between *M. fuliginosa* at the nymph or imago stages and *A. artemisiifolia*.

The stationary experiments demonstrated (adult species, n = 30; II–V instar nymphs, n = 30) that in a cage containing a combination of plants *A. artemisiifolia*, *Cirsium pendulum* and *Agrimonia striata*, some leaf-footed bugs preferred the habitual food plants: *Agrimonia striata* on days 1 and 2 and *C. pendulum* on day 3. By day 8, groups of leaf-footed bugs were found only on *Cirsium pendulum*; individual bugs were feeding on *A. artemisiifolia* and *Agrimonia striata*. One egg was laid on the lamina of *C. pendulum* (Fig. 4a). In the cage containing *A. artemisiifolia*, *M. fuliginosa* adults damaged the laminae, the top portions of stems (suction of phloem from vascular bundles), the base of shoots with leaves and those with developing inflorescences. Two eggs were laid on the stem and the lamina of *A. artemisiifolia* (Fig. 4b).

In the control cage containing *C. pendulum* and *Agrimonia striata*, we observed clusters of feeding leaf-footed bugs at 9:1 and 10:0 ratios. The feeding patterns slightly differed for the nymph and adult stages of *M. fuliginosa*. In the cage containing a combination of *A. artemisiifolia*, *C. pendulum*, and *Agrimonia striata* plants, II instar nymphs preferred feeding on *Agrimonia striata*, while III–V instar nymphs preferred *C. pendulum*. Although the plant condition and its feeding quality remained rather good (no wilting, drying off, or significant visual destruction was observed at the perforation sites), single individuals of leaf-footed bugs were feeding on *A. artemisiifolia* and *Agrimonia striata* (with the 6:2:2 ratio, respectively, on day 8 of being kept in the cage). Feeding bugs do not migrate over the plant during the daylight hours and stay in one part of the plant. In the control cage containing *C. pendulum* and *Agrimonia striata*, III–V instar nymphs also preferred *C. pendulum*.

Figure 5. Damage to *Ambrosia artemisiifolia*: a - drying of parts of shoots with leaf plates; b - disruption of the development of inflorescences.

In the stationary cages containing *A. artemisiifolia* only, II instar nymphs were feeding on the laminae (suction from the rachis was observed). III–V Instar nymphs were feeding on the laminae and stems (suction of phloem from vascular bundles), with preference given to the top parts, bases of shoots with a leaf, and some shoots with developing inflorescences. Furthermore, it was also noted that when feeding on the laminae, II instar nymphs did not damage the plant. We revealed no visual changes in plant appearance (perforation marks, dark spots emerging because of feeding, wilting or drying of the laminae or their fragments) after the insects had been removed. When III–V instar nymphs and adult bugs were feeding, the overall wilting of the plant was observed starting on day 4 (Fig. 5b). Perforation marks on leaf veins could be seen, while no visible marks of damage were revealed on the stems. Once the experiment was completed, the cages were removed, and the plants remained on their spots until the end of the season. By day 8 of constant feeding of bugs on *A. artemisiifolia*, parts of the plant continued to wilt and dry out at the sites of insect clustering (10 insects per plant). When the insects were feeding on *A. artemisiifolia* inflorescences, those were slowly wilting and subsequently drying out (Fig. 5 a, b).

Our experiments demonstrated that *M. fuliginosa* adults and nymphs at different instar stages feed on common ragweed without any harm to themselves. No insect death was observed in the cages; the nymphs developed normally and emerged to the next nymph stage after molting. This was possibly related to the chemical composition of the plants (namely, phytosterols and alkaloids, which play a crucial role in cellular metabolism of the phytophage (Telitchenko & Ostroumov, 1990; Useful wild plants of Russia, 2001). Accumulation of these substances protects the insects against predators (both vertebrate and invertebrate ones) and can be useful for them (Telitchenko & Ostroumov, 1990).

Conclusions

The studies conducted under natural conditions demonstrated that *M. fuliginosa* feeds on common ragweed in the ecotone plots of forest cenoses and is preferentially found in moderately insolated mesophytic plant cenoses. No leaf-footed bugs were found in the xerophytic biotopes and monodominant communities of common ragweed. The observations in stationary cages showed that nymphs of the leaf-footed bug feed on *A. artemisiifolia* only at some nymph instars, while this bug does not dwell on *A. artemisiifolia* during the full life cycle. By day 15–20 of cage keeping, the plant wilted because of the loss of turgor in cells, which makes further feeding of bugs impossible and may lead to their death. Under natural conditions, this forces *M. fuliginosa* switch to other plants. It is worth mentioning that ten *M. fuliginosa* individuals placed in a stationary cage cause death of the whole plant, while in nature two or three nymphs of leaf-footed bug (nymph instar V) make normal ontogenesis of the plant impossible as they cause wilting of individual plant parts, disturb the vegetative functions and inflorescence development, and lead to death of generative organs of the plant.

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