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Seasonal Abundance of Russian Knapweed Seedhead Fly, *Urophora xanthippe* (Dip: Tephritidae) and Identification of its Parasitoids in Cold Semi-Arid Climate

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Abstract

Russian knapweed (*Acroptylon repens* (L.) de Candolle) is a perennial plant and a native of Eurasia which is now spread in many parts of the world. It is essential to control this weed as it competes with crops for vital resources, which results in significant losses. Russian knapweed head flies (*Urophora xanthippe*) have been reported as one of the biological control agents of this species in Iran. In this study, adult head flies were captured using yellow sticky traps, and their gender was recorded. To investigate the potential parasitoids coincided with the start of each generation, 100 bolls of spotted knapweed were put in a box closed with mesh 1 % mm (20×15×15cm) and put in a growth chamber at 25 ± 1°C, 65 ± 5% RH and 16:8 h L:D.. Results showed that the gross and net fecundity rates were significantly different on rose cultivars. Species collected were sent for identification to the Iranian Research Institute of Plant Protection. Based on these results, the insects density occurred in nature from early May to early July. Evidence indicates that head fly density rises in nature from 5 June. In the weather of Birjand, the Density of head flies was maximized from 10 May to 25 June in Birjand. The peak appearance of head flies coincides with the emergence of the second generation. Two Hyperparasites pupae were identified as *Microdontomerus annulatus* (Spinola) and *Eurytoma* sp. belonging to the Torymidae and Eurytomidae family. Based on the information provided, it may be possible to find a more optimal time to use insecticide to control *Acanthiophilus helianthi* Rossi, which does not coincide with the peak flight of *U. xanthippe*.

Keywords: Seasonal abundance, Knapweed, *Urophora xanthippe*, Biological control

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1. Introduction

Today, food shortages and agricultural products for a variety of reasons, such as population growth, environmental degradation, and low production efficiency in developed and developing countries, reflect themselves on the world's growing population, and along with population growth, every effort has been made to increase production (Ogundipe et al., 2020). Cereals are the most important food crops on the planet and provide 70% of the world's food; generally, more than 75% of the energy and 50% of human protein is supplied by grains (Raheem et al., 2021). Of the approximately 1.22 billion hectares of cropland, about 17 percent of the crop area is wheat, which accounts for the largest share of the world's annual crop composition. Wheat production in 2013 was about 713 million tons. The EU ranks first with exports of 42.5 million tons out of the 179.9 million tons of wheat exported worldwide, followed by countries such as the United States and Australia with 33 and 17.7 million tons, respectively. Studies show that wheat provides 18.4% of energy and 20.5% of protein to more than 4.5 billion people in 94 developing countries, and at the global level, it is 19 and 20.8%, respectively. So special attention is paid to wheat as the leading human food worldwide (Ahmad et al., 2006; Erenstein et al., 2022).

Weeds cause severe agricultural losses due to competition between the crop and weeds for nutrition, light, and humidity, with a maximum estimated yield loss of 34%. The crop loss caused by weeds is estimated at more than $100 billion US dollars annually (Swanton et al., 2015). Crop yield relationships with the biotic and abiotic environment are based upon ecological principles such as the law of constant final yield (Weiner and Freckleton, 2010: McKenna et al., 2020) and processes of crop-weed competition (Gallandt and Weiner, 2015). Pathogens, insects, soil nutrients, soil moisture, temperature, and intra-and interspecific competition (e.g., row spacing, crop density and weeds) can all affect yield (Zimdahl, 2004; Haarhoff and Swanepoel, 2022). In most parts of the world, herbicides are the dominant technology and the most effective weed control tools ever developed for controlling weeds that infest crops, killing 90 to > 99% of the weeds targeted .Most weed control depends on the application of herbicides (Pacanoski, 2017). But, this golden age of herbicides was quickly cut short by detecting the first herbicide-resistant weeds in the early 1970s (khalil tahmasebi et al., 2017) .Also, Residual toxicity of herbicides has resulted in high incidences of cancer, hormonal and immunological disorders, and allergies, apart from the effects on reproductive ability. Therefore, an alternative eco-friendly and cost-effective method for sustainable weed management using living organisms or biocontrol agents is required (Chutia et al., 2006; Hasan et al., 2021). The challenge of feeding more people sustainably in the next century is among humanity's greatest (Godfray et al., 2010: Fróna et al., 2019). Nowadays, these problems necessitated to go back and searching for an alternate eco-friendly and cost-effective method of weed management through the biological approach in which microorganisms or their products could be used to suppress the growth or population of the weed species (Gnanavel and Natarajan, 2014; Fróna et al., 2019).

Russian knapweed (*Acroptilon repens* (L.) DC.) is a, deep-rooted perennial native to Eurasia. It was accidentally introduced into North America as a seed contaminant and spread rapidly. A single plant can produce about 1200 seeds per year, but 100 seeds per plant per year is more typical along roadsides (Schaffner, 2022). Seeds are viable for 2-3 years, but longer with proper storage (Zouhar, 2001). *A. repens* is a serious crop pest in its native range and elsewhere. It forms large monotypic stands that reduce diversity and degrade forage quality on rangelands (Gaskin et al., 2022). As it is allelopathic and survives under various conditions, it has become an invasive exotic wherever it is imported. *A. repens* is a major weed for grape, orchards and especially wheat crop in Iran. It has been declared a noxious weed in 30 provinces for wheat in Iran (zand et al., 2019). It has been declared a noxious weed in 18 US states (USDA-NRCS, 2016) and other countries in the world (USDA-ARS, 2003 and Schaffner, 2022). *A. repens* cause severe losses in wheat due to competition and allelopathic, with a maximum estimated yield loss of 35%.

Because of the importance of this weed, many steps have been taken to control and prevent its spread (Gaskin et al., 2022). However, the results of some studies on the control of this weed show that the methods used to control, are not very effective and are usually costly. Therefore, it seems that sustainable and effective control of this weed requires the integration of mechanical, biological, and chemical control, optimal farm management, and confronting the vegetative stage of this weed (Asadi et al., 2010).

Biological control is an important tool for weed management because plants in their natural habitat are frequently attacked by natural enemies (Khalil tahmasebi et al., 2012; Ani et al., 2018). Biological control of weeds is a method that, while observing ecological principles, can be extracted from natural enemies such as insects, mites, fungi, viruses, bacteria, nematodes, and plant pathogens. By reducing the rate of regeneration, reducing weed growth, and ultimately lowering the ability of weeds to compete with crops, they kept their populations below the level of economic loss (Gooden and Andres, 1999; Gaskin et al., 2022). Russian knapweed is a weed that has been extensively researched for its biological control worldwide, and by 2005, 38 species of its natural enemies had been identified. Out of these 38 species, the efficiency of two species (*Urophora*. *quadrifacita*, *U. affinis*) was more than other species (Linda et al., 2005; Minteer et al., 2016). Several species of *Urophora* have been introduced so far, but only two species of *Urophora affinis*, *U. quadrifacita*, have been used as biological controls in the world. *U. affinis* was first reported from Austria, France, and Australia, and *U. quadrifacita* was first reported from Colombia and Austria (Maddox, 1982; Minteer et al., 2016). These two species feed on the seeds of the crop weed, which is a stubborn weed. The species mentioned are specially fed to the seeds of various weed species. *U. Xanthippe* specie was first reported by Karimpour (2006) from the village of Urmia province in 2006 from two species of A. *repens* and A. *picris*. *U. Xanthippe* was first reported in 2011 in Birjand, Maud and Ghaen (Khalil Tahmasebi et al., 2016). *U. Xanthippe* hosting domain studies showed that this species has no activity on safflower, wild safflower, Milk thistle and Pot marigold and Russian knapweed is its only host.

So far, no estimate has been made of the seasonal abundance and peak flight of *U. Xanthippe* in Iran. The present study was conducted to identify the biology of this species and its hyperparasites agents in Iran.

2. Materials and Methods

In order to study the population dynamics of head flies (*U. Xanthippe*), a study was conducted in Birjand in 2017 under natural conditions. Five farms with a distance of 500 m were selected for the experiment, and the following methods were applied to achieve the objectives:

2.1 Population Dynamics of Biological Agents in Field Conditions

To determine the time of appearance, population fluctuations, and the number of generations of *U. Xanthippe*, 30 yellow sticky traps were installed vertically on the first *A. repens* at a distance of every 20 meters at the border of each of five farms. The installed traps were inspected weekly and the whole hunted insects were counted by sex and recorded in the relevant table. Thus, the date of appearance of whole insects by male and female and their density in time was determined. The sampling was performed periodically every seven days from the beginning of autumn to the end of summer (one year). Therefore, Population dynamics and the number of generations of *U. Xanthippe* per year were determined.

2.2 Collected Hyperparasites of U. Xanthippe

In each generation, 100 *A. repens* bolls were placed in plastic containers (20×15 × 15 cm) to collect parasitoids from populations of *U. Xanthippe* and covered with 0.1 mm mesh. Plastic containers were kept in an Insect growth chamber [25:18 h L:D., 16:8 h L:D. and 60% relative humidity] until the appearance of parasitoids. Species appearing in plastic containers were recorded daily due to differences in appearance. After taking notes, finally 10 of each species (5 males and 5 females) were placed in a microtube containing alcohol and sent to the Iranian Plant Protection Research Institute in Iran for identification.

Statistical analysis was conducted using the SPSS software ver 26, and graphing was performed by Excel software.

3. Results and Discussion

The number of *U. Xanthippe* trapped in yellow sticky traps is shown in Fig. 1. The results of this study showed that in the climatic conditions of Iran, the density of *U. Xanthippe*, regardless of gender, appeared in nature from early May and continued until the first half of July. Evidence shows that the density of *U. Xanthippe* will increase significantly in nature from 5 June. The highest recorded density of *U. Xanthippe* in Birjand climatic conditions was from 10 June to 25 June. Examination of yellow sticky traps showed that this species has two generations in Iran. The results also show that the peak of flight of seed-feeding flies coincides with the emergence of the second generation. Harris (1980) reported that if the weather conditions are favorable for early appearance, U. *affini* and U. *quadrifasciata* have more than one generation per year, which is consistent with the results of this study. There was no significant difference between collected male and female insects, with an average of 12.14 and 10.14, respectively.

The total number of male and female *U. xanthippe* collected during the sampling was 156, which included 71 males and 85 females (Fig. 2). Keyhanian (2008), in a similar study, showed that *Acanthiophilus helianthi* Rossi flies appeared in the climate of Qom from late April to early May, and their activities continued until the end of the first half of July in the fields. Namvar et al. (2020) reported the appearance of *Ceratitis capitata* in nature was from the beginning to the middle of April. The peak of population was in October and November; its lowest was from the end of December to the middle of February. The highest values of FTD of *Ceratitis capitata* were in October and November. Based on Spearman correlation coefficient, a significant correlation was observed between the changes in FTD values and the level of fruit contamination.

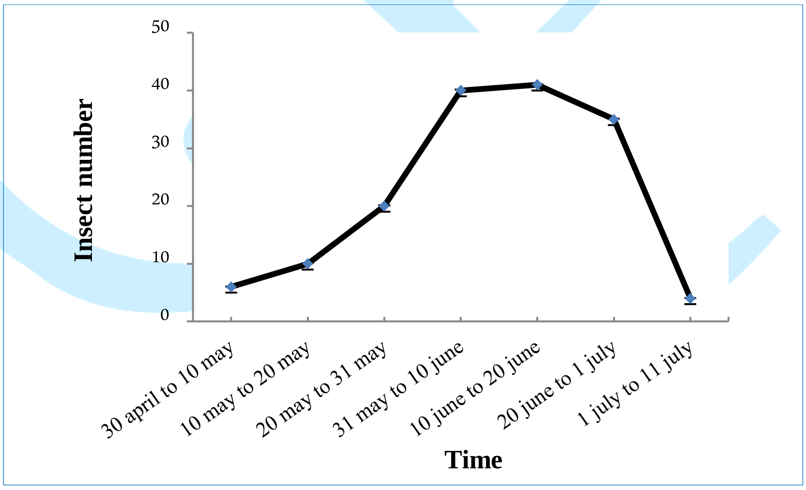

Fig. 1 Population dynamics of *U. xanthippe* in Birjand at different times in 2017 based on data from yellow sticky traps

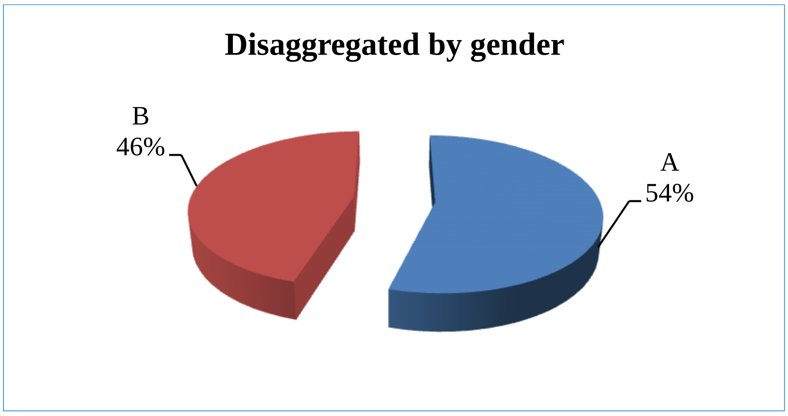

Fig. 2 Disaggregated of male and female of *U. xanthippe* in Birjand based on yellow sticky traps
A- Percentage of female flies **B-** Percentage of male flies caught

Data analysis showed that female to male U. xanthippe relativity was higher. Observations by the author and colleagues showed that a male *U. xanthippe* can mate with 2 to 3 females during its lifetime. This may explain why there are fewer male *U. xanthippe* than females. Spawning is done at one time by female *U. xanthippe* . The female *U. xanthippe* feed on the nectar of the flowers for a short time after spawning and die.

3.1 Collecting Hyperparasites

Experimental results showed that *U. xanthippe* undergo a complete metamorphosis with four distinct life stages – egg, larva, pupa, and adult. *U. xanthippe* has twice molt in the larval period and three instar larvae (Fig. 3). The last instar larva became pupae after receiving artificial signs that were artificially produced (Fig. 3). After two weeks, three species of *Urophora xanthippe* (Fig. 3) and two hyperparasites bees were observed from the pupae (Fig. 3 A and B2 and B3, respectively). According to the classification department of the Iranian Plant Protection Research Institute, two species of bees named *Microdontomerus annulatus (Spinola)* (Fig. 3-d) and *Eurytoma sp* (Fig. 3 A and B2 and B3, respectively) belonging to the families Torymidae and Eurytomidae, respectively, were identified and confirmed.

In a study by Keyhanian (2006) on the isolation of *Acanthiophilus helianthi Rossi* (Diptera: Tephritidae) hyperparasites wasps, only one hyperparasites wasp, *Antistrophoplex conthurnatus* Masi, from the Torymidae family, was isolated from the second generation of pests. Nematollahi and Bagheri (2018) reported *Antistrophoplex conthurnatus* is parasitoid of safflower shoot fly *Acanthiophilus helianthi*. *M. annulatus (Spinola)* and *Eurytoma are important because these parasitoids directly affect the number of U. Xanthippe that appear* from the pupae and ultimately significantly reduce the number of adult *U. Xanthippe*. The higher the parasitization rate, the lower the efficiency of *U. Xanthippe* in the biological control of Russian knapweed. The results of counting the ratio of produced bees to the number of *U. Xanthippe* produced indicate their activity (Fig. 4).

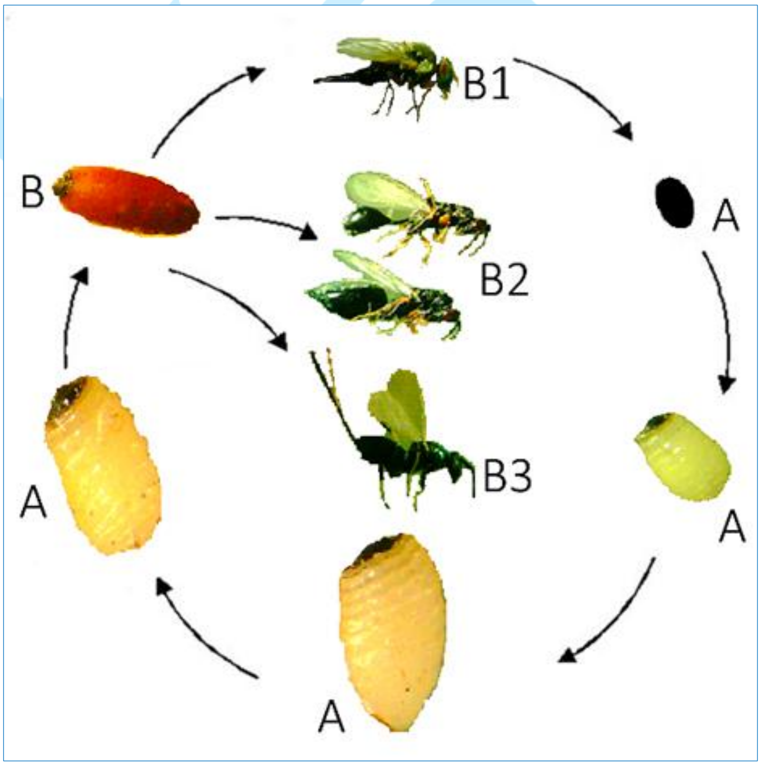

Fig. 3 different life stages of stage head flies *U. xanthippe* (A, B and B1) and the female and male Hyperparasite wasp *Eurytoma* sp. (B2) and hyperparasites wasp *Microdontomerus annulatus* (B3), (photo by the author)

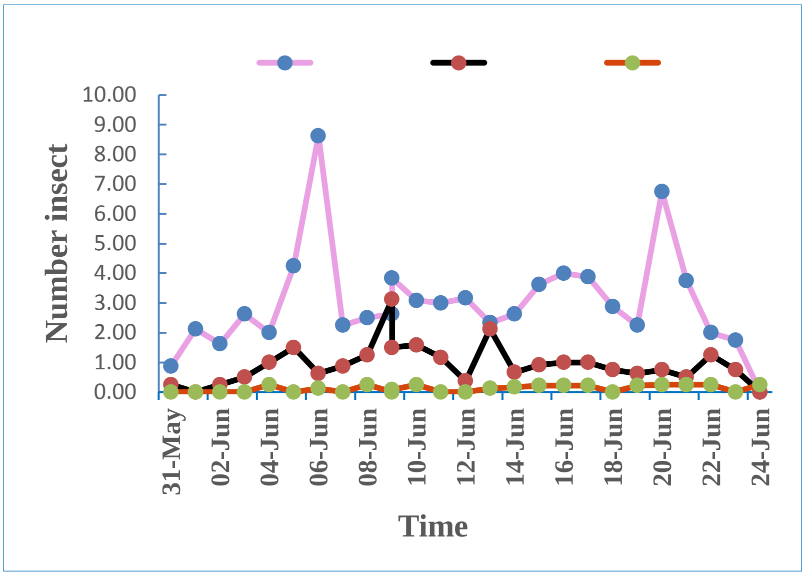

Fig. 4 Average abundance of Russian knapweed head flies (*U. xanthippe*) and yperparasites wasp (*Microdontomerus annulatus* and *Eurytoma* sp.) in sampling periods

The results of counting the number of insects removed in plastic containers in vitro for *U. Xanthippe* flies, and hyperparasites bees were 854 and 222, respectively. The Hyperparasites bees were equivalent to 26% of *U. Xanthippe* flies (Fig. 5).

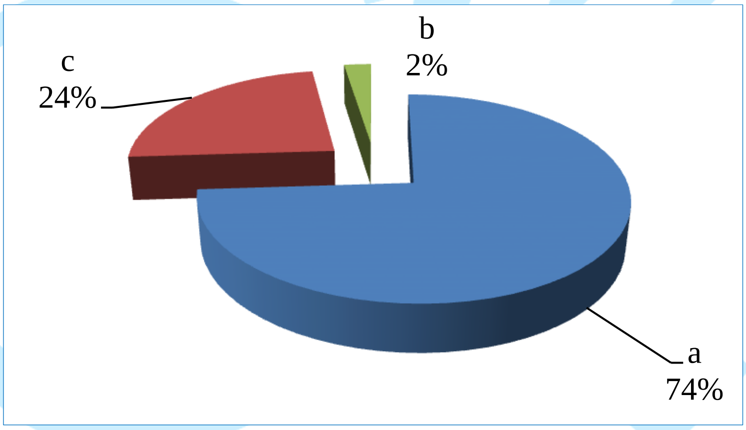

Fig. 5 parasitization percent crap head flies by two species of Hyperparasites wasps
(a) Not parasitized head flies *U. xanthippe* **(b)** parasitized by *Microdontomerus annulatus* **(c)** parasitized by *Eurytoma* sp.

Observations showed that the Microdontomerus annulatus (Spinola) population was more abundant than *Eurytoma sp.* The bee species *Microdontmerus annulatus* (Spinola) and *Eurytoma sp* make up 90.54 and 9.45% of the identified Hyperparasites population, respectively (Fig. 6).

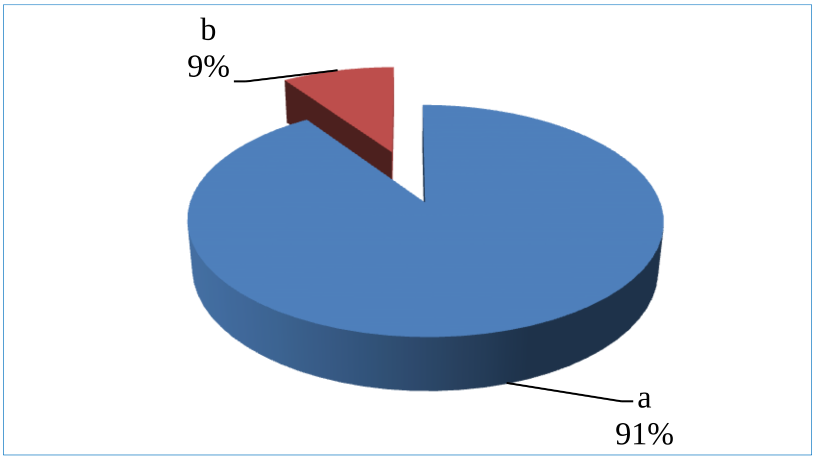

Fig. 6 Ratio of Hyperparasites bees of *U. Xanthippe* in laboratory samples
(A) *Microdontomerus annulatus* and **(B)** *Eurytoma* sp.

Objective results showed due to the appearance and small size of spawning *Eurytoma sp* is able to parasite only in the early stages of the *U. Xanthippe* larvae. Since larvae develop in the ovary, causing the plant to produce a gall instead of a seed; the gall Like Shelter acts as a nutrient sink and reduces the plants' seed production (Khalil tahmasebi *et al*., 2011). Observations showed that the straw- or cream-colored galls of *U. Xanthippe* are 1/9" long, thick and hard (woody), and tear-shaped (they form in the plant receptacle tissue and are relatively well-hidden when a seedhead is opened). Adults of *U. Xanthippe* are solid fliers and disperse far from where they matured. They spend much of their time on the host plant, and although it is possible populations of this fly spread strongly from sites of the initial release, its hosting range ends only with wheat (Khalil tahamsebi *et al*., 2016).

4. Conclusion

Acroptilon repens (L.) is a perennial weed of the Aster family that is propagated by seeds and rhizomes and considered an invasion and stubborn weed. Seeds are a significant factor in the spread of this weed over long distances (Gaskin et al., 2022). It is challenging to control *Acroptilon repens* after establishment. The impact of *A. repens* on co-occurring native plant species is more remarkable in North America than in the native range, suggesting biogeographic differences in the way it interacts with other species (Callaway *et al*., 2012) This invasive weed has invaded about 6.9 million hectares in the United States by 2005 (Duncan, 2005). It has also invaded 13.6 million hectares of paddock land in Montana, from which ranchers (goats and sheep) earn $ 155.5 million annually (Bucher 1984). Zouhar (2001) reported that in Montana, *Acroptilon repens* damage amounted to more than $42 million. Many factors naturally prevent the seeds' spread of *Acroptilon repens* to other places, one of the most important of which is the seedhead fly. Seedhead flies have a high ability to the destruction of the seed. Reviews have shown that the destruction of seeds by *U. Xanthippe* is more than 64% (Khalil tahmasebi *et al*., 2016). The experiment results indicated that greater the number of *U. Xanthippe* present in the *Acroptylon repens,* the greater the destruction of the seed. Studies have shown that this biological control agent only damages *Acroptylon repens* and is single-host, so its potential for biological control of *Acroptylon repens* can be exploited. Therefore, identifying and managing the limiting factors of *U. Xanthippe* is very important. Safflower flies *Acanthiophilus helianthi* Rossi, one of the most important pests of the safflower over the cropping areas of Iran. Therefore, farmers use insecticides to prevent damage to safflower crop, so it is necessary to study the biology of *Acanthiophilus helianthi* Rossi. and *U. Xanthippe* in areas where the two species coexist. The information obtained makes it possible to spray against *Acanthiophilus helianthi* Rossi. in the absence of *U. Xanthippe*. Lack of attention to the bio-control factors of Russian knapweed causes this plant to grow rapidly and due to favorable growing and distribution conditions for it, in the not-too-distant future, we will face a serious risk and invasion of Russian knapweed.

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Declaration of Conflict

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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