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BIOLOGICAL INTERACTIONS WITHIN TOBACCO BIOCENOSIS

VESNA KRSTESKA

University "St. Kliment Ohridski"- Bitola, Scientific Tobacco Institute Prilep
Kičevska bb, 7500, Prilep, F.Y.R. of Macedonia
E-mail: vkrsteska@yahoo.com

Abstract

Predatory hoverflies are useful pollinators in their adult stage and present a natural enemy of plant pests, especially of aphids in their larval stage.

The observations were made with the application of the following methods of catching: check of all leaves of 20 tobacco stalks; and check of 100 tobacco leaves in 10-day intervals during the entire period of vegetation of the tobacco. The collected material was further nourished and analysed under laboratory conditions. The tobacco crop was attacked only by *Myzus persicae* Sulzer and larvae were reared on tobacco leaves infested with *M. persicae* in Petri dishes.

During a period of three years *Sphaerophoria scripta* Linnaeus*, Sphaerophoria rueppelli* Wiedemann and *Scaeva pyrastri* Linnaeus are represented in great numbers. Under laboratory conditions the larvae from the species *S. scripta* consumed from 300 up to 365 aphids from the species *M. persicae;* the larvae from the species *S. rueppelli,* 280-321 aphids; and those of the species *S. pyrastri,* 280-563 aphids. These data show their great capacity for eating aphids. With their great voracity, and to preserve their own kind, they control the population of aphids on tobacco, and so have a great impact on the tobacco biocenoses. The aphidophagous Syrphidae are the main predators and they are ranged on the third trophic level of the food chain: tobacco leaf - aphids - aphidophagous hoverflies. In the complex food chain the aphidophagous Syrphidae are being parasitized by many species of parasites and because of that their ecological and economic use is lesser.

During the laboratory analyses from 2003-2005 we detected ten parasitic species of the order Hymenoptera, ecloded in the pupae of the aphidophagous Syrphidae: *Pachyneuron grande* Thomson, *Pachyneuron cf. grande* Thomson, *Syrphophagus aeruginosus* Dalman, *Diplazon laetatorius* Fabricius, *Diplazon* sp. 2, *Diplazon* sp. 3, *Homotropus* sp. 1, *Homotropus* sp. 2, *Sussaba* sp. 1, *Promethes* sp. 1.

The parasitism has a negative impact on reducing the population of the future generation of the species Syrphidae. The species *S. scripta, S. rueppelli* and *S. pyrastri* can be used as integral protectors of the tobacco leaf aphids, because of their great numbers and their great voracity.

KEY WORDS: tobacco, leaf aphids, aphidophagous hoverflies, parasites of Syrphidae.

Introduction

With the introduction of plants' integral protection, significant attention is paid to the knowledge of predaceous fauna. The presence and intensity of the aphidophagous species of hoverflies, in certain phases of phytocoenosis, is indicated by the presence of leaf aphid.

Aphids always appear in all tobacco-producing regions in the Republic of Macedonia and, when in calamity, they cause severe loss in the production of this crop (KRSTESKA *et al*., 2004). In tobacco fields *M. persicae* was recorded in great quantitative representations (KRSTESKA *et al*., 2003; KRSTESKA, 2007).

Ten predatory Syrphidae were found in tobacco: *S. scripta, S. rueppelli, S. pyrastri, Episyrphus balteatus* De Geer, *Eupeodes corollae* Fabricius, *Paragus quadrifasciatus* Meigen, *Paragus testaceus* Meigen, *Pandasyophthalmus tibialis* Fallén, *Syrphus ribesii* Linnaeus and *Melanostoma mellinum* Linnaeus (KRSTESKA, 2007, 2008). Aphidophagous hoverflies are attacked by a number of parasitic species (SCHNEIDER, 1950; ADASHKEVICH, 1975; GILBERT, 1986; JANKOWSKA, 2004; KRSTESKA, 2007).

The purpose of this study is to investigate biological interactions between tobacco plants, aphids, aphidophagous hoverflies, and parasites of aphidophagous hoverflies in a food chain.

Material and Methods

Field research

For the realization of the established aims, we applied the following methods during the studies:

a. Method of survey of 20 randomly selected tobacco stalks infested with aphids. Tobacco stalks were sampled from the whole area of the trial at 10-day intervals, from June 1 to the end of September. The examinations were performed on tobacco targets (leaves, tobacco flower and flower seed capsules). 10 checks were made by this method in each of the three years of investigations, i.e. 200 stalks per year, or 600 stalks in total. During the investigations 5813 tobacco leaves were examined in 2003, 5851 in 2004, and 5944 in 2005: in total 17608 tobacco leaves.

b. Method of Davies- survey of 100 randomly selected tobacco leaves infested with aphids. Tobacco leaves were sampled from the whole area of the trial at 10-day intervals, during tobacco vegetation. 10 checks were made by this method in each of the three years of investigations, i.e. 1000 leaves per year, or 3000 leaves in total.

These two methods of analysis are simple and safe to perform; there is an advantage when the data is collected by one person only. With these methods we can successfully monitor the number and types of composition of the leaf aphids, the qualitative and quantitative representation of hoverflies, their parasites and their relationship.

Laboratory research

The prime material was collected in natural conditions, then the collected material was further nourished, cultivated and analysed under laboratory conditions. The eggs, larvae and pupae of Syrphidae were placed

in special containers and raised until the eclosion of the adult. Larvae were reared on tobacco leaves infested with *M. persicae* in Petri dishes.

The first step is to use a little brush to separate the eggs and larvae gently from the colonies of plant aphids, and then put them individually on tobacco leaves in Petri containers infested with aphids. With this procedure we avoid cannibalism among these larvae, and once they are out of their eggs they have available food for other larval stages of their development. There is a filter paper inside the Petri containers which was previously dampened by distilled water. This gives enough moisture in the containers for the Syrphidae to develop.

If we want to determine the number of leaf aphids needed for the development of the aphidophagous hoverflies, we should feed the newly hatched larvae with leaf aphids and ensure that there are enough aphids in the same stadium. Accordingly, we increased the number of these so-called "meals" of leaf aphids proportionally with the larvae development stage and the aphids' requirements. Each day we calculated the number of consumed aphids and replaced them with new ones. The larvae feeding was prolonged until they converted in the next stadium of pupae.

The pupae which were brought up and those which were collected were separately placed in test-tubes shut with cotton pads, previously dampened in distilled water. In this way we ensured that the pupae had enough moisture for their development. Later we monitored the internal modifications of the pupae, the length of their developing stadium, the degree of eclosion of imago, the percentage of parasitism and the eclosion of different types of parasites.

The species, sexual index and morphological characteristics were determined in the adult hoverflies. Also, among ecloded parasites derived from the Syrphidae we determined their species and their morphological characteristics.

Results and Discussion

M. persicae has the highest potential for reproduction and development, and in the tobacco biocenoses of the region of Prilep it has approximately 8 generations. If *M. persicae* harshly attacks the tobacco it disrupts the physiological processes and reduces some of the chemical compounds in the tobacco leaves. The diet of the aphids on the leaves influences the reduction of carbon hydrates, the soluble sugars and glucoses that are especially represented in the top leaves (TODOROVSKI, 1965).

The predatory hoverflies have an important role in biological control of aphids in biocenosis. During the investigations six predatory hoverflies feeding on *M. persicae* were found in tobacco: *S. scripta, S. rueppelli, S. pyrastri, E. balteatus*, *M. corollae* and *P.quadrifasciatus.*

From the analyses conducted between 2003 and 2005 to determine the number of leaf aphids and the quantitative structure of hoverflies in the region of Prilep, we can conclude that over the several years there is variable quantitative coverage. The quantitative composition of the analyzed fauna is a sum of all stages of the representatives, that is, a sum of the eggs, larvae, pupae and adults as factual and potential predators of *M. persicae.*

Table I shows the numerical coverage of leaf aphids and predator hoverflies conducted in accordance with the method of 20 stalks. In the period between 2003 and 2005 the total preview of leaf aphids' predators was conducted on 600 stalks containing 17608 tobacco leaves. The population of leaf aphids grew from 48766 in 2003, to 79086 in 2004 and up to 95104 in 2005 or, in total, 222956 over the years.

Table I Quantitative representation of aphids and aphidophagous hoverflies (method of survey of 20 tobacco stalks).

Year	N of stalks	N of leaf aphids	*S. scripta*	*S. rueppelli*	*S. pyrastri*	*E. corollae*	*E. balteatus*	*P. quadrifasciatus*	Total
2003	200	48766	544	391	110	32	94	62	1233
2004	200	79086	528	758	661	31	218	18	2214
2005	200	95104	685	335	651	46	174	67	1958
Total	600	222956	1757	1484	1422	109	486	147	5405

The ratio of specific types of Syrphidae varied during the years. Five thousand four hundred and five (5405) hoverflies were determined. During the period of three years, percentage representation of *S. scripta* was 32.51%; *S. rueppelli*, 27.45%; and *S. pyrastri,* 26.31 %, and in ratio with other predatory hoverflies (Fig. 1) they have great impact in the tobacco biocenoses.

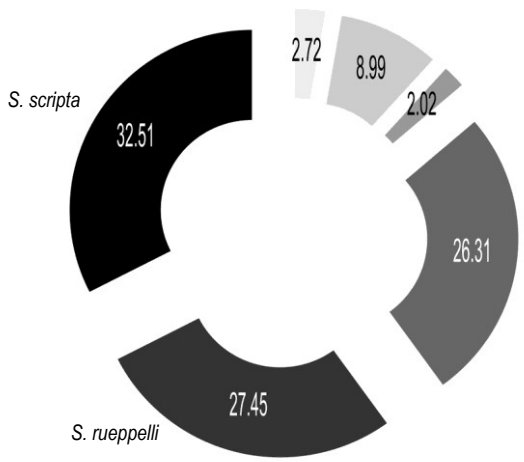

Figure 1. Percentage representation of aphidophagous hoverflies (method of survey of 20 tobacco stalks).

Table II. Quantitative representation of aphids and aphidophagous hoverflies (method of survey of 100 tobacco leaves).

Year	N of stalks	N of leaf aphids	*S. scripta*	*S. rueppelli*	*S. pyrastri*	*E. corollae*	*E. balteatus*	*P. quadrifasciatus*	Total
2003	1000	11548	221	172	37	5	23	18	476
2004	1000	28607	208	275	197	11	74	5	770
2005	1000	21571	217	87	160	13	49	10	536
Total	3000	61906	646	534	394	29	146	33	1782

Tab. II shows the numerical representation of leaf aphids and hoverflies according to the method 100 tobacco leaves. One thousand seven hundred and eighty-two (1782) hoverflies were determined. From this method we can see that the species *S. scripta, S. rueppelli* and *S. pyrastri* are represented in great number; percentage representation is 36.25%, 29.97%, and 22.11 % respectively (Fig. 2).

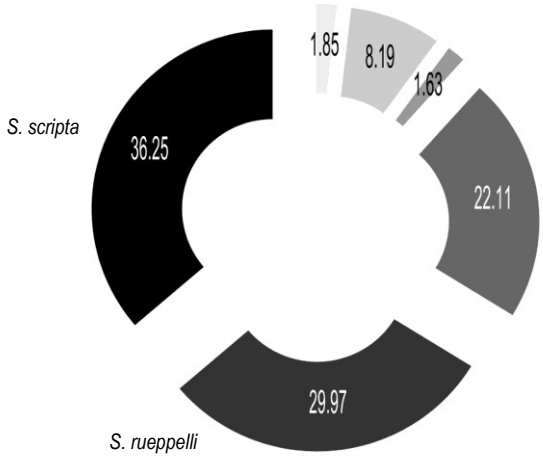

Figure 2. Percentage representation of aphidophagous hoverflies (method of survey of 100 tobacco leaves).

If we want to determine the predatory efficacy of the species S*. scripta, S. rueppelli* and *S. pyrastri* and their voracity in laboratory conditions, we should feed them with the larvae from the species *M. persicae,* which for many years has been an economically important pest of tobacco.

Under laboratory conditions the larvae from the species *S. scripta* consumed from 300 up to 365 aphids from the species *M. persicae*; the larvae from the species *S. rueppelli,* 280-321 aphids; and those of the species *S. pyrastri,* 280-563 aphids. From these data we can see that they have great capacity for eating aphids.

These species have an almost identical way of feeding and a similar way of catching their prey. After being hatched the new larvae immediately start their search for food. The first instar larvae (L1) suck the aphid at

an interval of an hour or hour and a half whereas the third instar larvae (L3) suck the whole aphid in a minute. Their voracity increases in the second, and especially in the third larval stage.

The relationship between eggs, hatched larvae, pupae and ecloded adults is a very variable factor affecting the population size of predatory hoverflies. Besides abiotic conditions, biotic interactions with other predators, parasites, and entomopathogen fungus may affect the survival of aphidophagous Syrphidae.

The hoverflies have significant impact on the regulation of the number of leaf aphids. The laboratory analyses on the predator species have shown that there is a large number of parasites ecloded in the pupae of different species of Syrphidae. Furthermore, we continued with our analyses in order to determine the possibility of using predatory hoverflies in the field of integral protection.

During these analyses we detected several species of parasite wasps ecloded in the pupae of the aphidophagous Syrphidae.

Parasites are rarely detected among the hoverflies species, whose larvae do not eat aphids. (GILBERT, 1986).

The female parasites lay down their eggs in larvae bodies of Syrphidae. When the host-hoverfly is in the stadium of pupa the parasite activates, and begins its feeding and development. During our analyses we determined that during their feeding time, the parasitized larvae are slow in motion and often the larvae are smaller. When they transform into pupa very often the size of the pupa is smaller.

At first the pupa of the hoverfly has its specific larval color. A few days later its color becomes more whitish and dull meaning that the larvae of parasites feed on the host's tissues and organs. The pupa becomes darker which is a sign of a parasitism.

During these laboratory analyses we detected ten species of parasites ecloded from the pupae of the aphidophagous Syrphidae:

- from the Pteromalidae family: *Pachyneuron grande* Thomson, 1878 and *Pachyneuron cf. grande* Thomson, 1878;

- from the Encyrtidae family: *Syrphophagus aeruginosus* Dalman, 1820;

- from the Ichneumonidae family: *Diplazon laetatorius* Fabricius, 1781, *Diplazon* sp. 2, *Diplazon* sp. 3, *Homotropus* sp. 1, *Homotropus* sp. 2, *Sussaba* sp. 1, *Promethes* sp. 1.

The species *P. grande, P. cf. grande* and *S. aeruginosus* develop a large number of imagoes ecloded in the pupae of aphidophagous Syrphidae. At the time of eclosion they make their way through pupae by making small holes on all sides of the pupae's cuticles. The pupa usually looks like lattice.

The representatives of the species *Diplazon, Homotropus, Sussaba, Promethes* eclode only one imago in the pupae of the aphidophagous hoverflies. Their imago at the time of eclosion make an abnormal hole in the front, curved side of the pupa. The identification of parasites can also be performed by analyzing those holes (GILBERT, 1986).

The parasite's eclosion time depends on the species, but it is from 10 up to 14 days.

The parasites of Syrphidae were established through over 3 years of analyses.

The presence of different parasites depends on the number of host hoverflies. While the presence and the evolution of the aphidophagous Syrphidae depends on the presence and evolution of peach leaf aphid *M. persicae* on the tobacco, climate changes also influence it.

If conditions are good, a great number of new generations of leaf aphids will develop on tobacco.

Table III. Numeric representation of pupae and parasited pupae in 2003-2005.

	S. scripta				***S. rueppelli***				***S. pyrastri***			
	100 tl		**20 ts**		**100 tl**		**20 ts**		**100 tl**		**20 ts**	
Year	**p**	**pp**	**p**	**pp**	**p**	**pp**	**p**	**pp**	**p**	**pp**	**p**	**pp**
2003	67	28	144	65	48	13	131	60	10	5	25	12
2004	52	18	144	49	77	25	189	73	55	19	151	64
2005	81	18	182	49	25	9	83	33	41	13	153	55
% of parasitism	25.31				27.81				27.86			

Legend: p - pupae, pp - parasited pupae; tl - tobacco leaves; ts - tobacco stalks

Table III shows the number of pupae and parasites among aphidophagous hoverflies in the tobacco in the period 2003-2005.

The population and the number of hoverflies varied simultaneously with the level of parasitism during the years.

The parasites of the hoverflies are polyphagous; they attack many different species of Syrphidae.

In 2003 we detected seven species of parasites ecloded from pupae of *S. scripta* and *S. rueppelli: P. grande, P. cf. grande, S. aeruginosus, D. laetatorius, Homotropus* sp. 1, *Sussaba* sp. 1, and *Promethes* sp. 1. The pupae from the species *S. scripta* were parasitized with 30.59 % and those of the species *S. rueppelli* were parasitized with 28.97 % (Figs. 3 & 4). Also out of the pupae of the species *S. pyrastri* 5 types of parasites were ecloded: *D. laetatorius, Diplazon* sp. 2, *Diplazon* sp. 3, *Homotropus* sp. 1 and *Homotropus* sp. 2. The rate of parasitism of pupae of the species *S. pyrastri* is 32.69% (Fig. 5).

In 2004 we detected five species of parasites ecloded from pupae of *S. scripta* and *S. rueppelli*: *P. grande, D. laetatorius, Homotropus* sp. 1, *Sussaba* sp. 1 and *Promethes* sp. 1. During this year the percentage of pupae of *S. scripta* parasitized is 25.48%, lower than the other years. The percentage of cocoons of *S. rueppelli* is much higher at 37.12%. Seven types of parasites were ecloded from the pupae of *S. pyrastri*: *P. grande*, *S. aeruginosus, D. laetatorius, Diplazon* sp. 2, *Diplazon* sp. 3, *Homotropus* sp. 1 and *Homotropus* sp. 2. The pupae of the species *S. pyrastri* have parasitism of less than 28.72%.

In 2005 we detected two species of parasites ecloded in the pupae of the species *S. scripta* and *S. rueppelli*: *P. grande* and *D. laetatorius*. That year the percentage among these two species was 20.30% for S. scripta and 28% for S. rueppelli, and compared with the previous years is very low. There were three types of

species ecloded from the cocoons of the species *S. pyrastri*: *Diplazon* sp. 2, *Diplazon* sp. 3 and *Homotropus* sp. 1. The pupae of the species *S. pyrastri* have the lowest percentage of parasitism with 25.95%, and compared with years 2003 and 2004 this is the lowest percentage of parasitism.

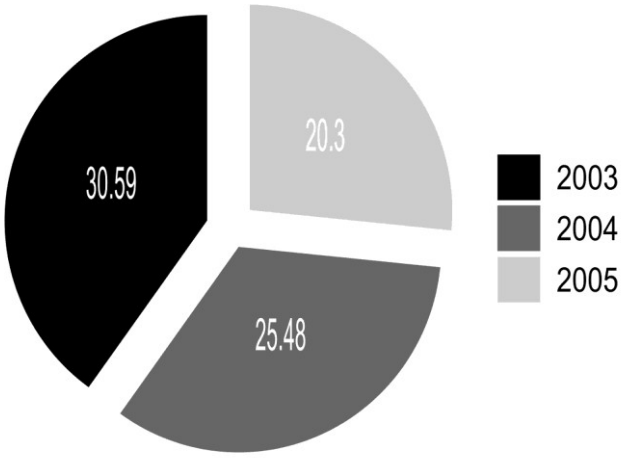

Figure 3. Parasitism of *S. scripta* through the years.

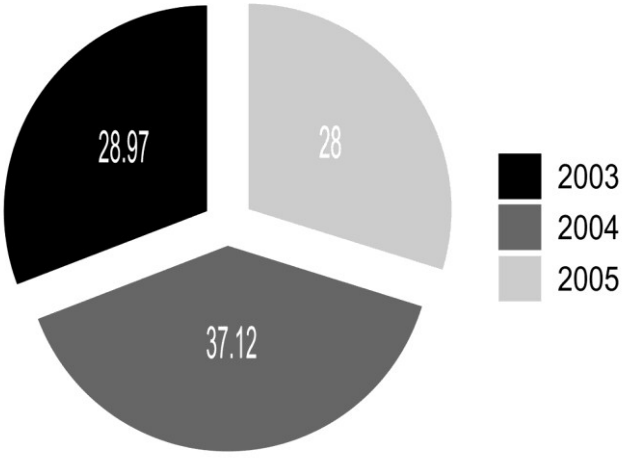

Figure 4. Parasitism of *S. rueppelli* through the years.

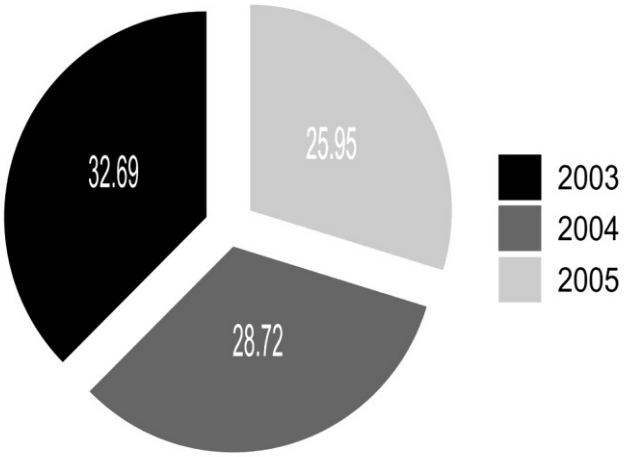

Figure 5. Parasitism of *S. pyrastri* through the years.

At the beginning of our analyses during the tobacco vegetation, the number of parasitized larvae was significantly low. Simultaneously with the increase of the number of hoverflies larvae, the percentage of parasitism went up. Tab. III shows that in 2004 the number of parasitized pupae was highest.

In the period 2003-2005 the number of hoverflies pupae and parasite pupae was high until the third decade of September then it began to fall.

During the three years of analyses we have established that the greatest number of parasites belongs to the family Ichenumonidae. The following species are represented with high percentages: *D. leatatorius* 41.46%, *Diplazon* sp.2 16.09%, *Diplazon* sp.3 8.78% and *Homotropus* sp.1 6.34%.

Furthermore, together with the others, the species *P. grande* (Pteromalidae) has high representation at 19.5%. Although the percentage of representation of this species is lower compared with *D. leatorius, P. grande* can produce many more imago out of one pupa of hoverfly.

The other parasites are represented with much lower percentages.

The parasitism has a negative impact on lowering the number of the future generation of the species Syrphidae.

Conclusion

The tobacco crop was attacked only by *M. persicae.* According to our analyses in all the years of our investigations leaf aphids were the most frequent pest of tobacco plantation.

During the investigations six predatory hoverflies feeding on *M. persicae* were found in tobacco: *S. scripta, S. rueppelli, S. pyrastri, E. balteatus*, *M. corollae* and *P. quadrifasciatus.*

The ratio of specific types of Syrphidae varied during the years. During the period of three years *S. scripta*, *S. rueppelli* and *S. pyrastri,* in ratio with other predatory hoverflies, had a great impact on the tobacco biocenoses.

Under laboratory conditions the larvae from the species *S. scripta* consumed from 300 up to 365 aphids from the species *M. persicae*. The larvae from the species *S. rueppelli* consumed 280-321 aphids and those of the species *S. pyrastri,* 280-563 aphids. From these data we can see that they have a great capacity for eating aphids.

During the investigations from 2003-2005 10 parasitic species of the order Hymenoptera from pupae of *S. scripta, S. rueppelli* and *S. pyrastri* ecloded: *Pachyneuron grande* Thomson, *Pachyneuron cf. grande* Thomson, *Syrphophagus aeruginosus* Dalman, *Diplazon laetatorius* Fabricius, *Diplazon* sp. 2, *Diplazon* sp. 3, *Homotropus* sp. 1, *Homotropus* sp. 2, *Sussaba* sp. 1 and *Promethes* sp. 1.

In order to have a natural balance in the food chain, predatory Syrphidae feed on the leaf lice and a large number of parasites feed on Syrphidae. At the beginning of analyses during the period of tobacco vegetation the number of larva parasites was low. The percentage of parasitism rose simultaneously with the number of hoverfly larvae. The parasitism of pupae was lower than 30%.

The parasitism has a negative impact on lowering the number of the future generation of the species Syrphidae.

The species *S. scripta, S. rueppelli* and *S. pyrastri* can be used for integral protection of the tobacco leaf aphids because of their great quantitative representation and their great voracity.

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БИОЛОШКЕ ИНТЕРАКЦИЈЕ У ДУВАНСКОЈ БИОЦЕНОЗИ

ВЕСНА КРСТЕСКА

Извод

Присуство и интензитет појаве предаторских сирфида у датој фитоценози увек указује на присуство лисних ваши. *Myzus persicae* Sulzer има високи потенцијал размножавања на дувану и у просеку има 8 генерација.

У току испитивања идентификовано је шест предаторских сирфида које се хране на *M. persicae*: *Sphaerophoria scripta* Linnaeus*, Sphaerophoria rueppelli* Wiedemann*, Scaeva pyrastri* Linnaeus, *Episyrphus balteatus* De Geer, *Eupeodes corollae* Fabricius и *Paragus quadrifasciatus* Meigen. Врсте *S. scripta, S. rueppelli* и *S. pyrastri* јављају se у већој квантитативној заступљености.

Ларва *S. scripta*, у лабораторији конзумира 300 до 365 ваши, *S. rueppelli* од 280 до 321 и S. *pyrastri* од 280 до 563 лисне ваши, што указује на то да ове врсте имају велики консумативни капацитет.

У истраживаном периоду, од 2003. до 2005. године, из лутке *S. scripta, S. rueppelli* и *S. pyrastri* еклодирало је 10 врста паразита из реда Hymenoptera: *Pachyneuron grande* Thomson, *Pachyneuron cf. grande* Thomson, *Syrphophagus aeruginosus* Dalman, *Diplazon laetatorius* Fabricius, *Diplazon* sp. 2, *Diplazon* sp. 3, *Homotropus* sp. 1, *Homotropus* sp. 2, *Sussaba* sp. 1 и *Promethes* sp. 1. У овом случају паразитизам игра негативну улогу у ограничавању величина популација Syrphidae у следећој генерацији или години.

Врсте *S. scripta*, *S. rueppelli* и *S. pyrastri* имају већу бројност и имају велику консумативну способност и стога могу да се користе у сврхе интегралне заштите против лисних ваши.

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