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BIOLOGICAL CONTROL POTENTIAL OF AN APHIDOPHAGOUS PREDATORS, CANNIBALISM AND INTERSPECIFIC COMPETITION

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Abstract
Aphidophagous predators have a great importance in biocenosis as bio-regulators of aphids. Specific relationships between predators, interspecific competition and cannibalism, have an important role in biological control. The focus of this study was on biological control potential of predators, interactions between larvae of *Coccinella septempunctata, Chrysopa carnea, Sphaerophoria scripta, Aphidoletes aphidimyza* and incidence of cannibalism. Observations were made in Prilep region (Macedonia) 2012-2015 and there was applied method of survey of 20 randomly selected tobacco stalks infested with aphids. Predatory larvae were reared with *Myzus persicae* in petri dishes under laboratory conditions. All experiments, prey consumption, interaction between predators and cannibalism were conducted in the laboratory. *C. septempunctata, C. carnea, S. scripta* and *A. aphidimyza* are primarily predators and they occupy the third trophic level of food chain (tobacco-*M. persicae* -predator). The growth of population of predators continuously follows the growth of aphid population on tobacco. During investigations, 5861 predators were determined in 2012 and 3788 in 2013. According investigations during 2013 they are voracious predators of aphids. In laboratory conditions, adults of *C. septempunctata* consume in average 468 and larvae 350 aphids, *C. carnea* larvae 458 aphids, *S. scripta* 333 aphids and *A. aphidimyza* 200 aphids. Interspecific competition and cannibalism are important factors in rearing conditions of predators. The outcome of interactions between predators 2014/2015 mainly depends on the body size of the competitors. In general large individuals behaved as predator while small individuals became prey. Eggs and L1 were extremely vulnerable in regard to larger larvae. Cannibalism in *C. septempunctata* and *C. carnea* occur when eggs or young instar larvae are kept together with larger larvae, in the absence of aphids. Predators are often used as biological control agents in Integral Pest Management, so mass rearing techniques must be developed which guarantee higher survival rates of predators.

Keywords: biological control, aphids, specific relationships.

Introduction

Constant progress in the field of biological control has raised the need to: create specific norms to allow its further development, produce biological control items, and make its use feasible by growers. Interest in the use of biological control techniques has grown worldwide as an alternative to chemical control, especially due to the adverse effects of chemicals on the environment and human health (Nogueira et al., 2016). Predation rate and numerical response are basic to any investigation of predator–prey relationships and key components in the selection of predators for biological control (Madahi et al., 2015). Predators and parasites keep the biocenosis balance and are able to reduce the calamity of pests on cultivated and weed plants. Aphidophagous predators have a great importance in biocenosis as bio-regulators of aphids (Vasilev, 1971; Kaitazov, et al., 1982; Vukovic, 1986; Vukovic, 1990; Harizanov and Babrikova, 1990; Simova-Tosic et al., 1989; Vujic and Radenkovic, 1995; Lazarevska, 1998; Janusevska, 2001; Krsteska, 2007). *C. septempunctata* has a broad ecological range. It is a polyphagous species; it mainly preys on aphids and other similar insects. The lady for whom it was named was "the Virgin Mary" and seven spots symbolize her seven

joys and seven sorrows (Lazarevska, 1998). The main predators of ladybirds are usually birds, but they are also the prey of frogs, wasps, spiders, and dragonflies. Attractive red colour warns would-be predators, that the ladybird is distasteful or toxic. *C. septempunctata* is predator of *M. persicae* on tobacco (Krsteska et al., 2004). The common green lacewing, *C. carnea*, is found in many parts of America, Europe and Asia. The adults feed on nectar, pollen and aphid honeydew, the larvae are active predators and feed on aphids, other small insects and also on caterpillars. *C. carnea* is predator of *M. persicae* on tobacco (Krsteska et al., 2005). Lacewings can be attracted by using certain companion plants and tolerating beneficial weeds. *C. carnea* adults consume pollen and nectar of Umbeliferrae species (Szentkirályi, 2001).
S. scripta is obligate aphidophagous species and has a worldwide distribution. It is useful pollinator in its adult stage and presents a natural enemy of plant pests, especially of aphids in its larval stage. *S. scripta* is most widely distributed in almost all tobacco growing regions in R. Macedonia (Prilep, Bitola, Krusevo, Kavadaci, Valandovo, Strumica, Radovis, Stip, Veles, Sv. Nikole). It is characteristic for all types of open habitats (Krsteska, 2008). Predatory midge *A. aphidimyza* is general aphid predator, attacking many different species of aphids on different host plants. Larvae are voracious native predators of over 60 species of aphids. Aphidoletes are used to control aphids indoors in commercial greenhouses and interior plants as well as outdoors in orchards, shade trees, roses and home gardens. *A. aphidimyza* played an important bio-regulatory role in the control of *M. persicae* on tobacco (Krsteska et al., 2003). Interspecific interactions among natural enemies also are named intraguild predation (IGP). Interactions between coexisting species of predators that share the same aphid prey resource in a patchy habitat often result in intraguild predation (Polis et al., 1989; Rosenheim et al., 1995, cit. Agarwala, 2003). In IGP the outcome of an encounter between two guild partners depends on their following characteristics: relative body size, mobility, vigour aggressiveness, defensive strategies and degree of feeding specificity (Szentkirályi 2001). In specific relationships between aphidophagous predators, cannibalism has an important role in biological struggle.
The focus of this study was on biological control potential of predators, interactions between *C. septempunctata, C. carnea, S. scripta* and *A. aphidimyza* (as the most present predators on tobacco) and factors that can induce cannibalism.

Material and methods
Observations were made on tobacco in region of Prilep during 2012-2015. To investigate population dinamics of predators, in 2012/2013 there was applied method of survey of 20 randomly selected tobacco stalks infested with aphids, in 10 days interval during vegetation. All experiments: the measurement of prey consumption (biological control potencial of predators), interaction between predators, cannibalistic behavior of predators were conducted in the laboratory. The eggs, larvae, are placed individually in petri dishes and were reared on tobacco leaves infested with *M. persicae* in laboratory conditions. Once hatched the larvae are subject to establishing the number of larvae stages, their voracity and their morphological changes. To determine the number of leaf aphids needed for the development of the predator, we fed the newly hatched larvae with leaf aphids in the same uniform age structure as food of predator in these experiments. Every 24 h the number of consumed aphids was recorded, the remaining aphids were removed, and new aphids were introduced into the petri dish. The larvae feeding were prolonged until they converted into pupae. We calculated “meals” of leaf aphids, in new hatched adults of *C. septempunctata*.
In 2014/2015 we study interaction between predators and cannibalistic behavior of predators in laboratory conditions. Larvae of the five species, that are in different stages of development were kept together (opposing one with another predator) to investigate interaction between predators. For measurement of interaction of predators, first step is gentle separation of predators, opposing one with another predator, out of the colonies of plant aphids, and on tobacco leaves in Petrie containers infested with aphids. To investigate the incidence of cannibalism in bigger petri dishes,

larvae of each species were kept together in plenty of food and in another experiment in absence of aphids.

Results and discussion

During investigation (2012-2015) of fauna species of the Aphididae family, it appeared that tobacco crop was attacked only by *M. persicae*. *C. septempunctata, C. carnea, S. scripta* and *A. aphidimyza* are primarily predators and they occupy the third trophic level of food chain (tobacco-*M. persicae*-predator).

C. septempunctata is one of the commonest ladybird predators of *M. persicae* in tobacco habitats. It occurs with the biggest quantitative representation of 5285 individuals. The seven-spot ladybird living almost anywhere there are aphids and during our investigations it reaches its maximum in mid-August. Adult are oval, red, with seven black spots on elytra. The head is black with a white or pale spot on either side of the head. Females lay eggs vertically in groups, near their prey. Eggs are oval, yellowish-orange. Larva is gray-black and on first, fourth and seventh abdominal segment has a bright orange stain. Pupae are black with orange patterns, unprotected by a cocoon. Both the adults and the larvae are voracious predators of aphids. Mandibles are used for chewing the aphids or another pray. During 2013 in laboratory conditions, adults of *C. septempunctata* consume in average 468 aphids. The larvae pass through four instars and consume in average 350 aphids.

Todoroski and Maceljski, 1983, study that larva consume 361 aphids and imago 756 aphids. According Mahyoub, 2013, the average number of aphids consumed by each of the four instar larva are 35, 63, 96, and 290, respectively, under laboratory conditions. Sattar et al., 2008, reported that single larva during 1st, 2nd, 3rd and 4th instars consumed 21.9, 55.9, 107.4 and 227.3 aphids respectively. When aphid colonies are small and prey is scarce cannibalism of eggs, larvae and pupae may occur, especially when the age structure among the larvae population was heterogeneous.

Cannibalism is adaptive in that it improves the chances of survival of this species. Adult exude repulsive smell as reflex-bleed from the tibio-femoral joints of its legs when it was disturbed, as defense mechanism against predators. A threatened adult of *C. septempuncatata* may play dead to protect itself. Stinking smell is found also among larva of *C. septempunctata*.

Larvae are mobile, and they may wander for pray. When acting as a predator, *C. septempunctata* used mandibles to tear large irregular holes in aphids. Adult coccinellidae were very aggressive and even in the presence of aphids attacked all larval stage of *C. carnea* (Szentkirályi, 2001).

A new method for *C. septempunctata* egg manipulation was developed in Egypt, which consists of a plastic cylindrical puts inside the rearing cages to lay eggs in it and after laying eggs-masses transferred to a separated machine to separate the egg-masses and to be ready to stick on the card to release (Mahyoub, 2013). *C. carnea* is dominant species of lacewing as bio-regulators of *M. persicae* on tobacco. Monitoring the dynamics of growth of populations revealed that it reaching its maximum in mid-August. Adults are green, with long antennae and membranous, pale green wings and golden eyes. In the evenings and at night they are attracted by lights and we often catch adults with light trap. In field we found laid eggs in groups underneath tobacco leaves near potential prey. Egg is oval and secured to the plant by long slender stalks as protection of cannibalism. The larvae are voracious predators of aphids and actively search for prey. The larvae are brown are known as "aphid lions". Larva grasps their prey with mandibles and suck out the liquidated body fluids of an aphid. During the larval stage it molt three times. Larvae can consume large numbers of aphids. In laboratory conditions, larvae consume in average 458 aphids. The mature larva secrete silk and build round white cocoon in concealed positions on tobacco plants and pupate.

Lacewings use various defensive methods for protection against natural enemies. Larva is mobile, when it was attacked by another prey, larva quickly increase the distance from the aggressor. Adults exude some strong, stink smell when were attacked (Szentkirályi, 2001).

Larvae of *C. carnea* are highly aggressive in confined petri dishes. In confrontation with another predator when *C. carnea* was predator, puncture holes from the mandible insertion could be

observed in the body of the prey. Same aged or older and stronger larvae of *C. carnea* killed the larvae of *C. septempunctata* in the absence of other aphid prey (Szentkirályi, 2001).
When food is scarce larvae of *C. carnea* turn cannibal. Cannibalism can ensure the survival of population. Usually cannibalism in all experiment occurs when eggs or young instar larvae are kept together with larger larvae. The extent of cannibalism even in the same size larvae or same instar larvae is high. In manipulation, *C. carnea* must be distributed as eggs, to avoid cannibalism.
S. scripta is important natural regulator of aphid population on tobacco. The adult is black, with yellow strips. Sexual dimorphism is present. The female has long body, broadened somewhere near the middle and ending conically. The male has a long, slim, cylindrical body, and the last two bands are often blurred. The male's eyes are touching. The female's eyes are not touching. After mating, the females lay eggs singly on the underside of tobacco leaves, among the aphid colonies. Egg is off-white, small rounded at both ends, with surface patterning. The larvae are green with a wide pale white dorsal band on either side of the heart line. During their growth, pass through three stages. Larva does not leave black excrement frequently, but only before pupation. Puparium is formed from the last larval skin and its color and pattern resemble the 3rd stage. Larvae kill a great number of aphids. They have strong mouth hooks, suitable for catching the prey, sharp mouthparts, strong pharynx and head muscles which help them to stab and suck the prey. In laboratory conditions, larvae consume in average 333 aphids. Larvae greed increases during the second, and particularly during the third larval stage. When hungry, the larva sucks the first aphids completely and as it becomes satiated, it doesn't eat it thoroughly, but goes to find another aphid. Cannibalistic tendencies in *S. scripta* are very low and we have not established a mutual attack.
The larvae with confrontation with another predator move very fast, always touching the substrate with their heads and excreting oral slime to defend themselves against the opponent. When *S scripta* larvae were superior in confrontations, the remaining body parts of the prey showed a rather large circular hole as a result from repeated mouth-hook insertions. There is a slime secretions on the whole body surface of pray.
A. aphidimyza is recognized as being an important naturally occurring control agent of aphids on tobacco. Adults are black, small, delicate midges (flies) with long legs and antennae. Adult midges are very efficient at locating aphid colonies. Females deposit tiny shiny orange eggs singly or in small groups among aphid colonies. Small, bright orange, slug-like larvae inject a toxin into aphids' leg joints to paralyze them and then suck out the aphid body contents through a hole bitten in the thorax. Larvae can consume aphids much larger than themselves and may kill many more aphids than they eat when aphid populations are high. In laboratory larvae of *A. aphidimyza* consume in average 200 aphids of *M. persicae*. Cannibalistic tendencies in Aphidoletes are very low and we have not established a mutual attack. Interactions between *A. aphidimyza* and *C. septempunctata, C. carnea, S. scripta* were asymmetric and *A. aphidimyza* larvae were always victims.
During investigations, 5861 predators were determined in 2012 and 3788 in 2013. The growth of population of predators 2012/2013 continuously follows the growth of aphid population on tobacco (Figure 1 and 2). Cannibalism is important factor in rearing conditions of predators. According our investigations, in plentiful populations of aphids, cannibalism among predators is rare feature. Hindayana, 2001, stated that in the absence of aphids, eggs of Syrphidae and Aphidolethes were heavily cannibalized by the conspecific larvae, especially by older larvae. In the absence of aphids, L1 was vulnerable to older larvae too. In general, the cannibalism on L1 (in both species) decreased significantly in the presence of aphids. According our investigations cannibalism among older larvae or larvae with equal size did not occure, neither in the absence nor in the presence of aphids. The larvae of Syrphidae and Aphidolethes would rather starve to death instead to eat the conspecific larvae. Cannibalism is common in *C. septempunctata*, especially when the aphid population is small or in the absence of prey (aphids) and when the age structure among the larvae is heterogeneous.
On the other hand, cannibalism in *C. carnea* frequently occurred and was high even when food was abundant and population density low (Ridgway et al., 1970; Phoofolo and Obrycki, 1998, cit. Hindayana, 2001).

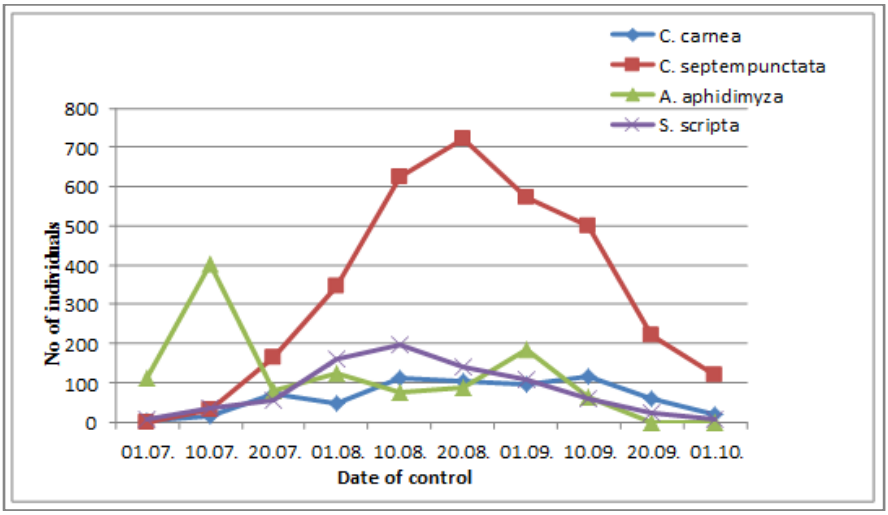

Figure 1. Population dynamics of aphidophagous hoverflies on tobacco in 2012

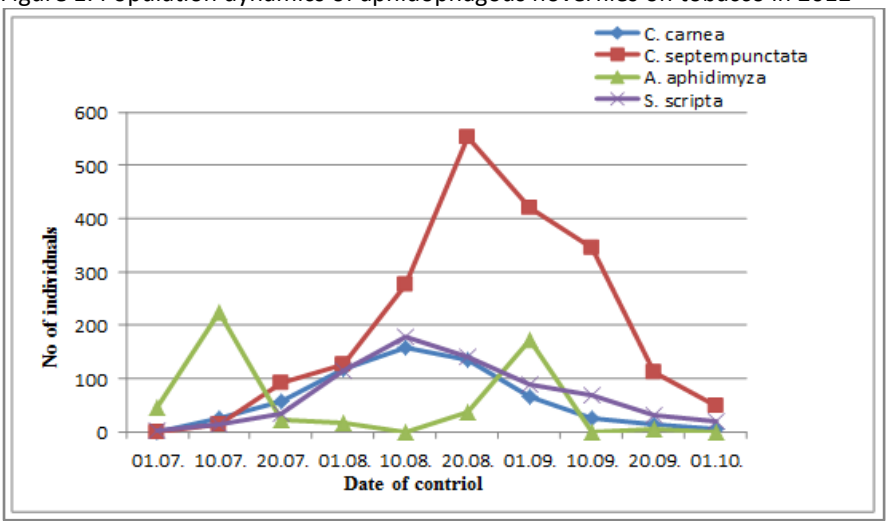

Figure 2. Population dynamics of aphidophagous hoverflies on tobacco in 2013

According our investigations, cannibalism in *C. carnea* occurs when eggs or young instar larvae are kept together with larger larvae. It was still high, even in the same instar larvae. Almost in all habitats with leaf aphids all variety of natural enemies of aphids is also present. Predators whose use similar, often limited resources, are potential competitors. *S. scripta, C. septempunctata, C. carnea* and *A. aphidimyza* share the same foraging habitat. The outcome of interactions between predators mainly depends on the body size of the competitors. In general large individuals behaved as predator while small individuals became prey. Eggs and first instar larvae were extremely vulnerable in regard to larger larvae. Mobility of predators also affects the occurrence of interspecific interaction. All instars of the rather small, immobile and defenseless *A. aphidimyza* larvae were always prey to *S. scripta, C. carnea* and *C. septemunctata* larvae.\Eggs and young instars of *S. scripta* were very sensitive to predatory behavior by *C. septempunctata* and *C. carnea*, and their larvae consume prey in all experiments without aphids. In L1, *S. scripta* did not show defense reactions or counterattack behavior in confrontations, unlike older larval stages (L2 and L3). In those cases where *S. scripta* larvae behaved as predator, L2 was the first developmental stage that was able to kill larvae of *C. septempunctata, C. carnea* and *A. aphidimyza*. However in competition, large proportion of *C. septempunctata* and *C. carnea* larvae survived an aggression, i.e., were not consumed by *S. scripta*. Larvae of both predators were killed depending on the relative body size of prey (*C. carnea* or *C. septempunctata*) and predator (*S. scipta*).

In experiments with *C. septempunctata* and *C. carnea*, the presence of aphids significantly decreased specific interaction between predators. *C. carnea* possess powerful mandibles and it is able to pierce the other predators. Spikes on the dorsal integument of *C. septempunctata* larvae prevent successful attacks of predaceous larvae. In *C. septempunctata* and *C. carnea* defense mechanisms were less pronounced in stages eggs and L1. Confrontations between predators of similar size often resulted in symmetric interactions where neither species consistently took on the role of the predator. In this experiment the large individuals behaved as predator while small individuals became prey. Interspecific competition is an adaptation due to the short-term nature of the colonies of the plant aphids. In laboratory experiments, both cannibalism and IGP decreased with increasing aphid density.

Conclusions

Aphidophagous predators compete for the same kind of victim or plant aphids in natural angorecosystems. *S. scripta, C. septempunctata, C. carnea* and *A. aphidimyza* are predators of *M. persicae* on tobacco. Under favourable conditions (temperature and food resource availability) there they may develop a large population. The growth of population of predators approximates roughly the period of mass reproduction of aphid population on tobacco. During the diet, predators often encounter various types of leaf aphid predators, from other families. In these situations, the same types of insects in the food chain can be predators and victims. The size of the predators determines the outcome of the interaction. Cannibalism in lacewings and ladybirds generally occurs when food resources are rare. Predators can be used in biological control of insect pests of crops. Interspecific competition and cannibalism are important factors in conditions of breeding and biological struggle.

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