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REGULARITIES IN THE FORMATION OF ENTOMOFAUNA IN ALFALFA AGROCENOSIS

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Abstract

The field work was performed at the experimental field of the Institute of Forage Crops, Pleven, Bulgaria during the period of 2006-2009. The share distribution of the orders Coleoptera, Thysanoptera, Diptera, Orthoptera and Hemiptera, suborder Sternorrhyncha, Heteroptera, Fulgoromorpha and Cicadomorpha was established for every four alfalfa growth cycles during the vegetation period for four years. It was found that the share participation of orders was determined primarily by the population dynamics of the dominant insect species and it was closely dependent on plant development following a characteristic course over the years. Depending on the food specialization and the division of the species into harmful and beneficial, the share of predators in regrowth corresponded to that of their prey. A clear trend was observed of increasing the share of harmful species and reducing the beneficial ones (Coleoptera, Thysanoptera, Hemiptera: Heteroptera) with the alfalfa development over the years.

Key words: alfalfa, entomofauna formation, regularities

INTRODUCTION

Alfalfa as an agrosystem with a direct impact of the anthropogenic factor is an artificially created system in which favourable conditions are created for the reproduction of many insect species (Pearson et al., 2008). Their high number is determined, on the one hand, by the monoculture cultivation of the crop (less often in mixed-grass associations) and directly or indirectly related insects to it. On the other hand, in the first and second years of alfalfa development, the populations of many insects migrate to crops and the amount of main species increases considerably in the following years.

The entomofauna of alfalfa is rich and diverse in species, which requires a more detailed study of the culture of different ages and regrowth (Pertseva, 2016). As a result of this diversity and productivity and the artificial dominance of this ecosystem, alfalfa fields have become a rich source of empirical data for a

number of theoretical issues. Those being in basic ecology, including parasite-host interactions (Saric-Krsmanovic et al., 2018; Louis et al., 2019), indirect effects (Pearson et al., 2008), intraguild predation (Iemma, 2015; Cividanes, 2021), diversity-ecosystem function relationships (Song et al., 2014), invasive species (Kajita & Evans, 2010), and plant defence theory (Lei et al., 2017; Dalling et al., 2020), regularities of formation of entomocomplexes in alfalfa agrocenosis.

In the process of studying the regularities of formation of entomofauna in alfalfa the following factors are taken into account - the biological characteristics and conditions of cultivation of perennial crops (Melikhov et al., 2018), associations between the eating of phytophages, zoophagous and phenophases of plant development (Pellissier et al., 2017; Shayestehmehr et al., 2017), the dynamics of insect population depending on the development of the crop by years and regrowth

(Pertseva, 2016), as well as the impact of cutting on insects (Mahsa et al., 2019).

Regularities of formation and functioning of entomocomplexes in alfalfa agrocenoses with rich biological diversity exposed to anthropogenic successions of various frequency require clarification. Therefore, the research was aimed to find regularities in the formation of entomofauna in *Medicago sativa* L.

MATERIALS AND METHODS

The field work was performed at the experimental field of the Institute of Forage Crops, Pleven, Bulgaria during the period 2006-2009. For this purpose in the spring of 2006 sowing of alfalfa, variety Pleven 4 was carried out on area of 300 m², sowing rate 25 kg ha⁻¹, row spacing 11.5 cm, oats predecessor without irrigation. The soil treatment included ploughing (22-25 cm) in autumn and two cultivations (15 cm) in spring. No pesticides were applied. To measure the abundance and dynamics of insects at a natural population density and composition of main species in alfalfa, net sweeping was carried out weekly during the growing season.

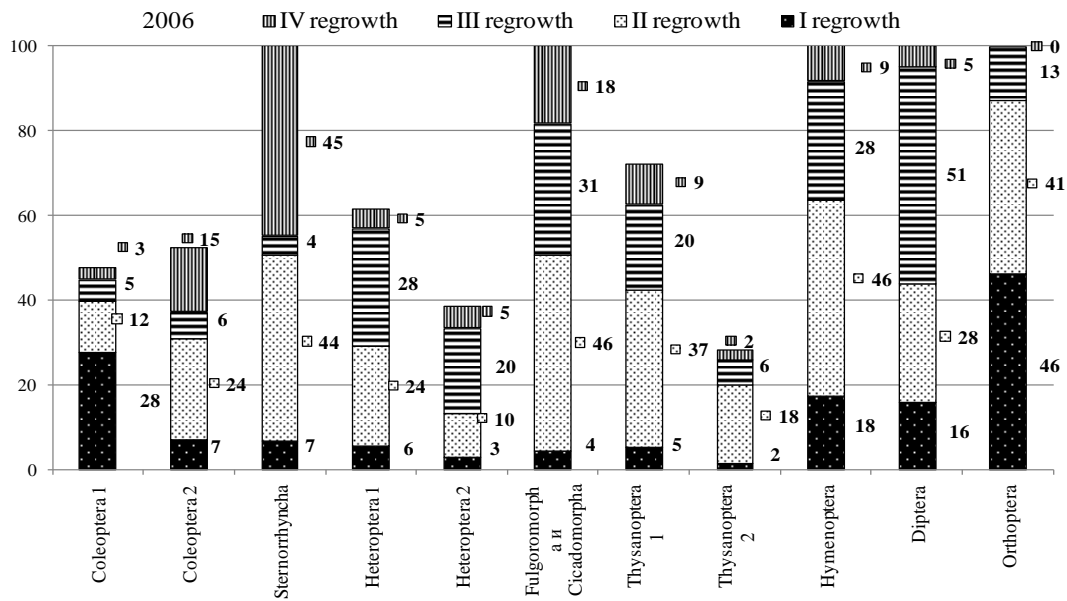
The quantitative participation of the representatives of the order Coleoptera, Thysanoptera, Diptera, Orthoptera and Hemiptera, suborder Sternorrhyncha, Heteroptera, Fulgoromorpha and Cicadomorpha was summed up before harvest for every four alfalfa growth cycles during the vegetation period in four years. Samples (ten per day) were taken along the diagonals of a crop from the plant growth to the harvest of each regrowth in the flowering stage. The share distribution of the presented orders and suborders in each regrowth was calculated as a percentage depending on their total quantitative participation during the year.

The share participation of the species of the orders Coleoptera, Thysanoptera and Hemiptera, suborder Heteroptera, depending on their feed preferences, was divided additionally into two groups: harmful (phytophagous) and beneficial (predatory) in each regrowth.

RESULTS AND DISCUSSION

In the first year of alfalfa development, the total number of the order Coleoptera (harmful and beneficial species by 35% share) and Orthoptera (by 46%) are characterized by the dominance of the numerical participation in the first regrowth compared to their proportion in the next cutting of the alfalfa. (Fig. 1). The first cutting was performed when plants reached the flowering stage, which occurred just in the early days of June (unlike subsequent years, when the first cutting was usually done around mid-May). That longer period favoured the passage of insects in alfalfa from neighbouring or more distant crops and habitats.

As seen in the figure, the Coleoptera order was represented by two groups: harmful and beneficial species and the harmful species were dominant with 28% in the first regrowth. The high participation of the order was determined by the biology and damage of the main phytophagous species. Those were *Hypera postica* Gyllenhal, species from the *Sitona* genus, among which the predominant species were *S. macularius* Marsham, *Catapion seniculus* Kirby and representatives of the *Tychius* genus (predominantly *T. flavus* Becker and *T. medicaginis* Brisout). *Subcoccinella vigintiquatourpunctata* Linnaeus and *Gonioctena fornicata* Bruggemann were less common species among Coleoptera. Their participation in the next regrowth was considerably less within 3-12%.



Legend: 1-harmful species; 2 – beneficial/beneficial species

Figure 1. The order and suborder ratio by regrowths in alfalfa agrocenosis in 2006 (first year of cultivation)

The beneficial species in the first regrowth had a minimal portion (only 7%), while their number in the second one reached the highest value of 24% followed by a fourth one (15%). The beneficial species were represented by predators belonging to the Histeridae, Cantharidae and Coccinellidae families, and among them the predatory ladybugs predominated - *Coccinella septempunctata* Linnaeus and *Hippodamia variegata* Goeze. The strong presence of predatory in the second, followed by the fourth regrowth was related to the numerous presence of their prey - aphids (Hemiptera: Sternorrhyncha) in both ones (44 and 45%, respectively).

In the first year of alfalfa cultivation, the presence of harmful species was the least pronounced and the ratio between the harmful and useful group was in favour of the predators with 53% against 47% share of phytophages, in total for the four regrowth.

The order Orthoptera dominated too in the first regrowth and was represented mainly by larvae of *Tettigonia viridissima* Linnaeus (Tettigonioidea), as, in the second, third and

fourth one the participation of the species decreased proportionally, although other species were caught, but in negligible density (*Calliptamus italicus* L. and *Dociostaurus maroccannus* Thunbg (Acrididae)).

The species of the order Hemiptera, suborder Sternorrhyncha (44%), Fulgoromorpha and Cicadomorpha (46%), order Thysanoptera (55% in total) and Hymenoptera (46%) had the highest share in the second regrowth. Among aphids (Sternorrhyncha) *Therioaphis trifolii* Monell dominated over other species, as the species specifically in the first year had the highest density for the studied period. According to the numerical dynamics, the spotted alfalfa aphid had two peaks in the second and fourth regrowth and it determined the high participation of Sternorrhyncha in both.

Cicadas (Fulgoromorpha and Cicadomorpha) were characterized by low numbers in the first regrowth (4%), with the predominating mainly of *Streptanus josifovi* Dlabola in the stem formation and budding stages. The common cicada entomofauna had the largest quantitative participation in the

second regrowth (46%), where the majority of the identified species of the suborder were presented. Cicada presence was considerable as well in the third regrowth (31%). The main cicada species with numerous presence were *Empoasca pteridis* Dahlbom, *Anaceratagallia laevis* Ribaut, *Austroagallia sinuata* Mulsant & Rey and *S. josifovi*. In the fourth regrowth, dominated primarily by *A. sinuata* and *E. pteridis*, which were with high density and forming 18% of Fulgoromorpha and Cicadomorpha.

The total number of thrips (Thysanoptera) was markedly highest in the second regrowth (55%), and the ratio between the harmful and useful species was considerably in favour of the harmful (37 and 18%, respectively). That ratio was clearly expressed in the third regrowth (20% harmful and 6% beneficial), where the total participation remained relatively high (26%). Among the harmful species, *Thrips tabaci* Lindeman and *T. atratus* Haliday dominated, which repeatedly exceeded the quantitative share of other thrips. The participation of predatory thrips was represented mainly by the dominant species *Aeolothrips intermedius* Bagnall. Considering the lower quantitative share of phytophagous compared to the following years, the nutritional activity of the predatory thrips was probably hampered. The absence of sufficient food during the year likely determined their weak presence with a total of 28%.

The Hymenoptera order was represented mainly by species belonging to the Ichneumonidae and Chalcididae family. Their numerical dynamics changed over the years because it was influenced by abiotic factors, the phenological development of alfalfa and the life cycle of the host insects. However, there was a clear trend in 2006, which was being researched in the following years. The order had a predominant presence in the second (46%), followed by the third regrowth (28%). Parasitoid species from the Chalcididae family began to settle, respectively to search for their

prey in May, in the first regrowth. Their numbers increased sharply and reached a maximum value in the second, followed by the third regrowth. During that period, plants were most strongly attacked by insect species with piercing-sucking mouthparts, such as aphids, heteropterous species and cicadas. The species of the Ichneumonidae family are adapted to specific stages of development of a certain insect - phytophagous with which they feed. Nutritional relationships from a cenological point of view were due to the participation of those species - parasitoids, only when there was an opportunity for their life and development in the alfalfa agrocenosis.

Representatives of the Heteroptera suborder, Hemiptera (total harmful and beneficial species with 48% share) and the Diptera order (51%) had the highest participation in the third regrowth. Accordingly, both harmful and beneficial heteropterous species had the highest share with 28 and 20%, followed by the second regrowth (24% harmful and 10% useful species). The main species of the suborder were the species *Adelphocoris lineolatus* Goeze and *Lygus rugulipennis* Poppius (Miridae), as well as *Piezodorus lituratus* Fabricius (Pentatomidae). The beneficial entomofauna was mainly represented by species belonging to the family Anthocoridae (*Orius horvathi* Reuter and *O. niger* Wolff) and Nabidae (*Nabis ferus* Linnaeus, *N. pseudoferus* Remane). The high number of harmful species during the year (63%) was accompanied by a relatively pronounced presence of beneficial ones (predators), whose total participation reached 39%. Considering that predators are polyphagous and feed on various pest species, including aphids and cicadas, which were numerous in the first year of the study, that determined the high participation of predatory bugs, emphasizing their exceptional role as bioregulators.

From the Diptera order, the Chloropidae family mainly predominated. Three beneficial species were identified from the Syrphidae

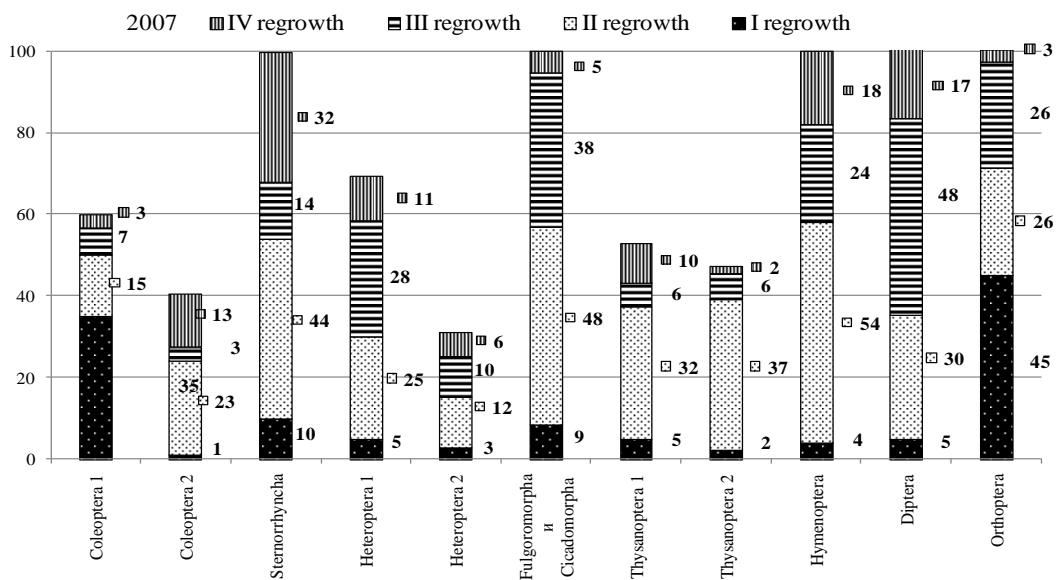
family (*Syrphus ribesii* Linnaeus, *S. vitripennis* Meigen and *Eupeodes corollae* Fabricius). They were not distributed in a separate group because of their very low share in relation to the total number of Diptera. Despite the high quantitative participation of the Chloropidae family, they belong to the group of alfalfa-indifferent insects. Their share was the highest during the flowering stage, which was explained by the attraction of more species from the flower nectar and honeydew secreted by aphids. Representatives of the Diptera order with the predominant presence in the third regrowth (51%) was due to the moving of flies into the alfalfa in the late June and July from adjacent areas sown with oats, looking for more favourable microclimatic conditions during the hot summer days. That trend continued in the following years. Harmful to alfalfa flies, represented by the *Liriomyza* genus (family Agromyzidae), which larvae mine the leaves, had a minimal presence and no damage was found on the plants. Therefore, it was not possible to track their numerical dynamics.

In the second year of alfalfa cultivation

(2007), the pests of the order Coleoptera (36% in total) and Orthoptera (45%) followed the established trend in the previous year. They occupied the highest share in the first regrowth (Fig. 2). It was observed that the participation of the harmful species from Coleoptera was relatively increased compared to 2006, reaching 35%. Among the phytophagous beetles, *H. postica* and *Sitona* genus had the highest numbers. They caused damage mainly in the first regrowth in accordance with their developmental cycle and their presence in the second regrowth was considerably reduced.

The participation of predators was most pronounced in the second (23%), followed by the fourth regrowth (13%) and fully corresponded to the dominant presence of aphids in the second (44%), followed by the fourth regrowth (32%).

The ratio between the harmful and beneficial beetles during 2007 changed in comparison to the previous year and the harmful ones predominated with 60% total participation. However, the predators share of 40% remained high.



Legend: 1-harmful species; 2 – beneficial species

Figure 2. The order and suborder ratio by regrowth in alfalfa agrocenosis in 2007 (second year of cultivation)

It should be noted that the established highest share of aphids in the second regrowth, with a pronounced difference compared to the fourth one, was due not only to the high numerical participation of the spotted alfalfa aphid, but also to the pea aphid abundance. *Acyrtosiphon pisum* Harris in 2007 had three times higher density reaching a peak value in the second regrowth compared to the previous year. Therefore, the common dynamics of aphids did not follow a characteristic course because of the minimal presence of the species in the fourth regrowth.

The trend of prevailing participation of cicadas in the second (48%), followed by the third regrowth (38%) was maintained, but their presence in the fourth one, unlike other years, was minimal. The numerical dynamics of one of the predominant species (*Empoasca pteridis*) reached the highest value in the fourth regrowth but due to its low density during the year, the presence of cicadas in the regrowth (due primarily to *E. pteridis*) reached only 5%.

The Thysanoptera order had the highest number in 2007 and there were clear trends. The dominant participation of thrips in the second regrowth was retained (69% in total), but the share of beneficial species (37%) was considerably higher than in 2006 (18%) and slightly exceeded the share of harmful species (32%). An equalized ratio was observed in the third regrowth (6% each). The high number of pest species was accompanied by the abundance of beneficial species during the year, as the total participation of phytophages with 53% slightly exceeded that of predators (47%). That indicated that the provision of sufficient food contributed to the favourable development and reproduction of predatory species that play an important regulatory role in the alfalfa agrocenosis.

The ratio between the two groups of Heteroptera was similar. The high share of phytophagous species in the third (28%), followed by the second regrowth (25%) was accompanied by a relatively pronounced

presence of predatory with 10 and 12%, respectively. The seasonal occurrence and abundance of predators (with predominant species of the genus *Nabis* and *Orius*) were associated not only with the high participation of phytophagous species, but also corresponded to the abundance of other pests such as aphids, thrips and cicadas in the second and third regrowth. Natural bioagents were influenced by the total quantitative participation of their prey and the density of predators was high because of the presence of sufficiently abundant and varied food. The total share of beneficial species during the year remained relatively high by 31%.

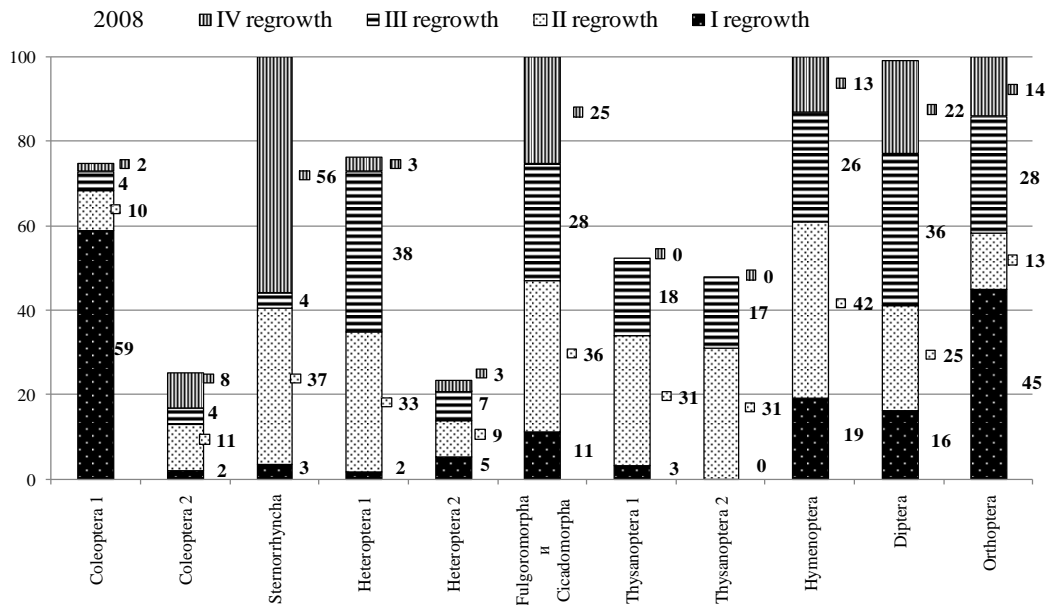
The representatives of the Hymenoptera order were characterized by pronounced participation in the second regrowth (54%), and Diptera predominated in the third (48%) with a relatively high presence in the second regrowth (30%).

The established tendency of increase of the harmful insects from the Coleoptera order and decrease of the beneficial ones **in the third year** of alfalfa development (2008) was confirmed (Fig. 3). For example, in the first regrowth, phytophages species retained the highest participation (59%) compared to other regrowth (as in previous years), but exceeded their number approximately twice compared to 2007 before the first cutting (35%). That was due to the abundance of *H. postica* and the *Sitona* genus as their number increased fivefold and one and a half times, respectively, compared to the first regrowth in the previous year. It was found a marked decrease in the number of beneficial species by 25% total share during the year compared to their participation in 2006 (53%) and 2007 (40%). Their presence in the second regrowth had the highest percentage (11%).

Orthoptera order maintained a relatively consistent high participation in the first regrowth in the first, second and third year of alfalfa cultivation, varying in the narrow range of 45 - 46%.

Aphids supported a high presence in the second and fourth regrowth, as prevailed in the fourth one with 56% share. The observed trend

was similar to that in 2006, but in 2007 it was much stronger and followed the characteristic course.



Legend: 1-harmful species; 2 – beneficial species

Figure 3. The order and suborder ratio by regrowths in alfalfa agroecosystem in 2008 (third year of cultivation)

Cicadas, as in the previous years, had the highest participation in the second regrowth (36%) owing to the rich species diversity and the high number of dominant cicadas (*A. laevis* and *P. spumarius*). A similar relatively high proportion of cicadas was observed in the third and fourth regrowth (28 and 25%, respectively). The reason for that was the higher number of other dominant species (*E. pteridis* and *A. sinuata*) during the year, which in accordance with their population dynamics followed a characteristic course and had a pronounced presence in the last two regrowths of alfalfa.

The tendency of emphasized dominance of the Thysanoptera order in the second, followed by the third regrowth was retained. The high amount of predatory thrips (mainly *A. intermedius*) corresponded to that of phytophagous (*T. tabaci* and *T. atratus*). The ratio between the harmful and beneficial species was almost equal in the second and third regrowth, as the total share of phytophages slightly exceeded that of predatory species (52

and 48%, respectively).

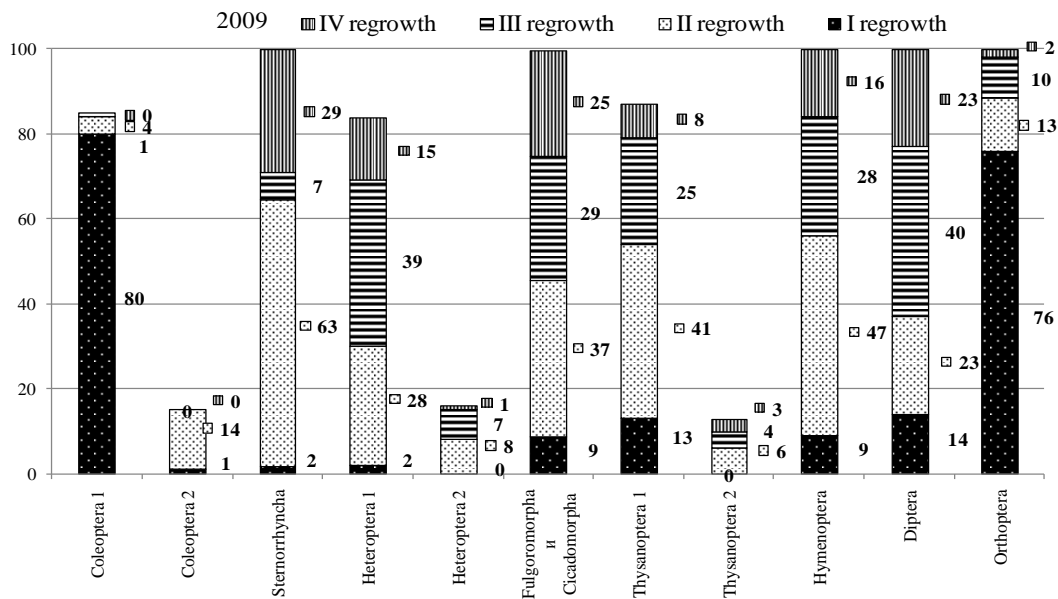
The share of harmful species from the Heteroptera order prevailed in the third (38%), followed by the second regrowth (33%), as their total participation of 77% during the year slightly exceeded the corresponding of the previous year (69% in total). The predatory species maintained their constant presence in the crop and a relatively high total share (23%) while their distribution by regrowth corresponded to the numerical dynamics of their prey.

The group of the harmful coleopteran species **in the fourth year** of alfalfa development reached the highest share of 80% in the first regrowth compared to previous years and in subsequent regrowth, their presence was negligible (Fig. 4). The beneficial species had the lowest share in the last year (15% in total), but nevertheless, they maintained the dominant trend in the second regrowth (14%), in accordance with the numerical dynamics of their prey (aphids).

Aphids markedly had the highest share in the second regrowth (63%), while their participation in the fourth one was weak (29%). There was a change in the ratio between the species in the two regrowths compared to previous years of research. The main reason was the unfavourable weather conditions in the first ten days of September (fourth regrowth) when usually, *T. trifolii* is the most numerous, but the amount of precipitation of 41.6 mm almost

washed aphids from the plants. Pea aphid had a minimal participation during the year and did not affect the share distribution.

As in the previous years, the quantitative share of cicadas prevailed in the second regrowth by 37% (primarily *E. pteridis*, *A. laevis* and *A. sinuata*), with an expressed share in the third and fourth regrowth (29 and 25%) owing to the high numbers mainly of *E. pteridis* and *A. sinuata*.



Legend: 1-harmful species; 2 – beneficial species

Figure 4. The order and suborder ratio by regrowths in alfalfa agrocenosis in 2009 (fourth year of cultivation)

The ratio between the harmful and beneficial thrips was greatly changed, and the total proportion of the phytophagous species substantially dominating over predatory, 87 and 13%, respectively. Despite the high share of phytophages during the year, their number in alfalfa crops occupied the lowest values for the four-year period (below 25 individuals/100 cuttings). The presence of beneficial species was severely limited due to the lack of sufficient food.

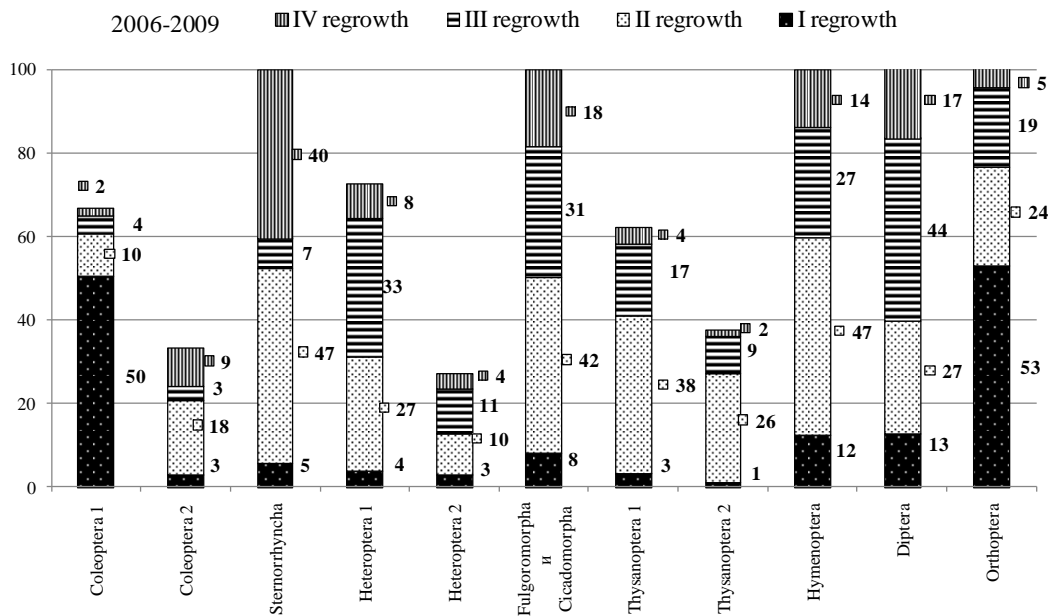
The tendency for the predominance of the representatives of the Heteroptera in the third regrowth was confirmed. On the other hand, the difference in the proportion between the harmful and beneficial species was the

biggest compared to the 2006-2008 period. The total share of phytophagous reached 84%, and predatory - only 16%. In the fourth year of alfalfa growing, the presence of Heteroptera, Sternorrhyncha, etc. insect species with piercing-sucking mouthparts was substantially reduced compared to the previous ones, which affected the number of beneficial heteroptera.

Like the harmful species of Coleoptera, Orthoptera reached the highest participation in the first regrowth of 76%. The predominant indifferent species from Hymenoptera maintained their high share in the second (47%) and from Diptera - in the third regrowth of alfalfa (40%).

The summarized results for the period 2006-2009 clearly showed that the order Coleoptera (53% in total) and Orthoptera (53%) had the highest share in the first regrowth - Fig. 5, which was determined by the dominant species. Among the beetle's species a constant and high participation had *H. postica*, *Sitona* spp. and *C. seniculus*. Similar results were reported by Pellissier et al. (2017). According to them, *H. postica* was one of the most problematic and main pests in the early season of alfalfa, prior to the first cutting of hay, although negative effects on the second crop can occur depending on the management practices

used. Although the majority of feeding damage was caused by larvae, the authors suggest that adults may still cause some damage by feeding on the new growth in the spring. In an earlier study, Rotrekl & Cejtchaml (2008) reported that species of the genus *Sitona* were the most numerous and caused the greatest damage in dry and warm weather when alfalfa was in the 2nd or 3rd leaf development stage. Also, Ivanova (2004) and Atanasova (2011) published similar to the current results for the conditions of the Plovdiv region, concerning the dominant species of Coleoptera and the periods of their mass appearance.



Legend: 1-harmful species; 2 – beneficial species

Figure 5. The order and suborder ratio by regrowths in alfalfa agroecosystem during the period 2006-2009

The overall ratio between the harmful and beneficial beetles was in favour of the harmful ones (67 and 33%), as the dominant participation of predatory ladybugs in the second and fourth regrowths corresponded to the numerical dynamics of their prey, that being mainly aphids.

In accordance to the dynamics of the main aphid species (*T. trifolii* and *A. pisum*), their percentage share in the second regrowth slightly exceeded the corresponding one in the fourth regrowth (47 and 40%, respectively).

Similar results were reported by Mahsa et al. (2019) according to which aphid and coccinellid population in alfalfa had two population peaks before the second and fourth cutting and their number changes and peaks seemed to be coincident and synchronous in different years. On the other hand, Iemma (2015) reported that *T. trifolii* was the most abundant aphid during the vegetation period, followed by *A. pisum*. According to the author the populational peaks occurred in September in the fourth regrowth of alfalfa.

The cicada participation predominated in the second (42%), but their share was considerable in the third followed by the fourth regrowth (31 and 18%). That defined them as permanent insect pests during the alfalfa vegetation. Their high share was determined by the abundance of the dominant and subdominant species *E. pteridis* (second and primarily third and fourth regrowths), *A. laevis* (second and third regrowths), *A. sinuata* (second, third and fourth regrowths) and *Philaenus spumarius* Linnaeus (second and third regrowths). Raupacha et al. (2002) found that the age of the host plant influenced the choice and preference of *Empoasca* species, such as adults greatly preferred more developed plants compared to younger in the later regrowths. Later, Kozhevnikova (2014) added that *A. laevis* was another of the most common species in *M. sativa*, as the cicada was most numerous before the second harvest and reached 6 - 10 individuals per leaf, in favourable for the species development conditions.

The dominant share of Thysanoptera in the second regrowth (64% in total) was clearly expressed as the harmful species had a comparative advantage. The percentage distribution of predatory thrips (mainly *A. intermedius*) fully corresponded to phytophagous ones (*T. tabaci* and *T. atratus*) and outlined their important regulatory role in the alfalfa agroecosystem. A similar conclusion about the strict trophic dependence between *A. intermedius* and *T. tabaci* and the important regulatory role of the predator in the alfalfa agroecosystem was reported by other authors (Conti, 2009; Abraham 2012).

Heteroptera order had a pronounced presence in the third (44% in total), followed by the second regrowth (37%) as harmful species had an emphasized higher share. The seasonal number of predators (mainly species of the *Nabis* and *Orius* genus) was related not only to the abundance of phytophagous species (*A. lineolatus* and *L. rugulipennis*, as well as *P. lituratus*), but also to the predominant share of

other pests such as aphids, thrips and cicadas in those two regrowths of *M. sativa*. Similar to the present results, Mahsa et al. (2019) reported that the dominant plant bugs in alfalfa fields were *A. lineolatus* and *L. rugulipennis*, having two population peaks in the second and third regrowths.

The Hymenoptera order had the highest share in the second (47%), and from Diptera - in the third regrowth of alfalfa (44%).

In a study of trophic relationships between terrestrial predatory beetles and phytophages in the alfalfa agroecosystem, Devyatkin (2006) reported similar results and revealed the synchrony between predator and prey. On the other hand, Popova (1965) found that the dynamics of the orders were based on dominant species, but there were differences both in terms of the main species and in terms of their mass occurrence.

CONCLUSION

A share distribution of the orders Coleoptera, Thysanoptera, Diptera, Orthoptera and Hemiptera, suborder Sternorrhyncha, Heteroptera, Fulgoromorpha and Cicadomorpha was established in the four regrowths from the alfalfa development.

The share participation of orders was determined primarily by the population dynamics of the dominant insect species and it was closely dependent on plant development following a characteristic course over the years.

Depending on the food specialization and the division of the species into harmful and beneficial, the share of predators in a regrowth corresponded to that of their prey.

A clear trend was observed of increasing the share of harmful species and reducing the beneficial ones (Coleoptera, Thysanoptera, Hemiptera: Heteroptera) with the alfalfa development over the years.

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