



**СЕЛСКОСТОПАНСКА
АКАДЕМИЯ**

ИНСТИТУТ ПО ФУРАЖНИТЕ
КУЛТУРИ - ПЛЕВЕН



AGRICULTURAL ACADEMY

Prof. Dr Ivelina Mitkova Nikolova

ENTOMOFAUNA OF ALFALFA (MEDICAGO SATIVA L.) IN THE PLEVEN REGION AND METHODS FOR CONTROL OF INSECT PESTS IN BIOLOGICAL FORAGE PRODUCTION

ABSTRACT

**FOR THE AWARD OF THE SCIENTIFIC DEGREE
"DOCTOR OF SCIENCES"**

**PROFESSIONAL FIELD 6.2 PLANT PROTECTION
(ENTOMOLOGY)**

SOFIA

2023





The dissertation is 461 pages long and includes 95 tables, 39 figures, 12 photos, and a list of literature from 886 sources, with 153 in Cyrillic and 733 in Latin.

The research was conducted at the Institute of Forage Crops in Pleven from 2006 to 2019.

The defence of the dissertation will take place on of hours in the Meeting Hall of in an open meeting before the members of the scientific jury.

The defence materials are available to those interested in the library at

Please send your feedback and notes to:

.....

ACKNOWLEDGMENTS

The realization of the studies became possible thanks to the care taken by the management of IFC-Pleven, for which I express my gratitude!

I am deeply grateful to my colleagues for their assistance in identifying insect species for my dissertation.

I am grateful to Professor Dr Nataliya Georgieva for her invaluable help and assistance!

I express my sincere gratitude to my colleagues from the Institute of Forage Crops, Pleven, who contributed to the implementation of the experimental activity, to the technical assistants Tanya Georgieva and Veselka Hristova!

I would like to express my sincere gratitude to my family for their unwavering support and understanding.



INTRODUCTION

Alfalfa, scientifically known as *Medicago sativa* L., is a high-yielding and nutritious forage crop widely cultivated for its commercial value. Due to its adaptability to various environmental conditions, nitrogen fixation capability, and agrotechnical properties, alfalfa is considered a strategic crop. However, as it is vulnerable to pests and diseases, there is a need for more efficient pest control measures to ensure its productivity and economic value (Tohidfar et al., 2013).

Comprehensive studies on harmful or beneficial entomofauna in alfalfa were mainly conducted in the 20th century for the Plovdiv and Sofia regions in our country. However, similar studies have not been carried out for the Pleven region, which makes it necessary to conduct such research. There is insufficient research on the trophic relationships between harmful and beneficial insects, determining the damaging activity of dominated species. They are missing subsequent biochemical plant changes after damage and methods to define the extent of their injury. There is a lack of data on the regularities patterns of the insect population in alfalfa crops. Knowledge of the enemy community inhabiting alfalfa as a main forage crop, as well as their predators and parasitoids, is essential for the development of environmentally sound methods for controlling the main phytophagous species in organic alfalfa production. Studies that seek to develop efficient control approaches for the most significant pests in alfalfa, with ecological, economic, and practical importance, are rare or non-existent. Solving this complex set of tasks will ensure high and consistent yields, preserve biodiversity, and achieve stable and quality production. That is the head goal of the present dissertation work.

AIM AND OBJECTS

The purpose is to study the entomofauna in the alfalfa agrocenosis in the Pleven region, the harmful activity of the main species, and to develop methods for controlling main insect pests in organic forage production.

The goal was achieved by completing the following tasks:

1. Study of species composition and numerical dynamics of main harmful and beneficial species;
 - 1.1. Study of morphological features, pest activity and numerical dynamics of *Catapion seniculus* (W. Kirby, 1808) (Coleoptera: Apionidae);
2. Study of regularities in the formation of the entomofauna in alfalfa agrocenosis;
3. Establishing nutritional relationships: aphids - aphidophagous;
4. Study of the harmful activity of a large alfalfa weevil, *Otiorhynchus (Cryphiphorus) ligustici* Linnaeus (Coleoptera, Curculionidae):
 - 4.1. Development of a method to determine the degree of damage by the larva of *Otiorhynchus (Cryphiphorus) ligustici* Linnaeus in alfalfa varieties;
 - 4.2. Modeling a scale of resistance to *Otiorhynchus (Cryphiphorus) ligustici* Linnaeus in alfalfa cultivars;
5. Development of methods to control main enemies in the organic forage production conditions:
 - 5.1. Biological testing of the efficacy of biological insecticides and biologically based products against the main insect pests in alfalfa;
 - 5.2. Influence of biological products on natural bioagents of main insect pests in alfalfa;
 - 5.3. Tolerance of alfalfa cultivars to attack by some main insect pests (*Catapion seniculus* Kirby; *Sitona* spp.);

- 5.4. Effect of perennial cereal-legume grass mixtures on damage by soil-habitat root insect pests;
6. Stability of alfalfa productivity after application of biological products;
7. Study of biochemical changes in plants attacked and damaged by main insect pests;
8. Forage quality and evaluation of energy nutritional value in alfalfa treated with biological products.

MATERIAL AND METHODS

The research studies were conducted at two different locations. The first location was the Second Experimental Field of the Institute of Forage Crops, situated near the village of Krushovitsa. It is located 15 km to the west of the city of Pleven. The second one was the First Experimental Field located in the Komudara area.

During the period 2006 - 2020, one laboratory and seven field experiments were carried out, three of which were at the First Experimental Field and the remaining four at the Second Experimental Field of the Institute.

SPECIES COMPOSITION AND NUMERICAL DYNAMICS OF INSECTS IN ALFALFA AGROCOENOSE

Studies were conducted between 2006 and 2009 and from 2012 to 2014 in the Second Experimental Field. These studies focused on the species composition and numerical dynamics of insects in the agrocenosis of alfalfa grown for forage. The bee fauna (Hymenoptera: Apoidea) was studied during alfalfa seed production in the First Experimental Field from 2015 to 2017.

1.1. Studies at the Second Experimental Field of the Institute of Forage Crops

Over four years, research was conducted on the entomofauna of alfalfa at the Institute's Second Experimental Field. In 2006, a spring sowing of alfalfa was planted with a sowing rate of 25 kg ha⁻¹, an area of 300 m², and a row spacing of 11.5 cm. The crop was grown under non-irrigated conditions with predecessor oats. From 2007 to 2009, the study focused on identifying the species composition of the representatives of the order Coleoptera and the harmful activity of *S. seniculus* and *O. ligustici*. From 2012 to 2014, a spring alfalfa crop was grown annually on an area of 200 m². The crop was used to identify the insect pest species of the order Coleoptera during the emergence and initiation of stem formation in alfalfa. During the growing season, no chemical control was carried out against the enemies, and the species insect composition was determined at a natural population density.

1.2. Studies at the First Experimental Field of the Institute of Forage Crops

To determine the species of pollinating bees (Hymenoptera), a study was conducted from 2014 to 2016 on a 250 m² area. Following alfalfa cultivation technology for seeds, alfalfa was cut in the first regrowth at the beginning of flowering, and the second regrowth, on which the relevant observations were made, was left for seed.

1.3. Entomological research methods

The following classic entomological methods were used to establish the species composition and dynamics of insects at natural population density:

- Method of mowing with an entomological bag;

- Method of the coloured sticky traps – yellow sticky traps (measuring 13.0 x 25 cm) were placed in the middle of the area after the emergence of the alfalfa and the beginning of stem formation. The traps were changed every week. The glued insect species were determined;
- Test plot method - by using test plots with dimensions of 0.5 x 0.5 m in 9 repetitions, the species and density of slow-moving insects during emergence and beginning of stem formation in alfalfa were determined;
- Soil excavation method – it was used to study the harmful activity of *Otiorhynchus ligustici*, tuber octopuses of the genus *Sitona* and *Catapion seniculus*. Through soil excavations, soil monoliths with dimensions of 20 x 200 x 40 cm were taken after mowing spring (first regrowth), summer (third regrowth) and autumn (fourth regrowth) regrowth in the flowering stage.

The analysis of the entomological samples is carried out in laboratory conditions with the help of a stereomicroscope, and the collected species are distributed, determined and counted. The classification of species was made according to Boychev (1975), according to which: dominant species with more than 15% participation, subdominant - from 5 to 15%, secondary - from 1 to 5%, tertiary - less than 1% participation compared to the total population density of enemies.

For the species identification, personal collections and collections of different groups of insects, determined by specialist entomologists, were used: Order Coleoptera - ch. as. Rumyana Kostova, PhD, Faculty of Biology at Sofia University "St. Kliment Ohridski". The new species *Catapion seniculus* (Coleoptera: Apionidae) was confirmed by Prof. Ivanka Lecheva, Agricultural University, Plovdiv; Order Hemiptera suborder Heteroptera - Assoc. prof. Nikolay Simov, National Museum of Natural History at BAS; Order Hemiptera, suborder Fulgoromorpha and Cicadomorpha - asos. prof. Venelin Pelov, IPAZR "N. Pushkarov". The cicadas *Anaceratagallia laevis* Ribaut and *Streptanus* (*Streptanulus*) *josifovi* Dlabola have been identified, and the remaining reported species of cicadas have been confirmed by ch. as. Iliya Gyonov, PhD – Faculty of Biology, Department of Zoology and Anthropology at Sofia University "St. Kliment Ohridski"; Order Thysanoptera - confirmed by Prof. Doctor of Agricultural Science Kiran Donchev, Institute of Forage Crops, Pleven; Order Hymenoptera, superfamily Apoidea - confirmed by Prof. Doctor of Agricultural Science Bojana Dochkova, Institute of Forage Crops, Pleven; Order Hymenoptera - Prof. Toshko Lyubomirov PhD, Institute of Biodiversity and Ecosystem Studies at the BAS - specimens are assigned to superfamilies and individual species.

To all the specialists who assisted about the species identification, I express my SINCERE THANKS!

2. ESTABLISHING THE NUTRITIONAL RELATIONSHIPS: APHIDS – APHIDOPHAGOUS

The following methods were used to establish the nutritional relationships between aphids and aphidophagous in the period 2006-2009: mowing with an entomological bag, square method, method of coloured sticky boards and method of route survey and visual observations (to detect parasitized aphids). To determine the composition and extent of aphids parasitized by parasitoids, mummified aphid specimens collected and transferred to laboratory conditions were sought. Data on parasitoid infection were obtained by rearing their larvae and pupae in the laboratory until imago emerged from the host's body. The phenology of the individual components in the aphid-aphidophagous association and the numerical dynamics of the species was followed by constant weekly sampling during the alfalfa vegetation.

3. DETERMINATION OF DOMINANT INSECT PEST DAMAGE

The studies were conducted during the period 2006-2009 with eight varieties of alfalfa included in the Official Varietal List of the Republic of Bulgaria (2005): Europe, Prista 2, Prista 3, Prista 4, Obnova 10, Pleven 6, Dara and Mnogolistna. A randomized block method with four replications and an experimental plot size of 10 m² was applied. The harmful activity of *O. ligustici* and tuber octopuses of the genus *Sitona* was determined by taking soil monoliths (20 x 200 x 40 cm) after harvesting the spring (first regrowth), summer (third regrowth) and autumn (fourth regrowth) regrowth in the flowering stage. In the same stages of the first and second boost, before mowing plants, with roots were taken together through the soil monoliths to determine the damage by *C. seniculus*.

3.1. DETERMINATION OF DAMAGE BY *OTIORHYNCHUS LIGUSTICI* LINNAEUS, 1758 (COLEOPTERA, CURCULIONIDAE)

The *O. ligustici* damage was found at natural population densities. It ranged from 0.2 (2007) to 0.6 adults/m² (2009). Root damage was recorded using the indicators: number, length and width of the gnawed deep longitudinal furrows along the main root of one plant, root neck diameter.

A methodology was developed to determine and compare the extent of *O. ligustici* root damage of alfalfa cultivars based on the following parameters: area of root damage, the area of the central root and the percentage of gnawed furrows. The first two quantities are involved in a formula for calculating the gnawed furrow percentage, as a final stage, determines the degree of damage (% damage).

Damage area was calculated as the sum of the product of the length and width of each gnawed root furrow of a plant. The root area was calculated to determine the proportion of deep longitudinal furrows on the root.

The area of the central root (S) was calculated by a modified formula for a face on the surrounding surface of a right circular cone whose shape corresponds to the shape of the principal root:

$$S=(\pi*d*L),$$

where: π - 3.14; d – root neck diameter, L – root length

The percentage of gnawed furrows (A) was the derivative of the root area and the damaged area and determined the degree of damage:

$$A = (x * 100) / y, \text{ where: } x - \text{area of the root; } y - \text{damaged area}$$

A six-point scale based on the number of furrows along a plant's root system was developed to measure damage over time to assess alfalfa variety resistance to alfalfa weevil, namely: Score 0 – healthy root; Ball 1 – single small gnawed furrows; Ball 2 – single long gnawed furrows or two furrows; Ball 3 – 3 or 4 gnawed furrows each; Ball 4 – 5 – 6 gnawed furrows; Ball 5 – over 6 gnawed furrows.

The scoring scale is involved in the damage index value calculation (In, %). The resistance of eight alfalfa varieties was determined by measuring their modified resistance scale values. The damage index (In, %) was calculated according to the McKinney formula:

$$In = \Sigma(a*b) \times 100 / n \times N, \text{ where:}$$

In - damage index .%; a – number of plants for a given bale; b – corresponding ball; n – total number of examined plants; N – highest point on the scale

As a final stage for determining the resistance of the investigated varieties based on In, a scale modified with the help of established resistance scales of IASAS and Donchev (1982) was used, where:

R (In=0-5) - resistant; MR (In=6-25) – moderately resistant; S (In=26-50) – sensitive; SS (In – over 50) – highly sensitive

3.2. DETERMINATION OF MORPHOLOGICAL CHARACTERISTICS AND PATTERN OF DAMAGE BY *CATAPION SENICULUS* KIRBY, 1808 (COLEOPTERA, APIONIDAE)

It measured the length of the beetle's body from the eyes to the tip of the elytra, as well as the length of its proboscis, to determine the adult size of *Catapion seniculus*. The study used material collected by mowing with an entomological bag during the growing season, and an average of 100 adults were assessed for each alfalfa regrowth.

Catapion seniculus damage studies were conducted at natural population densities of the species ranging from 4.1 to 4.9 adults/m². The damage was established by longitudinally cutting the stems and evaluating the indicators: number of healthy and proboscis-damaged stems with furrows/one plant, length, width and distance of the furrows from the root neck. The ranking of cultivars by degree of damage was done according to Lindansky (1988).

3.3. DETERMINATION OF DAMAGE TO SPECIES OF THE GENUS SITONA

There were determined nodule root formation and the damage degree by *Sitona* species by counting the indicators by indicator defined: number of healthy and damaged nodules, and hole number on the root system at a natural population density of the *Sitona* species, varying from 0.1 to 4.2 adults/m².

4. DEVELOPMENT OF METHODS FOR CONTROL OF MAIN INSECT PESTS IN THE CONDITIONS OF BIOLOGICAL FORAGE PRODUCTION

4.1. Biological testing of the efficacy of biological insecticides and bio-based products against main insect pests in alfalfa

The biological action of three insecticides was studied in the Second Experimental Field in field conditions during the period 2012-2014: two biological - Nimazal T/C and Pyrethrum FS EK and one synthetic - Nurele D, for the control of *T. flavus*, *A. lineolatus* and *A. pisum* – main insect pests in alfalfa seed production. The efficacy of insecticides was calculated according to the Henderson and Tilton (1955) formula on the first, fifth, seventh and ninth days after treatment.

Following the requirements for organic production, the field experience was located in an area with a two-year conversion period.

The neem and pyrethrum effects on the *Coccinella septempunctata* and *Aeolothrips intermedius* adults were studied to evaluate their toxicity. Treatments were carried out at the flowering stage of the second regrowth, and alfalfa was harvested for seed. According to the internationally accepted principles of the International Organization for Biological Control (IOBC), a four-point scale was used to evaluate the toxicity of the studied insecticides to beneficial insects under field conditions, according to which score 1 = harmless/non-toxic (<25%), score 2 = weak toxic (25 to 50%); score 3 = moderately toxic (51 to 75%), and score 4 = harmful/highly toxic (> 75%) (Hassan et al, 1994). The product's toxicity was measured on days one, five, seven, and nine using Henderson and Tilton's (1955) formula.

To establish the independent and combined action of one biological product with insecticidal activity - Agricol and two biological fertilizers - Nagro (nano fertilizer) and Litovit for the *Bruchophagus roddi* Guss., *A. lineolatus* and *A. pisum* control, and their impact

on the alfalfa productivity, field experience was based on the requirements of organic production. The study was conducted in the 2015-2017 at the Second Experimental Field. The treatment was carried out at the beginning of the flowering stage of the second regrowth. The insecticide efficacy was calculated according to the Henderson and Tilton (1955) formula on the first, fifth, seventh and ninth days after treatment.

Variety of experience: 1. Agricol; 2. Nagro; 3. Lithovit; 4. Agricol + Nagro; 5. Agricol + Litovit; 6. Control (water treated);

In laboratory conditions in 2018-2019, *Hypera postica* larval and adult forms originating from the natural population from the Second Experimental Field were tested. The study included three biological insecticides: Pyrethrum - Natura, Nimazal T/C and Rapax and one synthetic insecticide - Biscaya 240 OD for comparison. Each insecticide was applied in four replicates (15 larvae and 15 adults for each replicate). The treatment was carried out according to the method for testing the contact toxicity of plant protection products when applied to the substrate (Dochkova, 1982; Dochkova, 1987). Counting was done after 1, 5, 24, 48 and 72 h, and live, agonizing and dead individuals were enumerated. Corrected toxicity was calculated using the formula of Abbott (1925).

4.2. Effect of perennial wheat-legume grass mixtures on damage by soil-dwelling root insect pests

The trial was performed in the experimental field from 2014 to 2017. Sainfoin (*Onobrychis viciifolia* Scop.), bird's-foot trefoil (*Lotus corniculatus* L.) and alfalfa (*Medicago sativa* L.) were sown alone (100%) and in mixtures with cocksfoot (*Dactylis glomerata* Graebn.) (50:50%) (Table 1). Perpendicular to the main crops and in the mixtures in the first year (2014), spring forage pea was sown as a cover crop with a 75% sowing rate.

Table 1. Variants of the experiment

Variants	Ratio, %	Sowing rate, kg da ⁻¹
1 Sainfoin (<i>Onobrychis viciifolia</i> Scop.)	100%	12.0
2 Bird's-foot trefoil (<i>Lotus corniculatus</i> L.)	100%	1.5
3 Alfalfa (<i>Medicago sativa</i> L.)	100%	2.5
4 Sainfoin + cocksfoot (<i>Dactylis glomerata</i> Graebn.)	(50:50)	6.0 + 1.750
5 Bird's-foot trefoil + cocksfoot	(50:50)	0.750 + 1.750
6 Alfalfa + cocksfoot	(50:50)	1.250 + 1.750
7 Cocksfoot + Spring forage pea*	25:75*	0.875 + 3.0

The experimental plot size was 10 m² with four replications according to a methodology of field experience (Shanin, 1977). Four samples of each type of crop and mixture were analysed, i.e. a total of 24 samples in each year. To investigate the larval damage of these pests to the root system of the plants in the flowering stage – the beginning of pod formation of legume crops in the second regrowth – soil monoliths were taken. The following characteristics were recorded: percentage of nodules damaged by *Sitona* weevil larvae calculated as the ratio of the number of damaged nodules x 100 to the total number of nodules; the degree of damage caused by *O. ligustici* larvae as measured by the length of spiral furrows on the plant roots (cm); the productivity of dry aboveground biomass; dry root biomass (g/plant).

The experiment was performed in 2018-2020 in the experimental field. The characteristics of the individual perennial and grass-legume mixtures, as well as the applied ratios in the mixtures, are presented in Table 2. Sowing was done manually on October 2017. In the stage of flowering - the beginning of pod formation in the second cut of alfalfa, soil monoliths were

taken. After washing the plants with water, indicators characterizing the degree of damage by *Otiorrhynchus ligustici* were measured: number of spiral furrow per root, number of holes per root, furrows length (cm) spacing of the furrows from the root cervix (cm). The root length indicator does not take into account the entire length of the alfalfa root system. The height (cm) and the number of plant stems were also measured. For grass components, height (cm), number of stems, and root length (cm) were recorded.

Table 2. Variants of the experiment

Variants	Ratio	Sowing rate, kg da ⁻¹
1 Awnless brome grass (<i>Bromus inermis</i> Leys.), Nika cv.	100	3.00
2 Cocksfoot (<i>Dactylis glomerata</i> L.), Dabrava cv.	100	3.00
3 Alfalfa (<i>Medicago sativa</i> L.), Dara cv.	100	2.5
4 Alfalfa + Awnless brome grass	50:50	1.25 + 1.50
5 Alfalfa + Cocksfoot	50:50	1.25 + 1.50
6 Alfalfa + Awnless brome grass + Cocksfoot	50:25:25	1.25 + 0.75 + 0.75

5. STABILITY OF ALFALFA PRODUCTIVITY AFTER APPLICATION OF PRODUCTS WITH DIFFERENT BIOLOGICAL ACTIONS

A statistical evaluation was made by variance analysis and Fisher's parametric criterion F (Shanin, 1977; Barov, 1982) to characterize the representativeness and the reliable influence of the biological insecticides Nimazal and Pyrethrum on alfalfa productivity. Productivity stability parameters represented by stability variances (σ_i^2 and S_i^2) according to Shukla (1972) and equivalency W_i according to Wricke (1962) were calculated. They show how much of the variation associated with drug interactions and years is due to the specific variant. Kang's Yield Stability Summary Criterion (YSi) (1993) provides a measure for each variant that takes into account both its indicator value and stability.

6. FORAGE QUALITY AND EVALUATION OF ENERGY NUTRITIONAL VALUE OF ALFAFER (*MEDICAGO SATIVA* L.) TREATED WITH BIOLOGICAL PRODUCTS

Plant samples were taken from the above-ground biomass two weeks after applying Nimazal T/C, Pyrethrum FS EC, Agricol, Nagro, and Litovit biological products to assess the energy and protein nutritional forage value. The chemical composition was determined by standard methods according to the Weende system and included crude protein, crude fibre, phosphorus, calcium, water-soluble sugars and saponins. The content of fibrous components of cell walls or neutral detergent fibres, acid detergent fibres and acid detergent lignin were determined according to the systematic detergent analysis of Goering & Van Soest (1970), and the degree of lignification - by a coefficient = $KDL / NDV \times 100$. Enzymatic degradability / in vitro digestibility of dry matter (DMD) was determined as a percentage by the two-step pepsin-cellulase method of Aufrere, 1982 (Todorov, 2010). The energy and protein nutritional values were calculated based on the fibre components according to the French system (INRA, 1988).

The Statgraphics Plus (1995) programs for Windows Ver. 2.1 and PBSTAT were used to process the results at a confidence level of $p \leq 0.05$. Different tests were applied based on the type of values analyzed to ensure the reliability of the differences between average values and comparability of the results: One-way ANOVA for one-factor and multi-factor experiments,

Multivariate regression assay, Linear quadratic regression models and multiple regression analysis.

7. METEOROLOGICAL AND SOIL CHARACTERISTICS OF THE PLEVEN REGION

For the experimental four-year period (2006-2009), the average annual air temperature was 13.5 °C and higher at 1.6 °C than the previous 20-year period (11.9 °C). The hottest months for the Pleven area are June, July and August, when the average daily temperature reaches 22.4, respectively, 24.6 and 24.1 °C. Higher temperature fluctuations were recorded for June and July 2007, reaching 24.4 and 27.4 °C and in June 2006 and 2009, it dropped to 20.9 and 21.9 °C, respectively. These temperature differences affect the population density of enemies that appear and cause damage at sensitive stages of alfalfa development. Fluctuations in the amount of precipitation are significantly higher than those in temperature. During the study period, the lowest amount of rainfall was 2008 - 469.1 mm, and the year with the highest amount of precipitation was 2009 - 613.0 mm. Agrometeorological conditions during the 2012–2017 period also affected the insects and toxicity of the studied products, with 2013 and 2015 emerging as less favourable. The soil subtype is leached chernozem, with neutral soil acidity with pH (KCl) – 5.24 and an average stock of total nitrogen (N – 17.64 mg/1,000 grams of soil).

EXPERIMENTAL RESULTS AND DISCUSSION

1. SPECIES COMPOSITION AND NUMERICAL DYNAMICS OF INSECT PESTS IN ALFALFA AGROCOENOSE

1.1. ORDER COLEOPTERA

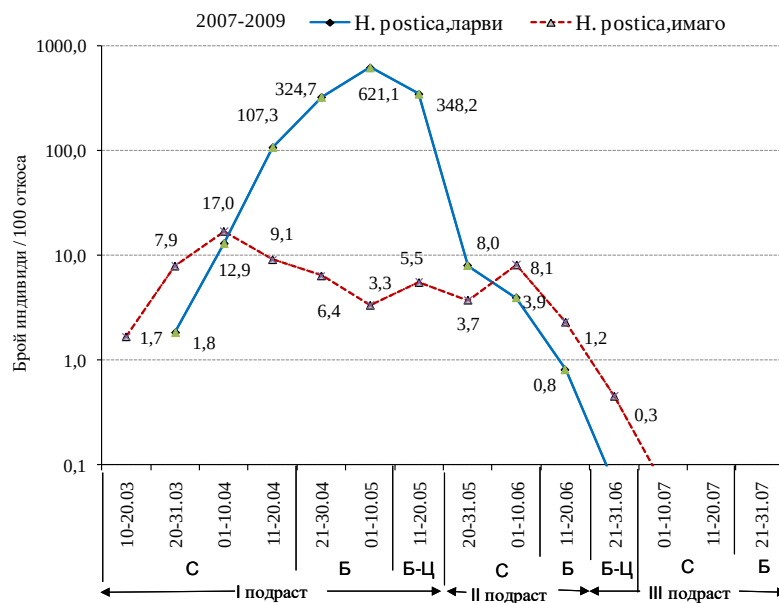
Main insect pests in the earliest and critical alfalfa development stage (sprouting and stem formation) were *Opatrum sabulosum* (24.30%), *Sitona* species (13.74%), *Agriotes lineatus* (13.43%), *Tanymecus dilaticollis* Gyll. (12.73%) and alfalfa leafhopper *Gonioctena fornicata* (10.40%).

During 2007-2009, 52 insect species from 42 genera and 13 families were found. Conventionally, species can be divided into three groups - harmful, useful and indifferent. The harmful species occupied an average of 86.0% of the total Coleoptera amount. Their participation increased over the years, and in 2009 was the highest - 92.2%. The highest species diversity was found in the family Chrysomelidae, represented by 15 species, followed by Curculionidae - 14 species.

Useful entomofauna averaged 13.8%. The main part of the species belongs to the family Coccinellidae, represented by the eight species. An exception to that family was *Subcoccinella vigintiquatuorpunctata*, which was a harmful species and occupied only 0.5% of the total number of stils. Indifferent types occupied 0.2%.

The dominant and most important species in the forage alfalfa crop was *Hypera postica*, with 69.6% on average of the total population density of the species. Its seasonal dynamics were distinguished by a high quantitative participation in the spring, which coincided with the development of the first alfalfa regrowth (Fig. 1). Overwintering adults appear in the second ten days of March to the first ten days of April when the plants were in the stem formation stage and reach a maximum in early April. A second peak in the adult insect number of the new generation was found again in the stem formation during the first ten days of June. The hatching of larvae was recorded at the end of March. Maximum values were recorded in budding and beginning of flowering before the harvesting of the first regrowth. After

mowing, the larvae number sharply decreased, reaching only 8.0 numbers/100 cuttings in the second regrowth.



Legend: S- stem formation; B – button stage; B – F– budding - flowering

Figure 1. Numerical dynamics of *Hypera postica* Gyllenhal in a alfalfa

Species of the genus *Sitona* were permanent subdominant insect pests in alfalfa agrocenosis. They account for 5.7% of the total amount of established coleopteran. Of these, the dominant species was *S. macularius* (4.4%). Overwintering adults emerged early in the spring, usually the second ten days of March (Fig. 2). Their number increased sharply and reached a maximum in the first regrowth in the first half of April (from 13.8 to 58.3 individuals/100 cuttings). In May and the beginning of June, the overwintering individuals died. Their population size falls to the limit of 0.3 - 5.3 individuals.

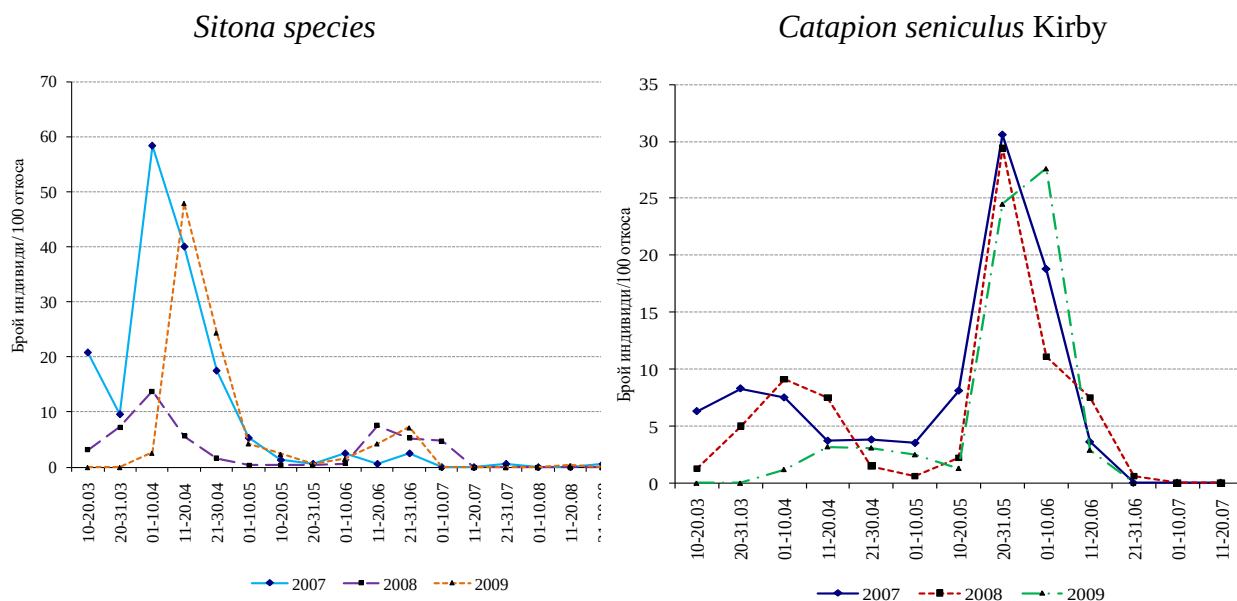


Figure 2. Numerical dynamics of genera *Sitona* and *Catapion seniculus* Kirby in alfalfa

During the second half of June to the first ten days of July, the number of adult individuals of the new generation increased considerably. The density of the population varied from 2.5 to 7.5 individuals, depending on the weather conditions of that particular year.

Catapion seniculus made up 3.4% of the total population of coleopteran insects. It was first reported as an alfalfa pest in Bulgaria.

The adult stem borer has a shiny black body covered with grey hairs, with a body length of 1.5 to 1.8 mm and a proboscis length of 0.689 to 0.737 mm. The stem borer goes through one generation and spends the winter as an adult in the topsoil or under plant debris. The damage to the alfalfa crop is caused by the larvae feeding inside the stem, creating a narrow channel along its length (as shown in Photo 1). The channels are typically found in the basal part of the stem, with an average length of 18.0 mm and a width of 1.40 mm. The overwintering larvae start appearing in the first half of March, during the early spring alfalfa crop (as depicted in Fig. 2).

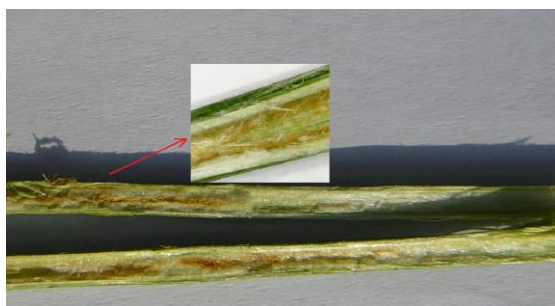


Photo 1. Stem damaged by the larvae of *Catapion seniculus* Kirby

The period of mass exit of the species from the wintering places was the third ten days of March and the first of April. The beginning of egg-laying coincides with the period of stem formation in the first regrowth of alfalfa and lasts until the second or third ten days of June. The maximum number was found in the second undergrowth in the stem formation and budding stage (the third ten days of May and the first of June) when the individuals of the new generation were en masse. The number varied from 27.6 to 30.6 depending on the climatic conditions during the year, after which it decreased, and in the third regrowth, single adults were found. During the first regrowth, *C. seniculus* caused considerable damage. In the second regrowth, single damage to plant stems were found, but they were not economically significant. As a result of the damage caused by the larva, the plants break and lie down. In the second year of cultivation, the stem borer damaged an average of 15.1% of the stems, in the third year – 23.3%, and in the fourth year – 8.7%.

1.2. ORDER HEMIPTERA, SUBORDER FULGORORMORPHA AND CICADOMORPHA

It has been identified that there are twenty-eight species of cicadas belonging to twenty-four genera and eight families. The Cicadellidae family was the most compositionally diverse, with 14 genera and 16 species. It represented 79.9% of the total cicada population density. Aphrophoridae occupied significantly lower participation – 11.4%, and the species of Cixiidae – 4.7%. The other families and species have low participation, are present accidentally in the alfalfa agrocenosis and do not threaten the plants from damage. During the four-year study, 11 new species were discovered on *Medicago sativa* (alfalfa), which have not been previously reported in Bulgarian entomological literature. These were the dominant species *Anaceratagallia laevis* (19.4%), the subdominant species *Streptanus* (*Streptanulus*) *josifovi* (10.4%), the secondary ones - *Allygidius* (*Allygidius*) *commutatus* (2.8%), *Psammotettix striatus* (3.4%), *Reptalus panzeri* (1.2%) and *Tettigometra sulphurea* (1.2%),

and tertiary species that are less than 1% of the total quantitative participation - *Tettigometra (Mitricephalus) obliqua* (0.5%), *Eupteryx atropunctata* (0.5%), *Agalmatum bilobum* (0.2%), *Zygina flammigera* (0.2%) and *Neoliturus fenestratus* (0.1%).

Dominant and subdominant species were economically important. *Empoasca pteridis* (22.0%) and *Anaceratagallia laevis* (19.4%) were dominant, and *Austroagallia sinuata* (13.6%), *Philaenus spumarius* (11.2%) and *Streptanus josifovi* (10.4%) were subdominant. Secondary species (*Selenocephalus obsoletus* – 3.8%; *Psammotettix striatus* – 3.4%; *Hyalesthes obsoletus* – 3.2%; *Allygidius commutatus* – 2.8%; *Macrosteles laevis* – 1.2%; *Tettigometra sulphurea* – 1.2%; *Reptalus panzeri* – 1.2%; *Asiraca clavicornis* – 1.0% and *Aphrodes costatus* – 1.0%) had a significantly lower participation from 1 to 5%, but some of them were carriers of viral and mycoplasma diseases, which affect the yield and quality of the obtained production. Species with less than 1% participation had no economic importance in the ecological conditions of the Pleven area.

Empoasca pteridis adults appeared in alfalfa in the second half of March at the beginning of the growing season (Fig. 3). Their number was minor in April and the first half of May in the first regrowth, moving in the range of 0.6-3.8 individuals/100 cuttings. The species had a low presence due to spring migration from its wintering grounds to its hosts. A considerable increase in density was observed in the second and third regrowth in the budding and beginning of flowering stages (from 3.0 to 18.8 individuals and from 2.5 to 22.4 individuals/100 cuttings, respectively), and after harvesting, values decreased. Upon each subsequent regrowth, adult individuals migrated back into the alfalfa crops from neighbouring areas, confirming the observed migration process.

The maximum number was observed in the second ten days of August in the stem formation-beginning of budding stages of the fourth regrowth when it reached 40.6 individuals/100 cuttings, then sharply decreased. Larvae of the species were established from the second half of May to the middle of June in the budding and flowering stages of the second regrowth.

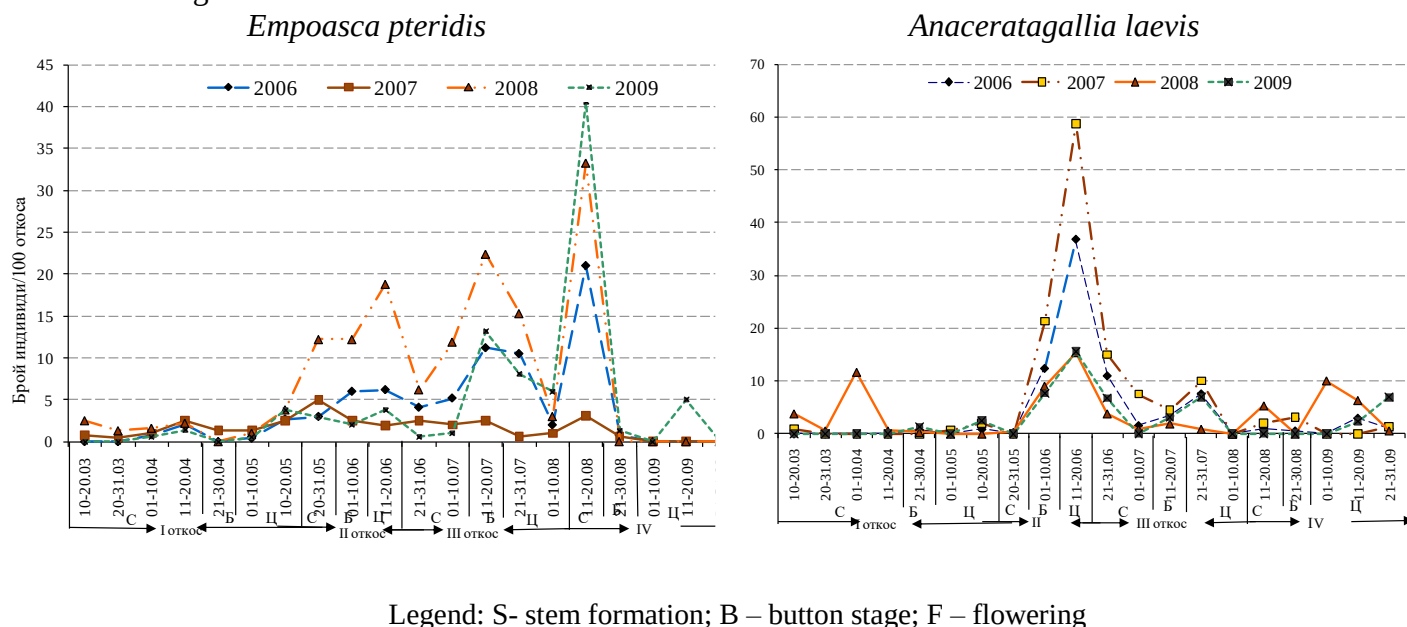


Figure 3. Numerical dynamics of dominant leafhopper species in forage alfalfa

The new species *Anaceratagallia laevis* has a body length of 0.9 to 1.2 mm. The cicada occurs in all regrowths during the growing season, with a clear maximum in the second ten days of June in the flowering phase of the second undergrowth (Fig. 3). The number over the years varied from 15.3 to 58.8 individuals/100 cuttings. Relatively high density was also

observed in the budding stage (7.6-21.3 individuals/100 cuttings), and in the following regrowths, it decreases markedly.

Austroagallia sinuata was present in alfalfa crops from the second half of April until the end of the growing season, and in the first regrowth, the number was insignificant. An increase was observed in the second half of June in the flowering and stem formation stages of the second and third regrowth. In the highest density, the species was found in the budding - beginning of flowering stages in the third and fourth regrowth during the third ten days of July and August (3.8 - 22.0 individuals/100 cuttings).

Philaenus spumarius appears in the second half of May until the end of September, with a distinct maximum in the second half of June in the flowering of the second regrowth, when it reaches 36.8 individuals/100 cuttings. It was also observed in higher numbers in September in the fourth regrowth.

The new species *Streptanus josifovi* has a moderately slender body and a highly rounded convex head. It measures between 5.3 and 6.2 mm. The transition from the head to the middle chest is rounded. In this species, the wing venation is darkly bordered, with vaguely defined vein tips. The leafhopper appeared in the crops in the second half of March and occurred until the second half of August, with a pronounced maximum in the second half of April in the stem formation of the first regrowth. The abundance in this period varied from 3.4 to 22.8 individuals/100 cuttings. In the following stages of the first and second regrowth, the density considerably decreases, with a comparative increase observed during the third ten days of June in the stem formation of the third regrowth.

The total cycad fauna on alfalfa had a significantly high quantitative participation in April, June, July and August, which was determined by the number of dominant and subdominant species.

Empoasca pteridis density growth and spatial distribution pattern depended to a large extent on the number of days to harvest and migration into crops through individual alfalfa undergrowths. The ratio of female to male individuals remained consistent over four years. Female individuals mainly predominate in the early development stage of each regrowth (stem formation), with the sex index being high and decreasing with the number of vegetation days increasing until mowing.

The share of female representatives in the first and second youth is the highest, and the indices also have the highest values. The average values of the sex index for the four years in the first adolescent varied between 0.69 - 0.76 and in the second adolescent - 0.68 - 0.77. In the third and fourth instars, the sex ratio approaches a similar participation of males and females as the plants develop. The proportion of female individuals in the stem formation in the third regrowth was considerably lower than the previous regrowths and varied from 0.60 to 0.65 over the years. The decrease in the numerical values of the indices in the fourth compared to the third sub-age was significant from 0.52 to 0.57, with the participation of the sexes almost equalizing (except for 2008).

It was found that the temperature factor had a proven influence on the density of female individuals of *E. pteridis* during the four years of the study (P-value did not equal and did not exceed 0.1). However, the amount of precipitation did not have a significant effect except for a negative impact observed in 2008.

The study of the sex index in *Empoasca pteridis* was relevant for prediction and signalling when the insect pest appears in high density.

1.3. ORDER HEMIPTERA, SUBORDER HETEROPTERA

Thirty-nine bedbugs belonging to twenty-five genera and twelve families have been identified. The most diverse in composition were the representatives of the Lygaeidae family,

represented by ten species, followed by Miridae - nine species and Pentatomidae - seven species. The highest contribution to the total population density of Heteroptera was Miridae (59.4%), followed by Anthocoridae (11.5%), Nabidae (10.7%) and Pentatomidae (9.7%).

Harmful species occupied 74.0% of the total amount of heteropteran insects. Their participation by year was as follows: 2006 – 74.5%, 2007 – 67.4%, 2008 – 73.9% and 2009 – 81.8%. Important pests from Miridae were the dominant species *Adelphocoris lineolatus* (29.7 %) and *Lygus rugulipennis* (19.1 %), from Pentatomidae the subdominant *Piezodorus lituratus* (5.2 %). Secondary ones were *Dolycoris baccarum* (3.4 %), *Polymerus cognatus* (3.2 %), *Adelphocoris seticornis* (2.6%), *Nysius helveticus* (2.3%) and *Camphylomma verbasci* (2.1%).

The *A. lineolatus* larvae appeared in the alfalfa crop in early May, at the beginning of the flowering stage of the first regrowth (Fig. 4). The first generation of the bug had the highest density in the second ten days of June in the second regrowth before mowing (from 10.6 to 36.3 individuals/100 cuttings).

The second generation developed in the third and fourth regrowth, reaching a maximum number in the third ten days of July, at the flowering of the third regrowth and reaching 178.8 pieces (2006). It was longer and more numerous than the first.

Lygus rugulipennis is a much wider polyphagus than *A. lineolatus*. The maximum number of the first generation was observed in the second ten days of June at the flowering stage of the second regrowth (from 1.9 to 52.5 individuals/100 cutting). A second peak in density was found in the second generation during the second ten days of July in the third regrowth (from 0.5 to 57.5 individuals/100 cutting), with values close to those of the first maximum. In 2006, the species was found in higher numbers and at the end of the beginning of the flowering stage of the fourth regrowth.

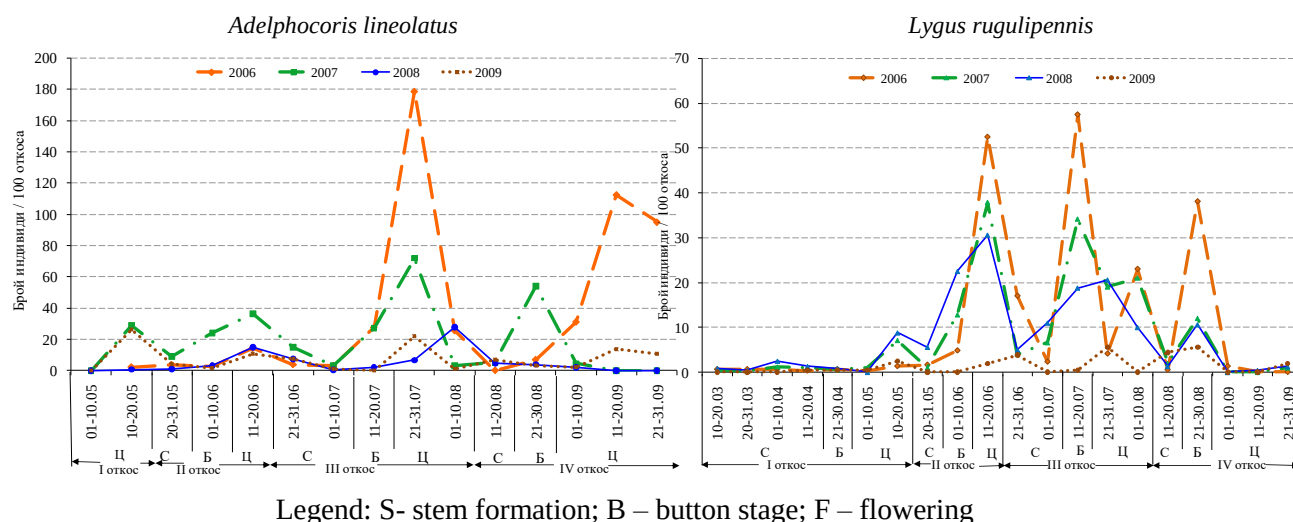


Figure 4. Numerical dynamics of a main phytophagous bug in alfalfa

Piezodorus lituratus appeared in higher density during the second ten days of May and June at the flowering stage, on the first and second regrowth, respectively. A clearly expressed maximum was established in the second half of July, at the end of budding - beginning of flowering stages of the third regrowth (from 3.0 to 9.4 individuals/100 cutting).

The numerical dynamics of the predatory species of the genus *Nabis* followed or corresponded to that of the harmful species, which emphasized their exceptional role as bioregulators. They were found in the crops from the end of March until the end of the growing season, with the highest numbers at the flowering stage during the second ten days of June in the second regrowth and the third ten days of July in the third regrowth (Fig. 5).

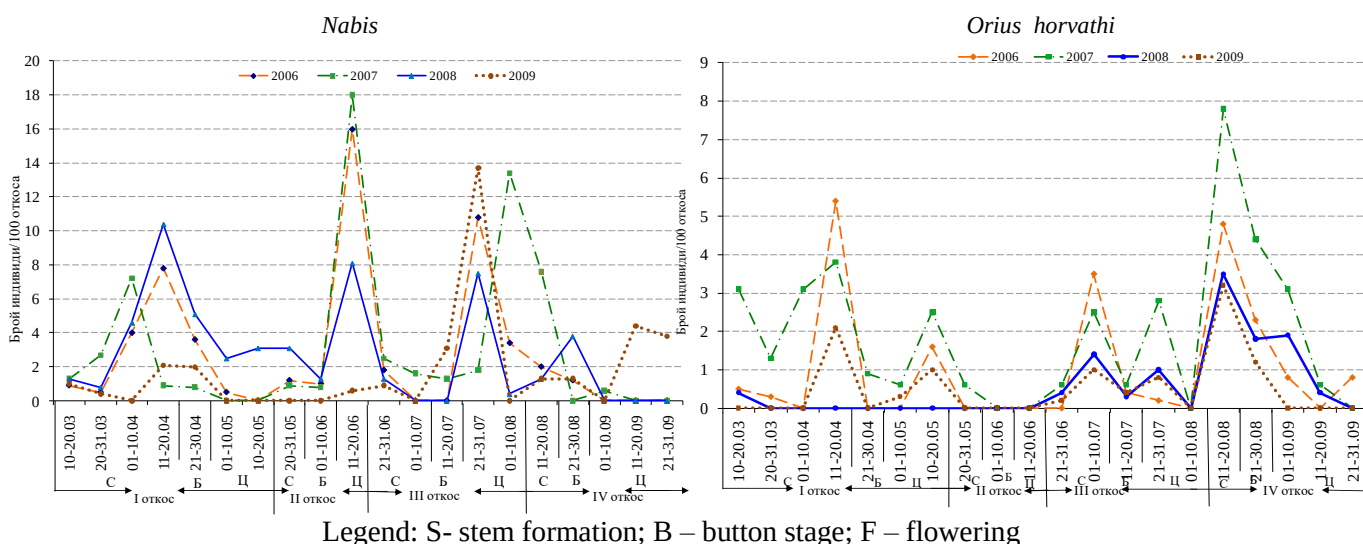


Figure 5. Numerical dynamics of predatory bugs of the genus *Nabis* and *Orius horvathi* Reuter in alfalfa

The *Orius horvathi* density was relatively high compared to other beneficial species. The species appeared in alfalfa in March and occurred until the end of the growing season. A high population was observed in the first, third, and fourth regrowth during the stem formation and budding stages.

The numerical dynamics of species of the suborder Heteroptera had a general course. Maximum density was found in the second and third regrowth in the flowering stage. After harvesting the individual regrowths, the number of bedbugs considerably decreases, after which an increase follows.

In alfalfa forage cultivation, the best time to control harmful bedbugs, if necessary, is during the budding stage of the second and third regrowth.

1.4. ORDER HEMIPTERA, SUBORDER STERNORRHYNCHA, APHIDOIDEA

A total of 23472 adult and larval aphids were caught in alfalfa crops, comprising one family and five species during the study period. The following species were identified: *Aphis fabae* Scopoli, *Aphis craccivora* Koch & C.L., *Acyrtosiphon pisum* Harris, *Therioaphis trifolii* Monell and *Macrosiphon avenae* Fabricius. With the highest participation was the spotted alfalfa aphid (*T. trifolii*), which was the dominant harmful species among aphids. The part of the pea aphid (*A. pisum*) was also considerable. The black bean aphid (*Aphis fabae*) occupied a smaller portion and belongs to the subdominant species with 6.2% participation. The abundance of *A. craccivora* (1.6%) and *M. avenae* (0.1%) was negligible, with *M. avenae* being an occasional species.

Therioaphis trifolii occupied 63.3% on average of the total population density of aphids. Newly hatched larvae appear at the beginning of May and reach relatively high values in the middle of the month in the first instar - Fig. 6.

Two distinct maximum in the number of wingless forms were observed, the first peak being in the second ten days of June, when the density reached 1253 individuals/100 cutting. After mowing, and given the high mid-day air temperature, the high part of the larvae and wingless died. During July and August, aphids were in the least quantity. The second maximum in numbers was found in the second ten days of September (685 pieces/100 cutting) when the conditions for the development of the species were favourable, and it occurred until late autumn. Winged aphids appeared in the colonies at the beginning of May

and were found until the end of September - the beginning of October, and in the second ten days of June, they were at their highest number (170 pieces/100 cuttings). On average, for the period of the study, two maximum were found in the population dynamics of *T. trifolii* - in the first and second ten days of June, when the plants were in the flowering stage of the second regrowth and the end of August to mid-September in the fourth regrowth of alfalfa.

Comparing the participation of the species by year, it was observed that the aphids were in the highest numbers in the first and second years of alfalfa cultivation.

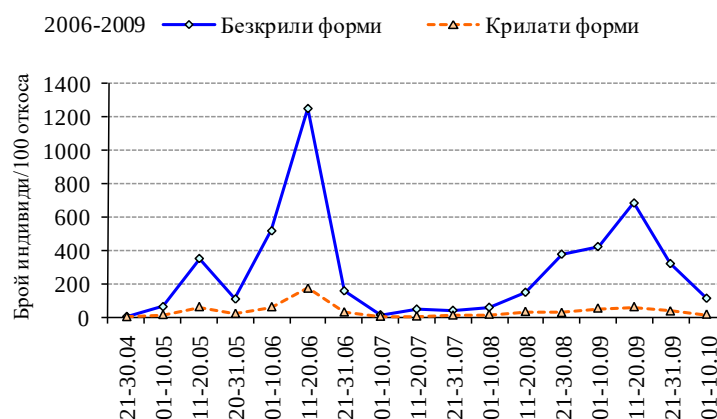


Figure 6. Numerical dynamics of *Therioaphis trifolii* Monell in alfalfa

Acyrtosiphon pisum occupied an average of 28.8% of the total population density of aphids. The first appearance of wingless forms was found at the end of March (Figure 7). A mass number of aphids from the first generation appeared in mid-April, with a peak in late May during the first regrowth. A second peak in numbers, twice as high as the first, was observed at the beginning of August in the third regrowth and flowering stage. The winged forms appeared at the end of April, with maximum numbers noted in mid-May in the first and mid-June in the second regrowth, just before cutting the plants.

On average, *A. pisum* occurred in the highest population density from June 1 to 20 in the second regrowth and the beginning of August in the third, depending on the specific conditions of the year.

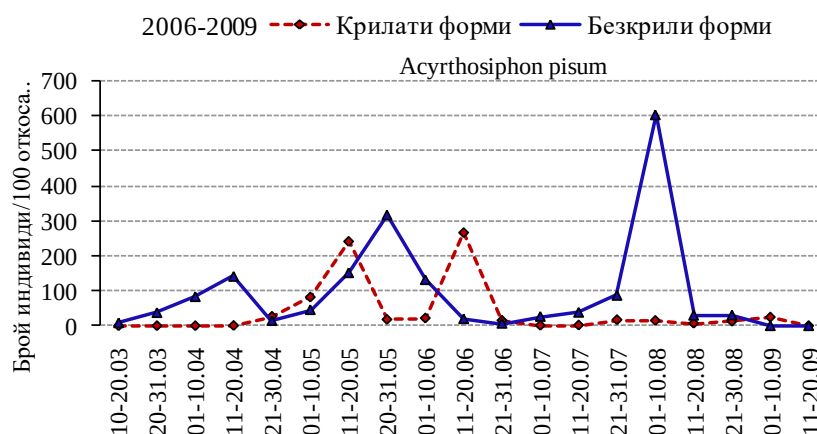


Figure 7. Numerical dynamics of *Acyrtosiphon pisum* Harris in an alfalfa crop

The pea aphid is at its highest in the second (total 762 individuals/100 cutting) and third year (total 1744 individuals/100 cutting) of alfalfa cultivation, as the agrometeorological conditions determine that.

1.5. ORDER THYSANOPTERA

During the period 2006-2009, a total of 2,410 specimens of adults and larvae were collected, with adults predominating. A total of 13 species from eight genera and three families were found. The number of thrips was significantly the highest in 2007, followed by 2008, 2006 and 2009 ($p < 0.05$).

The most diverse and numerous was the Thripidae family, which occupied 53.2% of the total population density of species, represented by five genera and ten species. In second place in terms of numbers was Aeolothripidae - 44.0% (with two species), and the participation of the Phlaeothripidae family was insignificant - 2.0% (with one species).

The established harmful species occupied the highest number (55.2%), of which the dominant species was *Thrips tabaci* with 37.4% participation, followed by the subdominant *Thrips atratus* – 9.7% and the secondary species *Haplothrips niger* – 2.0%, *Frankliniella intonsa* – 1.4% and *Odontothrips confusus* – 1.3%. The participation of useful thrips was considerably high, mainly represented by the dominant species *Aeolothrips intermedius* – 43.8% of the total population density of the order Thysanoptera.

The highest percentage of harmful species was *T. tabaci*. An increase in numbers was found in the second regrowth from the second half of May to the second half of June, with a maximum in the first ten days of June at the budding and beginning of flowering (from 25.5 to 291.4 number of individuals/100 cutting) – Figure 8. In the third and fourth regrowth, the species occurred in lower density. A slight increase in numbers was observed in the second half of July and the second half of August in budding and beginning of flowering, when it reached 51.2 and 100.6 individuals/100 cutting, respectively.

If necessary, treatment against *T. tabaci* can be carried out at the end of May - the beginning of June at the budding of the second regrowth.

Thrips atratus was a subdominant species and was present annually in alfalfa crops. The species appeared in late March-early April and occurred until the end of the growing season. It was observed in high numbers from the beginning of April to the beginning of June and a maximum at the beginning of budding and flowering stages of the first regrowth (reaches 21.3 and 15.0 individuals/100 cutting, respectively).

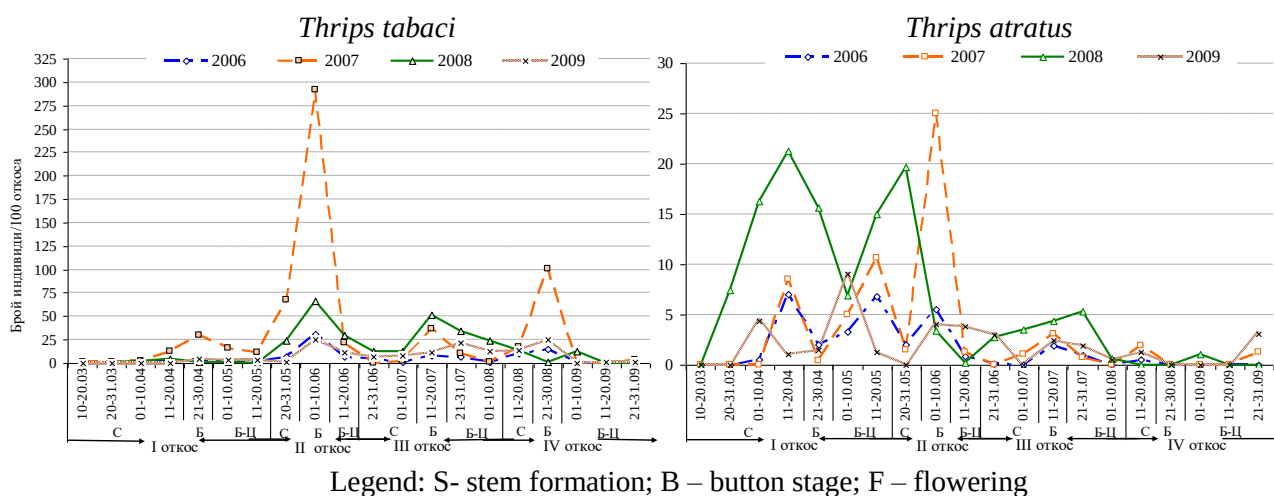


Figure 8. Numerical dynamics of *Thrips tabaci* Lindeman and *Thrips atratus* Haliday in alfalfa

A subsequent peak was found at the budding of the second regrowth (reached 25.0 individuals/100 cutting). In the third and fourth regrowths, thrips density was negligible.

It should be noted that depending on the critical level of spread in the crops and the profitability of control, treatment against *T. atratus* can be carried out at the beginning of budding of the first and second regrowths (early April and late May, respectively). Control can be applied simultaneously against *T. atratus* and *T. tabaci*.

Useful thrips included *Aeolothrips intermedius* (dominant species) and *Melanthrips pallidior* (tertiary species).

The population density of *A. intermedius* during the growing season averaged over the period varied from 0.4 to 267.5 individuals/100 cutting. In the first regrowth, its number was low, with a slight increase observed in the first half of May in the flowering stage. A considerable increase in density was found in the second regrowth of alfalfa, in budding and flowering, with a maximum in the second ten days of June in flowering. The number reached 267.5 individuals/100 cutting. After harvesting, the number of individuals decreased, with a more considerable increase observed in the third regrowth from late June to late July.

The preferred food of *A. intermedius* was the larvae of *T. tabaci*. Therefore, the dynamic of the predator almost corresponded to that of *T. tabaci*. That necessitates the selective and sparing beneficial insecticides used in tobacco thrip control.

It was observed that the number of harmful thrips participating was slightly higher than the number of useful ones.

1.6. ORDER LEPIDOPTERA AND HYMENOPTERA

Important insect pests of the order Lepidoptera were *Agrotis segetum*, *Euxoa temera*, *Autographa gamma*, *Heliothis maritima* and *Loxostege sticticalis*. In alfalfa grown for forage, the presence of *H. maritima* was more often observed. During the study period of 2006-2009, the species did not threaten a threat to the alfalfa plants as fourth and later instar larvae feed on plant generative organs and seeds, and alfalfa was mowed for hay. The remaining species were not present annually in crops and occurred in minimal numbers.

During the 2014-2016 period, by the technology, the first regrowth of alfalfa for seeds was mowed at the onset of flowering, while the second regrowth, on which the relevant observations were made, was left for seed production. Seven species of wild bees and one species of honey bee were found. The honey bee *Apis mellifera* L. was a perennial pollinator present annually in alfalfa. The highest density of the species was in 2016 during mass flowering (Table 3).

Table 3. Species composition and a number (number/m²) of the honey bee *Apis mellifera* Linnaeus (Apidae) in alfalfa crop in flowering stage

Species / Year	2014	2015	2016	Average
<i>Apis mellifera</i> Linnaeus	15.0	7.6	26.7	16.4

Regarding wild bees, on average, *Eucera longicornis* had the highest participation rate among wild bees, approaching 40% (Table 4). Second in number was the dominant species *Halictus maculatus*, which had maintained a relatively equal presence over the years. *Halictus quadricinctus* was also characterized by high participation compared to the total density of wild bees.

The flowering alfalfa period have an impact on the pollinating bee dynamics. The dominant bees *E. longicornis*, *H. maculatus* and *H. quadricinctus* appeared in the crop around mid-May at the first bud formation stage, and their density remained low until the end of the month. At the beginning of flowering, the bee number was below five bees/m² (21-31 May),

and it increased with the increase in the flower number and reached significantly higher values at the 50% flowering stage (01-10 June). During the mass flowering of plants, the density of all three species of wild bees reliably reached the highest values.

Table 4. Species composition and number (number/m²) of wild bees in alfalfa crop in flowering stage

Species	2014	2015	2016	Average,	%
¹ <i>Andrena dorsata</i> Kirby	1.8	2.0	2.7	2.2	6.4
¹ <i>Andrena falsifica</i> Perkins	3.7	0.6	4.2	2.8	8.4
² <i>Bombus sylvarum</i> Linnaeus	1.3	0.7	2.0	1.3	4.0
² <i>Bombus terrestris</i> Linnaeus	0.0	0.0	1.1	0.4	1.1
² <i>Eucera longicornis</i> Linnaeus	13.0	5.5	21.2	13.2	39.3
³ <i>Halictus maculatus</i> Smith	11.3	3.6	12.9	9.3	27.5
³ <i>Halictus quadricinctus</i> Fabricius	6.4	2.1	5.0	4.5	13.4

¹family Andrenidae; ² family Apidae; ³ family Halictidae

When following the influence of some factors of atmospheric conditions on the *E. longicornis* density, given dependencies were established (Table 5). For that purpose, at the 50 and 100% flowering stages, the average abundance of the species was calculated in two time ranges. The range from 12:30 to 15:00 was distinguished by a reliably higher temperature and significantly lower relative humidity and number of individuals, and from 8:30 to 12:00 - by a lower temperature and a reliably higher number and relative humidity ($p < 0.05$). The bee number correlated positively with relative air humidity ($r = 0.873$), while a negative trend was observed with air temperature ($r = -0.779$).

Table 5. Numbers of *Eucera longicornis* Linnaeus in different time ranges of the day depending on the temperature and relative air humidity during the period 20 May - 20 June

Year	Time range	Temperature, °C	Relative humidity, %	Numbers
2014	8:30 – 12:00	22.6 a*	66 b	13.8 b
	12:30 - 15:00	28.5 b	44 a	4.7 a
2015	8:30 – 9:30	22.2 a	64 b	4.1 b
	12:00 - 14:00	28.0 b	49 a	1.2 a
2016	8:30 – 9:30	23.1 a	72b	17.2 b
	12:00 - 14:00	29.3 b	46 a	3.9 a

* Columns with different letters are significant at $p < 0.05$

2. REGULATIONS IN THE FORMATION OF THE ENTOMOFAUNA IN ALFALFA AGROCENOSE

During the years of the study, a total of 54,201 insect specimens were caught, the majority of which were phytophagous. In the research process, certain regularities were established in the entomofauna formation in *Medicago sativa*. The quantitative participation of the order Coleoptera, depending on the alfalfa age, followed set rules (Table 6). During the first year of the crop's sowing, only 6.5% of the representatives participated in the order, compared to the total during the four years. That could be attributed to the migration of specialized insect pests from different habitats. In that way, the species populations directly related to the development of culture were formed. In the following years of cultivation, due to a continuous relationship between the previous year's numbers, the numerical presence of the order Coleoptera proportionally increased to 18.2 and 34.8% for 2007 and 2008, respectively.

In the fourth year of its development, alfalfa was characterized by the highest numerical participation of hardwing species compared to the total part of the order (40.6%).

The regularity established above in the alfalfa agroecosystem was not observed in the other orders. The representatives of the order Hemiptera with the three suborders were characterized by different food preferences and a high range of hosts, as a result of which they appeared mainly as polyphages and less often as oligophages. There was considerable fluctuation in the cicadas' number, bed bugs and aphids depending on the specific weather conditions over the years. In general, the highest quantitative participation in the first year - 34.7%, and the lowest - in the fourth year - 14.9%, compared to the total number of individuals for 2006-2009, was observed in the order Hemiptera.

Table 6. Quantitative participation of orders and suborders forming the entomofauna in alfalfa agroecosystem (by year and total for 2006-2009)

Orders and suborders	2006		2007		2008		2009		Общо	
	Number	%	Number	%	Number	%	Number	%	Number	%
Coleoptera	1159	6.5	3262	18.2	6255	34.8	7292	40.6	17968	33.2
Hemiptera, Fulgoromorpha и Cicadomorpha	336	26.7	250	19.9	315	25.0	357	28.4	1258	2.3
Hemiptera, Heteroptera	1463	53.4	552	20.1	391	14.3	336	12.3	2742	5.1
Hemiptera, Sternorrhyncha	7740	33.0	6509	27.7	5827	24.8	3396	14.5	23472	43.3
Hemiptera, общо	9539	34.7	7311	26.6	6533	23.8	4089	14.9	27472	50.7
Thysanoptera	395	16.4	1057	43.9	467	19.4	491	20.4	2410	4.4
Diptera	1230	46.8	824	31.4	159	6.1	413	15.7	2626	4.8
Hymenoptera	1500	53.3	927	33.0	147	5.2	238	8.5	2812	5.2
Orthoptera	420	46.0	145	15.9	87	9.5	261	28.6	913	1.7
Total	14243	26.3	13526	25.0	13648	25.2	12784	23.6	54201	100.0

Order Thysanoptera had the lowest participation in the year of sowing (16.4%), reaching a maximum value in the second year (43.9%), after which it maintained a relatively constant quantitative presence. The representatives of the order Diptera, Hymenoptera and Orthoptera were constantly present in the crop, and their part relative to the total quantitative participation for the period was highest in the first year (46.8, 53.3 and 46.0% respectively). Similarly to the previous series, no definite numerical regularity was established about the alfalfa age.

The summarized data on the numbers of the individual classes during the years of the study showed their relatively similar participation, varying within narrow limits from 23.6 (2009) to 26.3% (2006), while overall for the period 2006-2009, the year with the highest number stood out Hemiptera (50.7%), followed by Coleoptera (33.2%).

The process of formation of the entomofauna, considering the species diversity, shows an increasing trend (Fig. 9). A total of 90 species of insects were found in the year of establishment of the crop. In the following years, their number increased to 105 species in 2007 and 2008 and reached 119 number species in the fourth year of cultivation. The order of Coleoptera is indicative in this respect, and the formation of the species composition is relatively slow. In 2006, 26 species of alfalfa were found (mainly specific and permanent enemies), and their species diversity increased proportionally with the age of the crops and reached a maximum value of 50 species in 2009.

A similar trend was found in the order Thysanoptera, where in the first year, the species diversity was the least pronounced (7 species), while in the fourth year, 13 species of thrips were found.

In the case of cicadas, the species composition remained constant during the first three years (21 species), while in the fourth year, an increase in species identity was observed (24 species), while in the case of bedbugs (Hemiptera: Heteroptera), the species composition was formed already in the first year (32 species) and in subsequent years the species identity varied within narrow limits from 24 to 29 species. The trend is similar to aphids, whose species composition in the first two years includes permanent and occasional species (4-5 species), while in the third and fourth years, only the permanent enemies in alfalfa remain (3 species). Overall, Hemiptera species identity was relatively constant in 2006, 2007 and 2009 (ranging from 53 to 57 species), with a slight decrease in the third year (48 species).

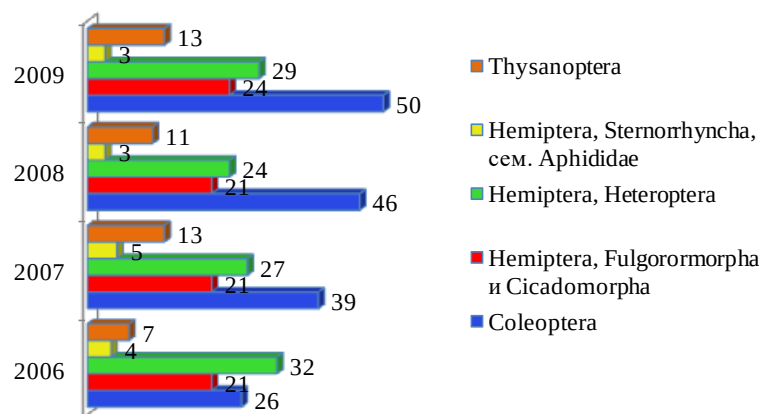


Figure 9. Species participation of orders and suborders forming the entomofauna in alfalfa agrocenosis

The percentage participation of insects from different orders was closely dependent on the development of alfalfa in separate regrowth and followed a characteristic course. The Coleoptera (total 53%) and Orthoptera (53%) order had a dominant presence in the first regrowth - Fig. 10.

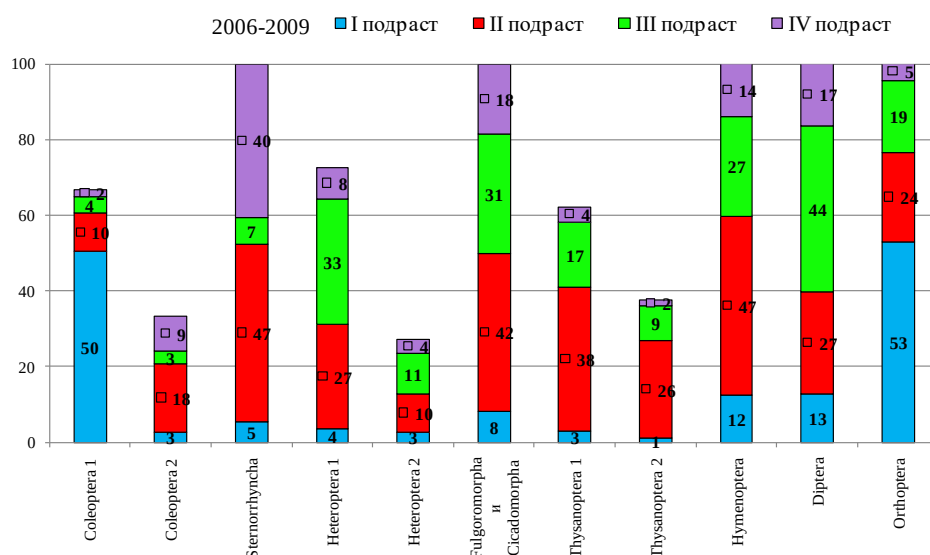


Figure 10. Ratio of ranks in the alfalfa acrocyanosis by regrowth in the period 2006-2009 (1 - harmful species; 2 - useful species)

The general ratio between harmful and useful coleopteran species was in favour of harmful ones (67 and 33%), and the dominant participation of predators in the second and fourth

instars corresponded to the numerical dynamics of their prey, mainly aphids (Sterorrhyncha). By the dynamics, the part of the main aphid species was the highest in the second and fourth regrowths, and their ratio in the second slightly exceeded the corresponding one in the fourth regrowth (47 and 40%). Cicada fauna (Fulgoromorpha and Cicadomorpha) predominated in the second ones (42%). The cicada part was significant in the third and fourth regrowths (31 and 18%), which defined them as constant pests during the alfalfa vegetation. The predominant proportion of Thysanoptera in the second regrowth was clearly expressed (total 64% compared to 26% in the third instar), where the considerably high percentage participation of useful thrips (26%) fully corresponded to their prey (38%) and outlined their important, regulating role in alfalfa agrocenosis. Heteroptera had a pronounced presence in the third (total 44%), followed by the second regrowth (37%), and the seasonal number of predators was related not only to the share of harmful bedbugs but also to the abundance of other prey, such as aphids, thrips and leafhoppers in both regrowths of *M. sativa*. Order Hymenoptera had the highest part in second ones (47%). It was mainly represented by species belonging to the Chalcididae and Ichneumonidae families, whose numbers reached their maximum in the second, followed by the third regrowth, when the plants were most attacked by sucking pests such as aphids, bedbugs and leafhoppers. Diptera occupied the highest proportion in the third regrowth (44%), with representatives of the family Chloropidae predominating, which, despite the high density, belong to the group of insects indifferent to alfalfa.

The proportional distribution of the different orders and suborders by regrowths followed certain regularities, determined above all by the population dynamics of the dominant species. When dividing the species into harmful and useful ones, depending on the food specialization of the predators, their part in regrowths corresponded to that of their prey. Nevertheless, with the development of alfalfa over the years, a clear trend of an increase in the participation of harmful species and a decrease in useful species has emerged.

3. ESTABLISHMENT OF NUTRITIONAL RELATIONSHIPS: APHIDS - APHIDOPHAGOUS

To establish the nutritional relationships between aphids and their aphidophagous, a total of 23,729 aphids (Aphididae), 265 predatory bugs (Anthocoridae and Nabidae), 148 individuals of the Chrysopidae family, 1,076 individuals of Coccinellidae, 345 number of syrphid flies (Syrphidae) and 339 numbers of parasitoids from the Hymenoptera order.

Analyzing the food system of aphids - aphidophagous was found that it was very diverse. There were three groups of aphidophagous:

1. Polyphagous predatory bedbugs from the Anthocoridae and Nabidae families;
2. Parasitoids from Hymenoptera, which form a complex of families (Aphelinidae, Aphididae, Pteromalidae and Ichneumonidae);
3. Oligophagous and polyphagous predators from the Coccinellidae, Chrysopidae and Syrphidae family.

Among the group of polyphagous predatory bedbugs, the most common were *Nabis ferus*, *N. pseudoferus*, *Orius horvathi* and *O. niger*.

The group of oligophagous and polyphagous predators was represented by species belonging to Coccinellidae, Chrysopidae and Syrphidae, and a large part of that community had a decisive influence on the density and control of aphids in alfalfa. Among them, Coccinellidae was dominant, and numbers contributed to more than two-thirds of the total number of that trophic level. *Coccinella septempunctata* and *Adonia variegata* were dominant species and accounted for 79.5% of the total number of predators at this trophic level.

Syrphidae had three species. It was less numerous than Coccinellidae, and among the dipterous aphidophagous, two were dominant. *Syrphus ribesii* had the highest participation,

exceeding 50% and was the main representative of syrphid flies in alfalfa. *Eupeodes corollae* was present in almost twice lower numbers, and *Syrphus vitripennis* was scarce and more often passed from neighbouring crops or nearby trees.

Chrysopidae included three species, with only the predominant species *Chrysoperla carnea* (88.5%) being constantly present over the years in the alfalfa crop. The density of the remaining two species (*Chrysopa phyllochroa* and *Ch. commata*) was considerably low. They did not occur every year.

The trophic structure of the food chain between aphids and parasitoids of the order Hymenoptera includes two relationships. The first link consisted of specialized aphid endoparasitoids. Parasitoids were found mainly in the Aphidiidae family (and less from the Aphelinidae), and the parasitized aphids were characterized by a more oval shape and light colour. The second link - the chain of oligophagous parasitoids included Hymenoptera species that specialized in parasitizing aphidophages. Among the parasitoids, individuals of the Ichneumonidae and Pteromalidae families were found, which parasitized the flies of the Syrphidae family.

The effectiveness of interaction between individual representatives of aphidophagous and aphids depended on the synchrony between the appearance and numerical dynamics of aphids with that of their aphidophagous. The analysis of the trophic structure was based on the dominance of species from five aphidophagous families: Chrysopidae, Coccinellidae, Syrphidae, Nabidae and Anthocoridae (Fig. 11). The numerical dynamics of *Therioaphis trifolii* and *Acyrthosiphon pisum* varied over the years, but the period and stage of the mass aphid appearance were relatively constant (in June and September, mainly in the second and fourth instars).

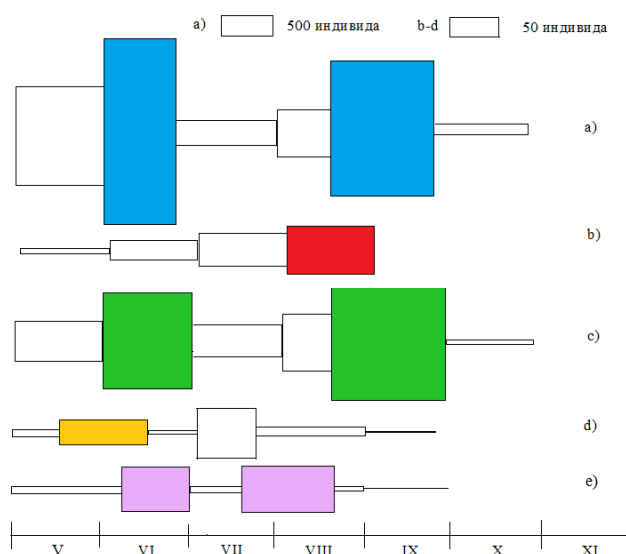


Figure 11. Dynamics of predators and aphids: a) *Therioaphis trifolii* Monell and *Acyrthosiphon pisum* Harris; (b) Chrysopidae; (c) Coccinellidae; (d) Syrphidae; f) Nabidae - genus *Nabis* and Anthocoridae - genus *Orius*

The appearance of adult predatory ladybirds and their larvae overlapped or coincided with the appearance of aphids and included the entire period from May to September. The summer and autumn increase in the number of aphids completely coincided with the increase in the predators of the Coccinellidae family, and synchrony in the numbers between predator and prey was observed. While only one of the periods of maximum values of the representatives of the Syrphidae family (around mid-June) coincided with a maximum in the aphid number, completely different responses were observed in the secondary parasitoids, whose numbers were more or less similar throughout the period (months V-IX) with a slight maximum in

August. Chrysopidae dominated in August and covered the period of aphid abundance at the end of the month. The mass appearance of predatory bedbugs from Anthocoridae and Nabidae was in June. That coincided with the multiplication of aphids during this period.

The trophic structure of the aphid-aphidophagous food system showed that aphid population control can be very efficient. In the period from mid-May to June, there was a sufficiently large potential of aphidophagous (family Coccinellidae, Syrphidae, Chrysopidae, Anthocoridae and Nabidae), while the predatory ladybirds of the Coccinellidae family can mainly control in September.

4. STUDY OF THE HARMFUL ACTIVITY OF OTIORHYNCHUS LIGUSTICI LINNAEUS

4.1. METHOD FOR DETERMINING THE DAMAGE DEGREE BY THE LARVA OF OTIORHYNCHUS LIGUSTICI LINNAEUS IN ALFAFER VARIETIES

In the year of seeding (2006), damage by the alfalfa weevil on the root plant system was not detected. During the study period, the percentage of plants damaged by the larvae increased by an average of 23.9% in 2007 to 79.8% in 2009. The indicator in question was not precise enough to determine the damage degree. It showed what proportion of plants have pest-damaged root systems but did not reflect the degree of damage.

For that purpose, a method (presented in material and methods) was developed to establish and compare the damage degree by *O. ligustici* on the roots of alfalfa cultivars, based on the indicators root damaged area, basic root area and percentage of gnawed furrows. The first stage to establish the degree of attack was to calculate the damaged area (Table 7). The values of the studied index increased successively during individual years on average from 5.6 to 42.1 cm². In the last year of cultivation, the area of damage increased almost eightfold compared to 2007, during which the attack was weakly expressed due to the low population density of species.

Table 7. Damaged area to the root system by *Otiorhynchus ligustici* Linnaeus in different varieties of alfalfa

Variety	Damaged area /10 roots, cm ²												
	2007				2008				2009				
	Regrowths												
	Spring	Summer	Autumn	Average	Spring	Summer	Autumn	Average	Spring	Summer	Autumn	Average	PD
1	3.08abc*	5.25ab	6.30b	4.88	7.09b	24.31bc	24.40b	18.60	32.66c	34.22b	38.42a	35.10	19.53
2	2.80ab	5.95bc	6.50bc	5.08	7.41b	22.01b	27.48cd	18.97	31.66b	33.72b	42.30b	35.89	19.98
3	3.48c	7.75f	8.70d	6.64	11.52c	24.30bc	28.79ef	21.54	33.60d	46.47e	61.28de	47.12	25.10
4	3.20bc	7.35ef	9.50e	6.68	16.01e	24.85bc	29.78fg	23.55	35.62f	45.21d	59.04d	46.62	25.62
5	3.40bc	7.00de	7.10c	5.83	7.18b	23.42b	26.69c	19.10	34.56e	44.31d	66.85f	48.57	24.50
6	2.80ab	6.60cd	7.00bc	5.47	7.72b	29.93d	30.59g	22.75	36.50f	37.86c	41.90b	38.75	22.32
7	2.47a	6.65cde	8.40d	5.84	12.75d	26.90c	28.23de	22.63	33.28cd	47.92f	56.53c	45.91	24.79
8	3.18bc	5.20a	5.50a	4.63	5.64a	13.49a	19.91a	13.01	24.00a	31.25a	61.83e	39.03	18.89
9	3.05	6.47	7.38	5.63	9.42	23.65	26.98	20.02	32.74	40.12	53.52	42.12	22.59

Legend: 1 – Europe; 2-Prista 2; 3-Prista 3; 4-Prista 4; 5-Obnova10; 6-Pleven 6; 7-Dara; 8-Mnogolistna; 9-Average; PD – average for the period; * Values in columns with the same letters are not significant, p<0.05

The indicator increased in ascending order from spring to autumn regrowth by an average of 130.6%. The feeding activity of the larvae was most active in the summer regrowth, where the area increased on average by 95.2% compared to the spring regrowth. In the autumn regrowth, the value increased to a lesser extent - an average of 20.5% compared to the summer.

The damage degree of the studied cultivars varied in individual years. In the second year (2007), variety Mnogolistna (4.63 cm²) distinguished itself with the smallest area of damage, followed by Europa (4.88 cm²) and Prista 2 (5.08 cm²), respectively by 17.8, 13.4 and 9.7% lower values than average for the year. The trend in 2008 was similar, with the decrease in Europa and Prista 2 being 7.1 and 5.3%, respectively. Variety Mnogolistna differed significantly by the lowest area – 13.01 cm². For the 2007-2009 period, the least preferred were Mnogolistna, Europa and Prista 2, which were 16.4, 13.6 and 11.5% below the average damage area for the study period.

Prista 4, Prista 3, Dara and Obnova 10 occupied leading positions in the individual regrowths and years, with a large area of damage, the values of which exceed the averages for the period, respectively by 13.4, 11.1, 9.8 and 8.5%.

The number of gnawed furrows along the root system was an important indicator complementing the damaged area. In separate years, it increased on average from 4.5 to 28.2 number/10 roots and increased sixfold during the study period. The most pronounced increase from spring to autumn growth was observed in 2007 (almost eight-fold), followed by two-fold in 2008 and one-fold in 2009. In addition, the values for the number of gnawed furrows corresponded to the damaged area and with the lowest number on average for the year 2007-2009, Mnogolistna, Prista 2 and Europa stood out, respectively with 15.7, 11.9 and 5.7% below the average value. A weak positive correlation between damaged area and furrow numbers ($r = +0.302$) in 2007 and a strong positive correlation in 2008 and 2009 ($r = +0.837$ and $r = +0.866$, respectively) were significant.

With the alfalfa development over the years, the intensity of growth in the furrow number decreased, while at the same time, the length increased. On average, it increased fourfold from 6.0 (2007) to 21.8 cm/10 roots (2009). As a result of the most active feeding activity of the larvae in the summer regrowth, the most pronounced increase in the length of the gnawed furrows was also observed - on average by 94.7% compared to the spring regrowth. A medium positive correlation was found between furrow length and damaged area in 2007 and 2008 ($r = +0.679$ and $r = +0.472$, respectively) and a strong positive correlation in 2009 ($r = +0.953$).

In addition, plants with a big root neck diameter were favoured by the beetle and, accordingly, with a larger area of damage. There was a medium positive correlation between the damaged area and root neck diameter ($r = +0.400$) in 2007 and a strong positive value in 2009 ($r = +0.853$).

It was necessary to consider the root area as a second stage of the developed method to determine the proportion of gnawed furrows on the root.

The area of the roots (Table 8) in the varieties in the autumn compared to the spring regrowth increased on average by 33.9, 11.6, and 16.6% for 2007, 2008 and 2009, respectively, which showed that the highest growth of the root system was observed in 2007. In the year with the most massive increase in autumn compared to spring regrowth and big root area, Pleven 6 and Mnogolistna stood out (respectively 39.4% and 39.1%).

The Europa and Dara varieties had the smallest root area. In 2008, there was a variation in the mentioned varieties in the spring and summer regrowth. In the autumn, the trend from the previous year was preserved. In 2009, the highest area values were Prista 4, Obnova 10 and Prista 3, and the lowest - Europa and Prista 2. In Prista 4, a pronounced increase in area was observed in the autumn compared to the spring regrowth.

Depending on the biological development and the specific features of the variety, the area growth rate had a different manifestation. Europe (60.7%) and Mnogolistna (59.9%) stood out with the fastest rate of growth of the root area over the years, and Prista 4 (52.6%) with the weakest.

The final stage for determining the damage degree was presented by the percentage of furrows as the derivative of the root area and the area of damage. The share of gnawed furrows increased in separate regrowth and years from 0.73 to 8.01% (from the spring regrowth of 2007 to the autumn in 2009, it increased almost ten times on average). Since larval activity was most active in the summer regrowth, the intensity of growth in the percentage of furrows was also most pronounced.

Regarding the years, the percentage of gnawed furrows increased with the highest intensity in the third year of cultivation (by 59.4% compared to 2007). In 2007, the lowest furrow percentage had Europa, Prista 2 and Mnogolistna, and the most preferred and with a high damage rate were Dara, Prista 4 and Prista. In 2008, a similar trend was observed, and in the last year of alfalfa cultivation (2009), the manifestation of the varieties was most indicative. An average negative correlation was found between the proportion of furrows and the root area. Europa and Multifoliate were distinguished by the highest intensity of area growth and a low percentage of gnawed furrows.

Table 8. Root area and percentage damaged by *Otiorhynchus ligustici* Linnaeus roots in different varieties of alfalfa

2007									
Varieties	Spring regrowth			Summer regrowth			Autumn regrowth		
	Root area, mm ²	% from roots with furrows	% of x	Root area, mm ²	% from roots with furrows	% of x	Root area, mm ²	% from roots with furrows	% of x
	1	2	3	1	2	3	1	2	3
Europe	232.84	0.69	-9.2	359.77	1.42	-24.9	367.14	1.72	-20.0
Prista 2	268.55	0.34	-55.3	381.67	1.68	-11.1	425.39	1.74	-19.1
Prista 3	261.37	0.77	1.3	379.16	1.85	-2.1	391.48	2.35	9.3
Prista 4	290.82	0.89	17.1	380.25	2.08	10.1	419.19	2.39	11.2
Obnova 10	317.86	0.98	28.9	383.71	2.24	18.5	394.00	2.26	5.1
Pleven 6	283.46	0.78	2.6	384.02	1.95	3.2	467.44	2.27	5.6
Dara	254.29	1.10	44.7	367.22	2.21	16.9	397.45	2.57	19.5
Mnogolistna	259.50	0.50	-34.2	380.96	1.65	-12.7	426.33	1.90	-11.6
$\overline{x}$	271.09	0.75	100.0	377.09	1.88	100.0	411.05	2.15	100.0
2008									
Europe	411.58	1.72	-30.1	423.12	5.75	19.8	471.79	5.17	-2.3
Prista 2	450.51	1.84	-25.2	480.26	4.58	-4.6	533.49	5.15	-2.6
Prista 3	476.10	2.42	-1.6	538.12	4.52	-5.8	505.70	5.69	7.6
Prista 4	484.74	3.30	34.1	490.00	5.07	5.6	495.34	6.03	14.0
Obnova 10	477.75	2.50	1.6	531.92	4.40	-8.3	535.76	4.98	-5.9
Pleven 6	435.13	2.77	12.6	504.60	5.93	23.5	507.90	6.02	13.8
Dara	463.15	2.75	11.8	494.31	5.44	13.3	505.23	5.59	5.7
Mnogolistna	413.07	2.37	-3.7	505.23	2.67	-44.4	534.98	3.72	-29.7
$\overline{x}$	451.50	2.06	100.0	495.94	4.80	100.0	511.27	5.30	100.0

Table 8. Root area and percentage damaged by *Otiorhynchus ligustici* Linnaeus roots in different varieties of alfalfa (continued)

Varieties	2009								
	Spring regrowth			Summer regrowth			Autumn regrowth		
	1	2	3	1	2	3	1	2	3
Europe	545.89	5.97	-0.2	560.33	6.11	-13.2	591.89	6.49	-19.5
Prista 2	511.03	6.20	3.7	540.00	6.24	-11.4	636.11	6.65	-17.5
Prista 3	538.82	6.24	4.3	567.47	8.19	16.3	702.71	8.72	8.2
Prista 4	539.14	6.61	10.5	602.02	7.51	6.7	712.91	8.28	2.7
Obnova 10	637.73	6.42	-9.4	650.33	6.81	-3.3	709.12	9.43	17.0
Pleven 6	528.31	6.91	15.6	560.18	7.76	10.2	641.08	9.54	18.4
Dara	532.39	6.25	4.5	589.06	8.13	15.5	641.71	8.81	9.3
Mnogolistna	565.04	4.25	-28.9	565.03	5.53	-21.4	647.49	6.55	-18.7
$\bar{x}$	549.79	5.98	100.0	579.30	6.91	100.0	660.38	8.06	100.0

4.2. SCALE MODELING OF RESISTANCE TO *OTIORHYNCHUS (CRYPHIPHORUS) LIGUSICI* LINNAEUS (COLEOPTERA, CURCULIONIDAE) IN ALFALFA VARIETIES

A six-point scale was developed to assess the resistance of alfalfa cultivars to *O. lygustici*, which was based on the number of root furrows per plant expressed in scores.

During the years and regrowths, the plant proportion (%) with a higher score increased proportionally. The most pronounced damage and the highest scores of four and five were found in 2009. When averaging the results, a clear trend emerged - in the less preferred varieties as Europe, Prista 2 and Mnogolistna plants by one and two scores predominated. Plants with scores of three and four occupied the lowest values (Table 9).

Varieties with a score of three and four exceeding the respective average by 7 to 170% were Prista 3, Prista 4 and Obnova 10.

Table 9. Damage by *Otiorhynchus ligustici* Linnaeus on the root system of scores in different varieties of alfalfa, average for 2007-2009

Varieties	% damaged root system of plants									
	score 1 % from x	score 2 % from x	score 3 % from x	score 4 % from x	score 5 % from x	score 1 % from x	score 2 % from x	score 3 % from x	score 4 % from x	score 5 % from x
Europe	15.5	3.3	14.0	2.2	12.2	-4.7	1.6	-33.3	0.5	-16.7
Prista 2	16.0	6.7	15.1	10.2	8.6	-32.8	0.8	-66.7	0.7	16.7
Prista 3	17.1	14.0	14.1	2.9	13.7	7.0	1.6	-33.3	0.0	-100.0
Prista 4	13.7	-8.7	14.7	7.3	13.7	7.0	2.9	20.8	0.9	50.0
Obnova 10	11.9	-20.7	10.3	-24.8	13.9	8.6	6.5	170.8	1.5	150.0
Pleven 6	16.6	10.7	13.2	-3.6	12.8	0.0	2.1	-12.5	0.6	0.0
Dara	14.1	-6.0	16.6	21.2	15.7	22.7	1.8	-25.0	0.0	-100.0
Mnogolistna	15.0	0.0	11.4	-16.8	11.7	-8.6	1.6	-33.3	0.4	-33.3
$\bar{x}$	15.0	13.7	12.5	2.2	0.6					

Score 0 – healthy root; Ball 1 – single small furrows; Ball 2 – single long or two furrows; Ball 3 – 3 or 4 furrows each; Ball 4 – 5 – 6 furrows; Ball 5 – over 6 furrows

A modified four-point scale to evaluate the resistance of different varieties based on the damage index was used (Table 10).

According to that scale, Europa, Prista 2 and Mnogolistna were moderately resistant to the *O. lygustici* damage. Other ones were sensitive. Resistant (R) or highly susceptible (SS) varieties were not identified.

Using resistant varieties like Europa, Prista 2, and Mnogolistna was crucial for protecting plant production. Their cultivation will contribute to the support of a stable alfalfa agrosystem in biological production.

Table 10. Damage Index (In, %) by *Otiorynchus ligustici* Linnaeus and resistance scale

Varieties	In, average for 2007-2009	Degree of resistance
Europa	21.7	MR (In: 6-25)
Prista 2	22.9	MR (In: 6-25)
Prista 3	25.7	S (In: 26-50)
Prista 4	29.1	S (In: 26-50)
Obnova 10	27.5	S (In: 26-50)
Pleven 6	27.2	S (In: 26-50)
Dara	29.4	S (In: 26-50)
Mnogolistna	23.1	MR (In: 6-25)

Legend: R-resistant; MR-moderately resistant; S-sensitive; SS-highly sensitive

5. DEVELOPMENT OF METHODS FOR THE CONTROL OF MAIN INSECT PESTS IN ORGANIC FORAGE PRODUCTION

5.1. BIOLOGICAL TESTING OF THE EFFICACY OF BIOLOGICAL INSECTICIDES AND BIOLOGICALLY BASED PRODUCTS AGAINST MAIN INSECT PESTS IN ALFALFA

5.1.1. Influence of the biological products Nimazal T/C and Pyrethrum FS EC on some insect pests of alfalfa

In a comparative test of biological insecticides against *Tychius flavus* on average over the period, Pyrethrum exhibited a significantly higher toxic effect, which provided good protection to plants seven days after its application with an efficacy exceeding 60% (Table 11). The Nimazal effect increased to 50.7% on the seventh day, followed by a decrease.

Table 11. Efficacy of some insecticidal products against *Tychius flavus* Becker, *Adelphocoris lineolatus* Goeze and *Acyrtosiphon pisum* Harris & M. (average)

Products	NIBT	1 DAT			5 DAT			7 DAT			9 DAT		
		E, %	NIAT	Sd	E, %	NIAT	Sd	E, %	NIAT	Sd	E, %	NIAT	Sd
Tychius flavus													
1. Nimazal	23.3	33.9	13.0c*	3.91	42.4	9.7 c	3.04	50.7	8.6 c	3.84	42.9	9.8 b	5.18
2. Pyrethrum	23.5	72.6	5.2 b	0.70	65.6	5.7 b	1.07	60.3	7.0 b	2.81	46.5	8.8 b	3.74
3. Nurele D	23.3	88.0	2.0 a	0.60	79.0	3.1 a	1.58	72.1	5.0 a	2.59	67.3	5.7 a	3.09
4. Control	25.1	-	23.4 d	7.75	-	19.3d	6.10	-	21.2d	11.79	-	19.3c	6.67
Adelphocoris lineolatus													
1. Nimazal	27.8	48.0	13.3 c	1.57	54.5	10.7 c	1.51	59.0	9.1 c	2.17	58.0	8.8 b	1.31
2. Pyrethrum	29.4	78.1	5.8 b	1.45	71.3	6.1 b	1.01	68.9	7.2 b	1.50	63.9	8.1 b	1.18
3. Nurele D	27.1	90.2	2.4 a	0.20	85.5	3.2 a	0.45	77.4	4.9 a	1.80	70.2	6.2 a	1.78

4. Control	29.9	-	28.4 d	9.75	-	26.4d	9.86	-	25.1d	12.06	-	24.1 c	10.5
<i>Acyrtosiphon pisum</i>													
1. Nimazal	230.3	54.2	92.3 c	37.60	61.0	83.1 c	37.94	66.1	69.9b	31.81	65.7	74.5a	38.11
2. Pyrethrum	233.9	82.1	33.9 b	4.27	76.9	46.9 b	9.15	73.1	52.9a	12.30	69.6	64.4a	18.04
3. Nurele D	240.9	92.4	14.0 a	3.94	85.5	30.3 a	9.70	79.4	43.4a	15.90	74.4	57.4a	23.59
4. Control	246.8	-	235.3d	54.7	-	245.6d	119.6	-	242.3c	122.9	-	247.3b	120.57

Legend: 1-Nimazal; 2-Pyrethrum; 3-Nurele D; 4-Control; DAT – Days after treatment; E-efficacy; NIBT-number of individuals before treatment of 20 m²; NIAT-number of individuals after treatment of 20 m²; Sd–standard deviation; *Values in columns with the same letters are not statistically proven, p<0.05

Both products reduce the pest number to a lesser extent than Nurele D, characterized by significantly higher efficiency and protected plants nine days after application.

Biological products were mainly shown to be insufficiently effective against *T. flavus* and would not protect crops from damage if necessary.

Biological products showed better control against *A. lineolatus*. Nimazal showed a satisfactory effect on the seventh and ninth day after treatment and an efficacy of 59.0, and 58.0%, respectively. Pyrethrum had a stronger toxic effect with significant differences, including on the seventh day after treatment, and provided considerably better plant protection than Nimazal. Nurele D had the highest efficiency.

Biological insecticides were most effective in controlling *A. pisum*. The increasing efficacy of Nimazal reached satisfactory values of 66.1 and 65.7% on the seventh and ninth days after its application. Pyrethrum exhibited high efficacy throughout the reporting period in the 69.6-82.1% range with significant differences compared to Nimazal from the first to the seventh day inclusive. Compared to the standard, its toxic action was significantly weaker during the first reporting days. On the seventh and ninth days, it approached that of the synthetic insecticide with insignificant differences.

Applied products with insecticidal action had a positive effect on seed alfalfa productivity. Treatment with the organic products Nimazal and Pyrethrum was associated with an increase in yield by 16.3 and 36.7%, respectively, on average over the study period (221.1 and 260.0 kg ha⁻¹), with confidence relative to the control established only after treatment with Pyrethrum. The synthetic insecticide Nurele D was associated with the highest productivity, but compared to the seed yield realized after the Pyrethrum use, the established differences were unreliable.

5.1.2. Influence of the biological product with insecticidal action - Agricol and biological fertilizers - Nagro and Agricol on insect pests in alfalfa seed production

During the 2015-2017 period, the alone and combined action of the biological product with insecticidal action, namely Agricol and two organic fertilizers - Nagro and Litovit for the control of *Bruchophagus roddi*, *A. lineolatus* and *A. pisum* was studied. The established trends in the manifestation of the products during the three years were identical. Therefore, first-year results were presented. In a comparative test of the biological products applied alone, Agricol exhibited the significantly highest toxic effect and provided excellent plant protection one week after its application (Table 12). The product had a fast initial action against the insect pests (91.2 – 100.0% efficacy on the first day) and a long-lasting effect (66.7 – 89.2% - on the seventh day), successfully controlling the density of the three insect species. The bioinsecticide exhibited higher efficacy against the pea aphid, providing excellent control of aphid populations one week after treatment and sufficient protection on the ninth reporting day.

Table 12. Efficacy of biological products with insecticidal action (alone and in combination with biofertilizers), average

Products		<i>Bruchophagus roddi</i>											
NIBT	E, % 1 DAT	NIAT	Sd	E, % 5 DAT	NIAT	Sd	E, % 7 DAT	NIAT	Sd	E, % 9 DAT	NIAT	Sd	
1	21.5	85.4	3.0 a	0.36	79.3	3.9 b	1.50	61.8	8.4 ab	1.34	31.3	13.4 b	2.26
2	19.7	70.1	5.6 b	1.52	63.1	6.3 c	1.41	50.7	9.9 bc	1.23	21.8	14.1 b	1.45
3	21.9	32.9	14.1 c	1.32	27.1	13.7 d	1.96	21.1	17.3 d	2.25	10.0	18.0 c	1.38
4	22.2	90.2	2.2 a	1.96	86.9	2.7 a	1.62	72.1	6.5 a	1.95	49.2	9.8 a	1.00
5	21.3	83.4	3.6 a	1.75	73.7	5.3 c	1.67	50.7	10.9 c	1.35	23.2	15.1 b	1.59
6	21.4	-	20.5 d	0.94	-	18.5 e	1.75	-	21.4 e	2.76	-	19.7 c	2.64
<i>Acyrtosiphon pisum</i>													
1	143.3	86.0	18.6 b	3.06	83.1	23.2 a	5.35	76.8	36.3 b	4.38	58.9	64.9 b	4.11
2	138.5	58.1	45.4 c	3.28	56.1	51.5 b	4.28	54.3	63.1 c	5.85	19.8	106.2 c	6.40
3	139.5	50.1	64.0 c	4.62	27.9	93.9 c	4.39	21.7	105.6d	11.85	5.4	121.1 d	7.30
4	139.5	91.9	9.9 a	2.32	88.6	14.6 a	5.01	86.3	18.9 a	8.50	73.6	35.5 a	8.72
5	133.2	87.6	14.5 ab	3.18	79.8	25.5 a	5.50	66.5	41.7 b	6.21	56.8	54.4 b	4.04
6	152.2	-	135.1 d	47.65	-	152.6 d	21.75	-	140.8 e	15.52	-	140.4 e	8.85
<i>Adelphocoris lineolatus</i>													
1	26.6	88.9	2.7 b	1.02	80.2	4.9 b	0.50	65.2	8.8 b	1.49	51.6	13.0 ab	0.50
2	24.5	64.6	8.7 c	1.30	62.8	8.4 c	1.70	51.0	11.3 c	1.07	33.9	16.2 b	1.60
3	25.6	38.0	15.1 d	1.78	26.7	17.4 d	1.00	21.3	19.0 d	1.64	8.6	23.4 c	3.62
4	26.1	95.3	1.1 a	1.10	89.5	2.1 a	1.97	73.5	6.4 a	0.55	56.4	11.3 a	1.20
5	23.7	84.2	3.5 b	0.50	68.8	5.2 b	1.49	58.2	9.7 bc	1.25	40.0	14.3 ab	0.32
6	24.8	-	24.8 e	3.55	-	23.5 e	2.45	-	23.3 e	1.27	-	24.5 c	4.71

Legend: 1- Agricol; 2- Nagro; 3- Litovit; 4- Agricol + Nagro; 5- Agricol + Litovit 6-Control; DAT – Days after treatment; E-efficacy; NIBT-number of individuals before treatment of 20 m²; NIAT-number of individuals after treatment of 20 m²; Sd–standard deviation; *Values in columns with the same letters are not statistically proven, $p < 0.05$

The biological nano fertilizer Nagro exhibited insecticidal properties against insect pests, and the toxic effect reliably exceeded that of Litovit, exceeding 60% one week after treatment. It provided enough control against alfalfa seed chalcid and pea aphid. Efficacy on the alfalfa plant bug reached a value approaching 60% on the seventh day and also determined an average level of control. The effect on all three pests during the last reporting day was unsatisfactory. Despite the lower values compared to Agricol, Nagro provided good plant protection for one week after application.

The combined application of the organic products was associated with an increase in efficacy compared to their application alone. Using Agricol with Nagro led to excellent pest density control and toxicity exceeding 80% one week after treatment (at *A. lineolatus* value almost levelled off at 80% on the seventh day). The toxic effect of the applied mixture remained high on the ninth reporting day for *A. risum*, while for *A. lineolatus* and *B. roddi* varied in 60-80% and provided a good level of control. Concerning *A. lineolatus*, a significantly higher toxic effect of the combination compared to the independent Agricol use was observed after the first reporting day, and about Nagro - throughout the reporting period. Regarding the other species, reliable differences between the mixture and Agricol were observed on the ninth day after application, and regarding Nagro - from the first to the ninth day inclusive. The observed synergistic effect of the combination was defined as subadditive synergism.

The efficacy of Agricol in combination with the biological fertilizer Litovit was less pronounced.

The results of an additional study showed that treating alfalfa with the biological products also determined a significant increase in plant leaf size with values ranging from 11.4 to 27.5% (average 83.53 cm² stem⁻¹). A maximum was observed after using Agricol in combination with Litovit, followed by Agricol + Nagro. The amount of net productivity of photosynthesis (NPP) was of great importance in the formation of biological and economic yield, as it increases daily between 1.65 and 1.79 g of dry matter from 1 m² of leaf area (4.1 to 8.5%). The application of Agricol resulted in the highest NPP due to a smaller leaf area. Agricol and Nagro combination had the highest content of plastid pigments.

A high positive effect of the used products on the vegetative and generative development of alfalfa was found (Table 13), and in the direction for forage, Litovit stood out with a significantly higher favourable effect on the growth and development of alfalfa compared to Nagro. When they were jointly imported with Agricol, they provided the most pronounced increase in yield by 30.6 and 27.5% respectively. Alfalfa seed production was more affected by the application of Agricol, which was associated with an increase in seed yield by 24.4% and a significant increase in productivity after using fertilizers. The Agricol and Nagro combination provided the significantly highest yield and an increase of 29.7%.

Table 13. Forage and seed productivity in alfalfa after treatment with biological products, average for the period 2015-2017

Variants	Dry mass kg ha ⁻¹		Differences to C, %	Seeds kg ha ⁻¹		Differences to C, %
Control (C)	2557.38	a*	-	549.4	a	-
Agricol	2918.64	b	14.1	683.3	d	24.4
Nagro	3102.35	c	21.3	656.0	c	19.4
Litovit	3224.16	d	26.1	627.3	b	14.2
Nagro + Agricol	3259.46	d	27.5	712.7	e	29.7
Litovit + Agricol	3339.30	d	30.6	680.7	d	23.9

*Values in columns with the same letters are not statistically proven, $p < 0.05$

5.1.3. Biological testing of the efficacy of biological insecticides against *Hypera postica* Gyllenhal (Coleoptera: Curculionidae) (laboratory experience)

The effect of the tested biological insecticides Pyrethro-Natura, Nimazal and Rapax (Biscay - standard) against the larvae of *Hypera postica* showed that it had fast initial action and toxicity of 54.0% showed only the synthetic insecticide Biscay one hour after treatment (Table 14). Its action increased with increasing hours and reached the highest average value of 94.7% 72 hours after application. The toxicity of Biscay was reliably highest compared to the other products from the 1st to the 48th hour after treatment.

Biological insecticides showed no toxic effect one hour after treatment, and a weak effect was detected at the 5th hour. Among them, only Pyrethro-Natura showed a significantly stronger effect (11.0% toxicity), with the larvae being in a state of agony. In the following hours, the mortality rate in that variant significantly increased and 24 hours after treatment the proportion of dead individuals reached 79.3%. The significantly highest toxicity was found with Pyrethro-Natura, followed by Nimazal T/C and Rapax at the 24th, 48th and 72nd hours. Pyrethro-Natura provided complete control with high toxicity against *H. postica* larvae from the 24th day after its application. It was necessary to note that at the 72nd hour, its action approximated that of Biscay and no credibility was established between the two products.

The toxic effect of Nimazal increased comparatively in a more pronounced degree from the 24th to the 72nd hour compared to the other two biological insecticides and reached a satisfactory toxicity of 67.4% 72 hours after treatment.

Rapax increased its activity with increasing hours, but the toxicity at 72 hours remained unsatisfactory at 40.7%. Agony in larvae treated with Rapax continued for a long time, including 72 hours after treatment.

Table 14. Corrected toxicity of insecticidal products against larvae and adults of *Hypera postica* Gyllenhal, %

<i>Hypera postica</i> larvae										
Products	1h	NIAT	5 h	NIAT	24 h	NIAT	48 h	NIAT	72 h	NIAT
Pyrethro-Natura	0.0	0.0 a	11.0	13.4 b	79.3	3.1 c	85.0	2.3 c	89.0	1.7 c
Nimazal T/C	0.0	0.0 a	1.7	14.8 a	44.7	8.3 b	54.7	6.8 b	67.4	4.9 b
Rapax	0.0	0.0 a	0.4	15.0 a	28.0	10.8 a	33.0	10.1 a	40.7	8.9 a
Biscay 240 OD	54.0	6.9 b	62.0	5.7 c	87.7	1.9 d	93.4	1.0 d	94.7	0.8 c
<i>Hypera postica</i> adults										
Pyrethro-Natura	0.0	0.0 a	0.7	14.9 a	52.0	7.2 c	57.0	6.5 c	63.2	5.5 c
Nimazal T/C	0.0	0.0 a	0.0	0.0 a	22.7	11.6 b	35.7	9.7 b	41.4	8.8 b
Rapax	0.0	0.0 a	0.0	0.0 a	11.7	13.3 a	14.4	12.9 a	19.7	12.1 a
Biscay 240 OD	23.4	11.5 b	31.2	10.3 b	61.0	5.9 d	67.0	5.0 d	72.7	4.1 d

Legend: NIAT-number of individuals after treatment; 1 hour – one hour after treatment; 5 h, 24 h, 48 h, 72 h - corresponding hours after treatment; *Values in columns with the same letters are not statistically significant, $p < 0.05$

The results against the adults showed that the products had a weaker toxic effect compared to the larvae, but the trend regarding the strength of their activity was maintained. Biscay showed an initial action with a long-lasting impact (average 72.7% at 72 hours) and the significantly highest toxicity throughout the tested period. Pyrethro-Natura had satisfactory toxicity 24 hours after treatment (52.0%), which increased and reached an average of 63.2% at the 72nd hour. Compared to Biscay, the product toxicity during the reported period was significantly weaker.

Nimazal and Rapax showed unsatisfactory action, which at 72 hours reached only 41.4 and 19.7%, respectively. Species were in a state of agony. As Nimazal and Rapax showed lower toxicity under field conditions, they were not considered suitable for controlling *H. postica* adults. The larvae number and adult forms in wet and dry controls remained unchanged (15 specimens in each dish) during the reported hours after treatment.

5.2. INFLUENCE OF BIOLOGICAL PRODUCTS ON THE NATURAL BIOAGENTS OF MAIN insect pests IN ALFALFA

Useful entomofauna in the study period (2012-2014) was represented by species belonging to the Thysanoptera, Coleoptera, Hymenoptera orders and Hemiptera: suborder Heteroptera. *Aeolothrips intermedius* and *Coccinella septempunctata* were presented in high numbers in the crops, which made it possible to evaluate their response to the biological products Nimazal and Pyrethum.

Bioinsecticides manifested to be harmless to seven-spot ladybird adults (Table 15).

Table 15. Toxicity of biological products to *Coccinella septempunctata* Linnaeus and *Aeolothrips intermedius* Bagnall

Products	1 DAT	5 DAT	7 DAT	9 DAT
----------	-------	-------	-------	-------

	NIBT	T,%	NIAT	Sd	T,%	NIAT	Sd	T,%	NIAT	Sd	T,%	NIAT	Sd
<i>Coccinella septempunctata</i> 2012													
1. Nimazal	148.5	8.00	115.8b*	2.00	15.50	107.5b	2.28	20.60	106.5b	1.97	22.40	130.9a	2.25
2. Pyrethrum	152.1	26.30	93.1 c	3.22	23.60	108.8c	2.16	20.40	111.8b	2.51	18.00	130.0a	2.65
3. Nurele D	150.9	91.70	10.5 d	6.82	85.00	33.3d	2.00	70.6	45.8c	2.40	63.80	64.3b	2.20
4. Control	149.0	-	143.7 a	-	-	148.2a	-	-	140.8a	-	-	147.6a	-
2013													
1. Nimazal	94.5	6.20	74.0b	1.71	10.20	75.9b	2.71	13.10	56.8a	2.50	11.00	63.9b	6.20
2. Pyrethrum	100.0	16.10	70.0c	2.61	14.50	76.5b	3.19	9.60	62.5a	2.16	8.30	69.7b	16.10
3. Nurele D	86.4	80.70	13.9d	2.77	64.00	27.8c	3.67	50.20	29.8b	2.71	48.70	33.7c	80.70
4. Control	105.7	-	88.2a	-	-	94.6 a	-	-	73.1a	-	-	80.3a	-
2014													
1. Nimazal	128.1	8.50	114.8b	2.25	10.60	105.1b	2.62	18.30	100.4b	3.29	20.20	89.6b	3.37
2. Pyrethrum	135.5	20.90	105.0c	2.80	20.60	98.8c	2.16	16.10	109.1b	2.80	15.40	100.5b	2.27
3. Nurele D	121.4	82.30	21.0d	2.61	80.10	22.2d	2.80	75.60	28.4c	3.67	57.40	45.3c	2.43
4. Control	130.6	-	127.9a	-	-	119.9a	-	-	125.3a	-	-	114.5a	-
<i>Aeolothrips intermedius</i> 2012													
1. Nimazal	57.6	19.15	44.1b	4.45	27.20	35.8b	3.11	24.10	39.7b	1.27	11.00	53.2c	1.41
2. Pyrethrum	63.1	36.50	37.9c	2.69	28.10	38.7b	2.69	22.20	44.6b	1.31	13.70	56.5b	1.84
3. Nurele D	54.5	92.80	3.7dd	3.96	77.80	10.3c	3.11	67.90	15.9c	2.97	57.00	24.3c	4.24
4. Control	71.2	-	67.4a	-	-	60.8a	-	-	64.7a	-	-	73.9a	-
2013													
1. Nimazal	32.2	10.50	27.8b	2.12	14.55	32.1a	1.48	25.73	24.3b	1.03	14.95	31.2a	4.17
2. Pyrethrum	35.3	23.47	26.0c	2.59	20.35	32.8a	2.48	16.74	29.9a	2.07	13.03	34.9a	2.87
3. Nurele D	28.9	79.91	5.6d	2.11	71.90	9.5b	2.40	57.35	12.5c	2.19	39.10	20.0b	1.84
4. Control	30.5	-	29.4a	-	-	35.6a	-	-	31.0a	-	-	34.7a	-
2014													
1. Nimazal	46.8	18.50	37.4b	0.71	25.65	37.1b	1.34	29.13	30.9b	2.64	24.02	34.7b	1.44
2. Pyrethrum	51.0	35.62	32.2c	2.24	30.70	37.6b	3.25	23.70	36.2b	2.69	21.57	39.0b	2.15
3. Nurele D	47.6	89.52	4.9d	2.29	84.95	7.6c	2.62	75.50	10.9c	3.54	57.58	19.7c	3.65
4. Control	49.1	-	48.2a	-	-	52.3a	-	-	45.7a	-	-	47.9a	-

Legent: DAT – Days after treatment; T – Toxicity; NIBT-number of individuals before treatment of 20 m²; NIAT-number of individuals after treatment of 20 m²; Sd–standard deviation; *Values in columns with the same letters are not statistically proven, $p < 0.05$

In a comparative analysis, the toxic effect of Pyrethrum was proven to be higher than Nimazal on the first day after application ($p < 0.05$), which is due to the established fast "knockdown" effect. A similar trend was observed on the fifth day in 2012 and 2014. Based on the internationally accepted scale for evaluating toxicity, Pyrethrum's impact on useful species was more than Nimazal. However, it did not exceed 25% and was determined as harmless. As a result of the evaluation, bioinsecticides were safe, with a toxicity level of less than 25%.

Nurele D stood out as having the highest toxic effect demonstrated. It was highly toxic (> 75%) on the first and fifth days and moderately toxic (51 to 75%) on the seventh and ninth days after treatment.

Biological products had a more pronounced toxicity on the predatory thrips *A. intermedius*. Nimazal was mainly harmless and had one score during the reporting days. Higher values in its toxic effect, slightly exceeding 25%, were found on the fifth day after its application in 2012 and 2014 and on the seventh day in 2013 and 2014. Pyrethrum caused significantly higher mortality of thrips than Nimazal only on the first day after treatment ($p < 0.05$), and the differences in toxicity between the two products during the remaining days were statistically insignificant. Compared to Nurele D, the toxicity of biological products was three to four times lower.

Nurele D exhibited a similar effect to Pyrethrum. It had a fast initial effect but was

distinguished by a reliably high toxic effect on predatory thrips. It should be noted that predatory thrips were more sensitive to the Nimazal and Pyrethrum impacts than predatory ladybugs.

According to the accepted principles of the International Organization for Biological Control (IOBC) for evaluating the toxicity of studied insecticides on useful insects, Nimazal appeared to be harmless with toxicity not exceeding 25% against adult individuals of *C. septempunctata* and in 67% of cases against *A. intermediate*. Slightly toxic with a score of 2, not exceeding 30% mortality, it had on the fifth and seventh day after treatment on predatory thrips. Pyrethrum was harmless to predatory ladybirds and in 67% of cases against *A. intermedius*, with a safety score of 1. It caused mortality in thrips and was defined as slightly toxic, with a score of 2. Nurele D was highly toxic in 50% of cases and moderately toxic to both predatory species in the remaining 50%.

5.3. TOLERANCE OF ALFALFA VARIETIES TO DAMAGE BY SOME INSECT PESTS(*CATAPION SENICULUS* KIRBY; *SITONA* GENUS)

5.3.1 TOLERANCE OF ALFALFA CULTIVARS TO *CATAPION SENICULUS* KIRBY AND FACTORS DETERMINING IT

The *Catapion seniculus* damage in the different alfalfa varieties was most pronounced in the first regrowth. Damage during the second regrowth was single and had no economic importance. The extent of damaged plants increased gradually and reached the highest value in the third year of alfalfa cultivation (2008). On average, the damage reached 37.9% (Table 16). The varieties least affected by the species, with values below the average for the experiment (37.9%), were Europa (23.6%) and Multileaf (21.1%).

Catapion seniculus was damaged Europa and Multileaf minor, and on average over three years, the damaged stems were 9.10 and 8.2%, respectively, and were 6.3 and 7.2% less than the average value. When ranking varieties, the most stable positions had Europa and Mnogolistna, which by year and on average for 2007-2009 had first and second positions. These varieties had the shortest height of the stems before harvesting the first regrowth, which affected the *A. seniculus* choice. A strong positive correlation was found between the percentage of damaged stems and their height ($r = + 0.830$). The pest strongly preferred Prista 3, Obnova 10 and Pleven 6.

In the spring season, they exhibited a faster growth rate and had taller stems than other varieties. That provided a better feeding environment and suitable conditions for the development of the species, which was associated with a higher damage rate.

The number of stems per plant was a parameter that varied more narrowly than height but also affected *A. seniculus* preference. A strong negative correlation was found between the two indicators ($r = - 0.703$). In varieties with slower growth rates, such as Europa and Mnogolistna, the distance of furrows from the root neck was shorter. On the other hand, in the varieties with faster growth rates and greater pest sensitivity, the distance was longer. There was a positive, average correlation between the degree of the damaged stems and the furrow distance from the root neck ($r = + 0.665$).

Studied varieties did not show *C. seniculus* resistance, but Europa and Mnogolistna stood out with sufficient tolerance.

Table 16. Damage by *Catapion seniculus* Kirby in different alfalfa varieties and correlations between percentage of damaged stems and some parameters, average 2007-2009

Varieties	% of plants with damaged stems	% damaged stems	Rank	Number of stems per 1 plant	Stem height, cm	Distance of the course from the root
-----------	--------------------------------	-----------------	------	-----------------------------	-----------------	--------------------------------------

					neck, mm	
Europe	23.6	9.1	2	3.5	61.1	22.7
Prista 2	39.5	15.5	3	2.3	67.3	27.3
Prista 3	45.8	20.0	8	2.7	67.7	31.9
Prista 4	33.8	16.6	4	3.5	62.4	30.5
Obnova 10	36.3	18.2	6	2.9	64.2	29.1
Pleven 6	36.2	18.2	7	2.4	66.2	28.0
Dara	32.8	17.0	5	2.6	65.3	27.7
Mnogolistna	21.1	8.2	1	3.5	60.5	28.6
$\bar{x}$	37.9	15.4	-	2.9	64.3	28.2
r =	-	-		- 0.703	+ 0.830	+ 0.665

Legend: $\bar{x}$ – trial mean, r – correlation

5.3.2 Tolerance of alfalfa cultivars to *Sitona* genus

Nodulation in alfalfa in the first cultivation year differed among regrowth with an increase in the furrow number from spring to autumn regrowth (mean 46.5, 60.4 and 86.3 furrow numbers/10 roots). In the following years, the formation of tubers by undergrowth did not follow the trend indicated above, and the values varied as follows: for 2007 – on average from 78.1 to 91.6 furrow numbers/10 roots; for 2008 – average from 24.1 to 113.1 furrow numbers/10 roots; for 2009 – on average from 10.1 to 10.9 pieces/10 roots. Regarding alfalfa varieties, nodule formation was most pronounced in Europa, Obnova 10 and Mnogolistna (on average for 2006-2009 – respectively 71.2, 66.8 and 65.1 nodule number/10 roots). Larva-damaged nodules grow from spring to fall regrowth in different years. In 2006, the percentage of nodule damage varied on average from 0 (for spring regrowth) to 6.4 % (for autumn regrowth); in 2007 – from 2.8 to 4.9%; in 2008 – from 9.3 to 23.7% and in 2009 – varied from 16.7 to 24.3%. On average, the proportion of damaged nodules increased from 2.9% (2006) to 21.4% (2009).

Sitona species showed given preferences for the studied alfalfa cultivars. Over the years and on average during the research period, the Dara, Pleven 6, and Prista 2 varieties stood out with a high percentage of damaged nodules, above average for the experience - 11.6, 12.7 and 12.8%, respectively. Prista 3, Europe and Prista 4 (average 10.5, 9.1 and 9.3%) had values close to the average for the experience. Obnova 10 and Mnogolistna were manifested as tolerant to mages of the *Sitona* genus, and the damage percentage was an average of 6.8 and 7.9, respectively. The gnawed hole amount was 20.0 and 22.2% below the average value for the experiment. Cultivars were characterized by a higher saponin content in the plant roots, which was one of the probable reasons for the low beetle preference for them.

5.4. EFFECT OF PERENNIAL CEREAL-LEGUM GRASS MIXTURES ON SOIL DWELLING ROOT INSECT PESTS

5.4.1. Effect of perennial wheat-legume grass mixtures on damage by *otiorhynchus ligustici linnaeus* and *sitona* genus

Individual crops with perennial leguminous crops, as well as wheat-leguminous grass mixtures, had different nodule formation potential (table 17). The nodule location on the root system was mainly on the lateral roots, with a 91.0% proportion. The nodule-forming activity was reliably highest in bird's-foot trefoil, followed by the mixture with cocksfoot (respectively 17.4 and 10.6 total number of nodules/root). The alone sowing with alfalfa, as

well as in a mixture with the wheat component, had a less pronounced process - respectively 2.4 and 2.1 total nodule numbers. In the independent crops, the nodule formation proved to exceed the corresponding one in the mixtures with cocksfoot, as follows: in sainfoin with 57.8%, bird's-foot trefoil with 61.7% and alfalfa with 28.2%.

The most preferred and frequented by the species of the *Sitona* genus was the bird's-foot trefoil crop, where the degree of damaged nodules reached an average of 75.4%. The damage in the mixture with cocksfoot was significantly lower - 25.9%. sainfoin, grown independently was characterized by a lower degree of damaged nodules - 36.6%, and the mixed crop - by 27.3%. Crops with alfalfa occupied an intermediate position (with 70.3% damage in the single crop and 62.9% - in the mixture). A clear tendency to reduce the nodule damage in the mixed versus single crops was established by 25.4% (sainfoin+cocksfoot), 65.6% (bird's-foot trefoil+cocksfoot) and 10.5% (alfalfa+cocksfoot).

Otiorhynchus ligustici caused damage to varying degrees depending on the legume type. Alfalfa grown in a pure crop and a mixture was distinguished as the most attractive crop to the pest, with the highest number of gnawed furrows and holes, and length of spiral furrows. Lowly affected and with the lowest damage degree were the bird's-foot trefoil crops. A marked preference of the species for the independent legume crops was established, and in the mixed ones the amount of gnawed furrows by the larvae was reduced by 30.7; 44.4 and 33.3%, the amount of holes – with 5.9; 50.0 and 20.0%, and the furrow length – with 20.0; 14.3 and 13.3%, respectively, in the sainfoin mixtures, bird's-foot trefoil and alfalfa with cocksfoot.

Table 17. Nodulation and degree of damage of species of the *Sitona* genus and *Otiorhynchus ligustici* Linnaeus in stand-alone and mixed perennial legume crops, average for 2015-2017

	Total nodule number		Number nodule damaged		Nodule damaged, %	<i>Otiorhynchus ligustici</i>						Length of root, cm	
	Central root	Lateral root	Central root	Lateral root		Legume stem number	Number of spiral furrows	Numbers, holes	Furrow length, cm,	Furrow distance from the root neck, cm	Legumes / Cereals		
1	2.4 d*	7.7 c	0.7 b	3.0 b	36.6	3.5 a	1.3 bc	1.7 b	2.0 bc	2.6 bc	18.3 b	-	
2	0.9 c	16.5 e	0.8 b	12.3 c	75.4	5.0 b	0.9 ab	1.4 b	1.4 ab	1.8 a	16.9 a	-	
3	0.0 a	2.4 a	0.0 a	1.7 a	70.3	3.1 a	2.4 d	3.5 d	3.0 d	1.9 a	20.9 c	-	
4	0.5 b	4.9 b	0.1 a	1.6 a	27.3	3.4 a	0.9 ab	1.6 b	1.6 ab	2.0 ab	18.1b	11.7a	
5	0.4ab	10.2 d	0.0 a	2.7 b	25.9	5.8 b	0.5 a	0.7 a	1.2 a	2.7 c	17.4ab	14.0b	
6	0.2ab	1.9 a	0.0 a	1.3 a	62.9	3.0 a	1.6 c	2.8 c	2.6 cd	3.0 c	22. d	11.4a	

Legend: 1- Sainfoin (*Onobrychis viciifolia*) – full flowering stage; 2. bird's-foot trefoil (*Lotus corniculatus*) – full flowering stage; 3. alfalfa (*Medicago sativa*) – full flowering stage; 4. Sainfoin + cocksfoot (*Dactylis glomerata*) – full flowering stage; 5. Bird's-foot trefoil + cocksfoot – full flowering stage; 6. Alfalfa + cocksfoot – full flowering stage (the stage is determined by the leguminous crop); * Values in columns with the same letters are not significant ($p < 0.05$)

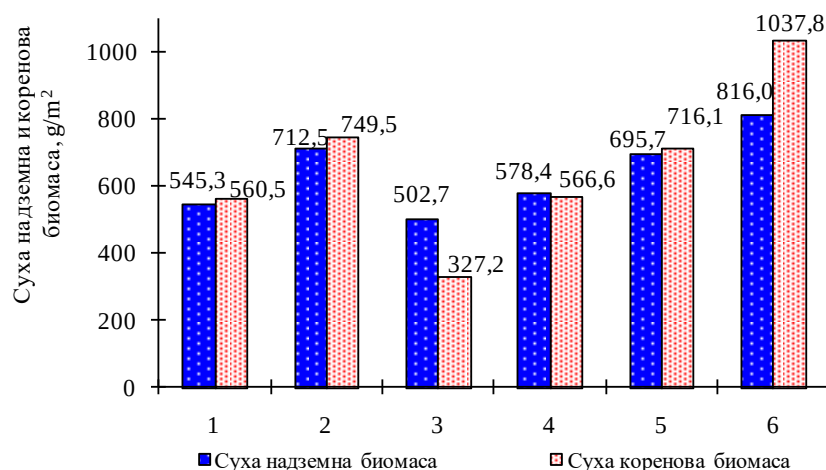
The distance between the spiral furrows at the root neck increased, and it was higher in the mixtures as the root system of alfalfa grew more actively during cultivation.

Otiorhynchus ligustici damage suppressed plant growth, and plant height negatively correlated with damage indicators. Regression analysis showed that the length of the gnawed furrows had the highest and most reliable negative impact on the leguminous plant height in the independent and corresponding mixed crops (- 13.1). A weaker significant negative effect had the hole amount on the roots (-5.7).

The productivity of perennial legume crops can be increased depending on the cropping system and technology. Intercropping with cocksfoot significantly exceeded the weight of plant biomass than corresponding stand-alone perennial crops (Fig. 12). Productivity increase in the sainfoin mixture was the most pronounced, with an average of 30.7%, followed by that

of the alfalfa mixture - by 18.7%, and the bird's-foot trefoil mixture - by 15.0%.

A similar trend was observed regarding root biomass productivity. The productivity of dry biomass in the independent leguminous crops slightly exceeded the corresponding leguminous components in the mixed crops. The cereal plants in the mixed crops had lower productivity compared to the cocksfoot crop. The productivity differences were significant at 38.5%, 47.0% and 51.7% for the mixtures of sainfoin, bird's-foot trefoil, and alfalfa, respectively. A trend in the root biomass productivity was similar to aboveground mass.



Legend: 1-sainfoin; 2-sainfoin + cocksfoot ; 3-bird's-foot trefoil; 4-bird's-foot trefoil + cocksfoot ; 5-alfalfa; 6-alfalfa + cocksfoot

Figure 12. Productivity in individual and mixed crops, average for 2015-2017

With the development of the plants and advancing age, the dry above-ground and root mass values also increased, and both masses magnified most intensively from 2015 to 2017 in sainfoin (respectively by 66.5 and 331.6%), followed by bird's-foot trefoil (65.2 and 169.4%) and alfalfa (44.1 and 131.8%). The dry above-ground mass of the leguminous crop amount and the cereal component reliably increased with the years of cultivation in mixed crops. The most pronounced productivity expansion of the leguminous component in the mixture from 2015 to 2017 was found in the bird's-foot trefoil (by 1.6 times), and in the cocksfoot - in a sainfoin mixture (by 1.5 times).

Damage by *O. ligustici* had the highest negative impact on the above-ground biomass yield. Growing the crops in mixed crops had a positive effect on the chemical composition, increasing the content of crude protein, calcium and phosphorus compared to individual crops.

5.4.2. Effect of alfalfa cropping system on damage by *Otiorhynchus ligustici* Linnaeus

Cultivation of alfalfa in cereal component mixtures with the smooth brome, bird's-foot trefoil and triple mixture led to a reliable reduction of the root damage of alfalfa by the *O. ligustici* larvae than the independent crop. The greatest extent that was established in the mixed bird's-foot trefoil crop (Table 18). In the mixture, the furrow and hole damage, and furrow length decreased by 70.5, 71.4 and 23.1%, respectively. The decrease in the triple mixture was significant by 49.8, 56.5 and 31.6%, respectively. Despite the shorter furrow length in the triple mixture than alfalfa combination with smooth brome, differences were insignificant.

The mixed cultivation of leguminous and cereal crops had a positive effect on the development of the cereal components than their independent cultivation. The values of the

indicators: stem height, stem number and root length reliably increased in the mixtures. On average for the period, the co-cultivation of the smooth brome with alfalfa was distinguished by the significantly higher amount of formed stems and an increase of 45.4% to independent wheat crop. The smooth brome mixture had the highest increase in stem height (by 52.1%) and root length (26.8%) than alone cultivation. The highest values according to the three indicators, smooth brome in the mixture with alfalfa stood out.

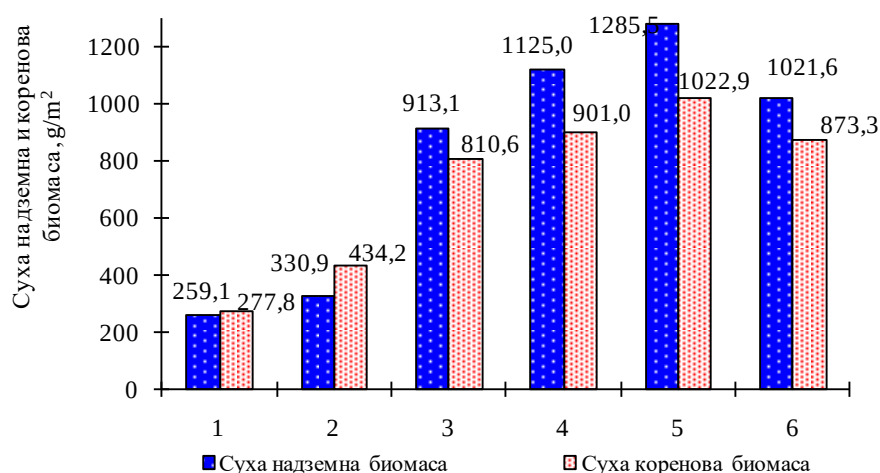
Table 18. Damage rate of *Otiorhynchus ligustici* Linnaeus in perennial stand-alone and wheat-legume grass mixtures crops, average

B	Legumes							Cereals		
	Height, cm	Stem number /plant	Number of spiral furrows /plant	Number holes /plant	Length of 1 furrow, cm	Distance from the root neck, cm	Root length, cm	Height, cm	Stem number /plant	Root length, cm
1.	-	-	-	-	-	-	-	28.7 a	6.3 bc	14.2 a
2.	-	-	-	-	-	-	-	36.1 b	6.1 b	19.3 d
3.	74.9 a	6.2 c	3.5 c	3.5 c	3.2 b	5.9 c	29.0 a	-	-	-
4.	83.9 c	4.1 a	2.1 b	1.9 b	2.9 ab	3.4 a	33.3 b	43.7 c	6.9 c	18.0 c
5.	83.1 c	4.9 b	1.0 a	1.0 a	2.5 a	4.5 b	32.0 b	52.6 d	8.9 d	21.8 e
6.	78.7 b	4.9 b	1.8 b	1.5 b	2.2 a	4.7 b	29.8 a	43.2 ^{SB}	52.3 ^{CF}	6.0 ^{SB} 4.9 ^{CF} 16.7 ^{SB} 19.0 ^{CF}
x	80.1	5.0	2.1	2.0	2.7	4.6	31.0	c d	b a	b d

Legend: V – Variants; 1. Smooth brome (*Bromopsis inermis*); 2. Cock's-foot (*Dactylis glomerata*); 3. Alfalfa (*Medicago sativa*); 4. Alfalfa+ Smooth brome; 5. Alfalfa + Cock's-foot; 6. Alfalfa + Smooth brome + Cock's-foot; x– average; ^{SB}- Smooth brome; ^{CF}- Cock's-foot; *Values in columns with the same letters are not statistically significant, $p < 0.05$

The mixture cultivation of alfalfa with cock's-foot stood out with the smallest area of damage and reduction with 76.2%, followed by the triple mixture - with 62.8% and the smooth brome mix - with a 31.3% reduction compared to alfalfa sowing.

As a result of the weak preference, a reliable increase in the productivity of the above-ground mass by 40.8% was found for alfalfa with a cock's-foot compared to the independent cultivation of the legume crop (Fig. 13).



Legend: 1. Smooth brome; 2. Cock's-foot; 3. Alfalfa; 4. Alfalfa+ Smooth brome; 5. Alfalfa + Cock's-foot; 6. Alfalfa + Smooth brome + Cock's-foot

Figure 13. Productivity of dry above-ground and root biomass of perennial stand-alone and wheat-legume grass mixtures, kg/da

An intermediate position is occupied by the mixture containing Smooth Brome (with a 23.2% increase), and the weakest increase is found in the triple one (11.9% increase).

Root biomass productivity was significantly higher in mixed crops than in the corresponding single crops. The alfalfa values in a mixture with smooth brome, cock's-foot and the combination of the legume crop with the two wheat components increased by 11.2, 26.2 and 7.7%, respectively, compared to the independent alfalfa crop.

The mixed cultivation of alfalfa positively impacted its chemical composition, increasing crude protein content from 3.7 to 15.2% and phosphorus content from 3.3 to 18.8%.

Alfalfa cultivation in grain-grass mixtures is a modern approach to organic forage production and is related to reducing damage from enemies, increasing productivity and quality, and protecting the environment.

6. Stability of alfalfa productivity after application of biological products

It was found that the years had a significant impact, through a variance analysis concerning seed yield, on this indicator - 22.1% of the total variation in the tested variants (treatment with Nimazal, Pyrethrum and Nurele D). The preparation types had the biggest impact, accounting for 54.1%. The study demonstrated a significant effect with a confidence level of $p \leq 0.01$, indicating the impact of years of preparation.

The interaction between the factors year x preparation (AxB) was significant at the confidence level $p \leq 0.5$ and allows to evaluate the stability of the manifestations of each variant about the years (Table 19) based on variances of stability σ_i^2 and S_i^2 according to Shukla, ecovalence W_i by Wricke and stability criterion YS_i by Kang.

After interpreting the stability indicators, it was concluded that only the untreated control was unstable. With it, the values of stability variances σ_i^2 and S_i^2 according to Shukla and of equivalence W_i according to Wricke were the highest. There was an instability of linear type - the values of σ_i^2 were credible, while those of S_i^2 were insignificant in the control.

Table 19. Stability parameters of alfalfa seed yield options after treatment with synthetic and biological products in terms of years

Variants	$\bar{x}$	σ_i^2	S_i^2	W_i	YS_i
Nimazal	221.1	-133.8	-6.1	40.6	0
Pyrethrum	259.9	485.8	80.7	660.2	4+
Nurele D	286.0	-111.3	35.4	63.1	7+
Control - untreated	204.8	1154.3*	155.8	1328.7	-2

Kang's YS_i summary stability criterion gives a negative score to the control variant, rating it as the least productive and unstable. On the contrary, treatment with Nurele D stands out as technologically most valuable, followed by treatment with Pyrethrum. These variants combine high yield and very high stability in different years.

7. STUDY OF BIOCHEMICAL CHANGES IN PLANTS ATTACKED AND DAMAGED BY MAIN ENEMIES

7.1. Chemical composition of root mass in alfalfa varieties and correlations with the degree of damage by *Otiorhynchus ligustici* Linnaeus

A result of *O. ligustici* damage to the alfalfa root system was a change in the chemical

composition. The data collected between 2007 and 2009 showed a decline in crude protein levels (-1.0% to -4.5%) and crude fibre (-1.0% to -6.1%) in the roots damaged by proboscis. The extent of that decline varied depending on the cultivar. The details are provided in Table 20.

An exception was found in the medium-resistant varieties Europa and Mnogolistna, characterized by the weakest preference and attack by *O. ligustici*. In Europa, crude protein increased by 3.9%, while in Mnogolistna it did not change and crude fibre increased by 1.9 and 2.9%, respectively. That was most likely a defensive response by the plants to the attack. In all damaged roots, the content of calcium, phosphorus, sugars and saponins increases.

It was established that in the moderately resistant varieties to the pest, the increase in the concentration of water-soluble sugars was less pronounced (by 2.0 and 4.0% in Europa and Mnogolistna), and in the sensitive varieties - significantly stronger (from 9.5% in Prista 4 to 15.4 % at Prista 3). About saponins, the trend was the opposite - the increase was less pronounced in sensitive varieties (from 12.7% in Dara to 19.0% in Obnova 10 and Pleven 6) and bigger - in moderately resistant varieties (21.7% - Mnogolistna, 22.6% - Prista 2 and 23.2% - Europa). The increase in the content of saponins, as anti-nutritional substances, to a greater extent in Mnogolistna, Prista 2 and Europa, was due to the defensive reaction of the plants to compensate for the losses from the *A. seniculus* damage.

Table 20. Biochemical composition of root mass in alfalfa, 2007-2009

Indicators	Root type	Europe	Prista 2	Prista 3	Prista 4	Obnova 10	Pleven 6	Dara	Mnogolistna
CP g/kg	1	120.3	123.6	120.7	128.3	126.2	130.7	126.4	127.4
	2	125.0	122.4	117.1	123.0	121.5	127.7	123.2	126.5
CF g/kg	1	258.7	266.4	270.2	280.4	263.4	275.6	262.8	254.3
	2	263.6	264.1	259.0	271.2	258.4	259.7	256.2	261.6
Ca g/kg	1	7.78	7.78	7.63	7.56	7.72	7.31	7.46	7.58
	2	7.79	8.06	7.44	8.01	8.36	8.30	7.68	7.89
P g/kg	1	1.99	2.09	1.89	1.78	2.05	2.08	1.91	1.90
	2	2.19	2.31	2.03	2.13	2.21	2.08	2.11	2.21
WSS, %	1	8.10	7.55	7.22	7.58	7.67	8.31	8.34	7.96
	2	8.26	8.99	8.33	8.30	8.30	8.67	9.27	8.28
S, %	1	2.28	2.26	2.27	2.34	2.52	2.42	2.52	2.53
	2	2.72	2.62	2.77	2.81	3.00	2.88	2.84	3.08

Legend: 1 - healthy roots; 2 - damaged roots; CP - crude protein; CF - crude fibers; Ca – calcium; P – phosphorus; WSS - water-soluble sugars; S - saponins

The correlation dependence between the damaged area and some indicators characterizing the chemical composition of the damaged root system was established. The correlations related to the content of CP and CF over the years were negative and varied. The dependence had a negative middle effect for CP ($r=-0.357$) but a weak negative effect value for CF on average for the period. A positive correlation prevailed between the damage degree and the amount of calcium, and the correlation concerning phosphorus was medium negative ($r=-0.663$).

The relationship between the damaged area and the sugar amount was positive and varied in the range of 0.126 - 0.596.

7.2. Above-ground chemical composition of alfalfa cultivars and correlations and stability with the degree of damage by *Catapion seniculus* Kirby (Coleoptera: Curculionidae)

Catapion seniculus damages mainly the first alfalfa regrowth, which was why a

biochemical evaluation of the above-ground mass of the first regrowth was made. Varieties Europa and Mnogolistna occupied stable positions and stood out with the highest content of crude protein (CP) (exceeding by 6.2 and 5.8%, respectively, compared to the average value) and with the lowest content of crude fibre (CB) (decrease of 4.7 and 4.2%) and calcium (decrease of 6.3 and 2.5% respectively) (Table 21). The content of saponins exceeded the average value by 1.9 and 11.4%. The trends in Europe and Mnogolistna about phosphorus and water-soluble sugars were unclear.

Sensitive to the damage of *S. seniculus* Prista 4, Pleven 6, Dara and Obnova 10 had a lower CP content. The reduction in those variants varied from 2.0 to 3.2% compared to the average value. They were also distinguished by a relatively higher content of CF, exceeding the average value from 1.6% (Prista 4) to 5.2% (Dara).

The calcium content was higher than the tolerant varieties and exceeded the average. The saponin amount varied within narrower limits but did not exceed the average value (except - Dara). No clearly expressed trends are observed about the content of phosphorus and water-soluble sugars. A strong negative correlation was found between the degree of damage and the CP content ($r = -0.937$) and a medium and strong positive correlation with the CF and Ca indicators ($r = 0.673$ and $r = 0.772$ respectively). The correlation coefficient for phosphorus, water-soluble sugars and saponins was negative with weak or moderate interaction ($r = -0.098$, $r = -0.256$ and $r = -0.591$, respectively).

Table 21. Biochemical characteristics of alfalfa varieties, I regrowth and correlation dependence between the damage degree by *Catapion seniculus* Kirby and chemical component content, on average for 2007-2009

Varieties	Damaged stems, %	CP g/kg	CF g/kg	Ca g/kg	P g/kg	Sugars, %	Saponins,%
Europe	9.10	191.0	244.7	19.24	3.05	2.71	1.07
Prista 2	15.54	178.6	259.1	20.49	3.12	3.00	1.03
Prista 3	20.03	177.2	252.0	19.68	2.56	2.76	1.01
Prista 4	16.63	174.2	261.1	21.31	2.63	2.61	1.01
Obnova 10	18.21	176.4	257.2	21.73	3.21	2.83	1.02
Pleven 6	18.24	175.3	264.9	20.98	2.81	2.89	0.91
Dara	16.98	176.1	270.3	20.84	3.06	2.90	1.16
Mnogolistna	8.16	190.3	246.0	20.03	2.73	3.06	1.17
$\bar{x}$	15.36	179.9	256.9	20.54	2.90	2.84	1.05
r	-	-0.937	0.673	0.772	-0.098	-0.256	-0.591

Legend: CP - crude protein; CF - crude fibers; Ca – calcium; P – phosphorus

To analyze the stability and adaptability of cultivars to *C. seniculus* damage, several statistical methods were employed to describe the effects of cultivar $\times$ environment interaction. According to the analysis of variance of the investigated indicators - degree of damaged plants and damaged stems, crude protein and fibre, soluble sugars and saponins, statistically significant differences ($p < 0.001$; $p < 0.05$) were found for all characteristics of the environment (E), cultivars (G) and cultivar $\times$ environment interaction, as well as the involvement of different gene systems in their control.

Damaged plants: Prista 4 and Dara had the highest stability score according to both analysis methods for that indicator, followed by Prista 2 and Prista 3. Prista 4 and Dara had a relatively low degree of damaged plants and exhibited better specific adaptability to low and high-damage environments, respectively (Fig. 14 a).

Degree of damaged stems: The values of the stability parameters regarding that indicator determined as the most valuable Mnogolistna variety, according to the regression analysis of Eberhart and Russell ($b_i = 1.17$), Tai ($a_i = 1.066$) and Theil ($T = 1.18$). Prista 4 ($b_i = 1.06$)

was also evaluated as a valuable and stable variety. Mnogolistna was least preferred by *C. seniculus* (low degree of damaged stems) (Fig. 14 b), showing high tolerance to proboscis damage and better adaptability in any environment. Europa had an unstable response type, which suggests its high sensitivity to changing environmental conditions. However, it had better adaptability in adverse environments and a low percentage of damaged stems. Prista 4 appeared as a stable variety with a responsive response to the environment ($b_i = 0.06$).

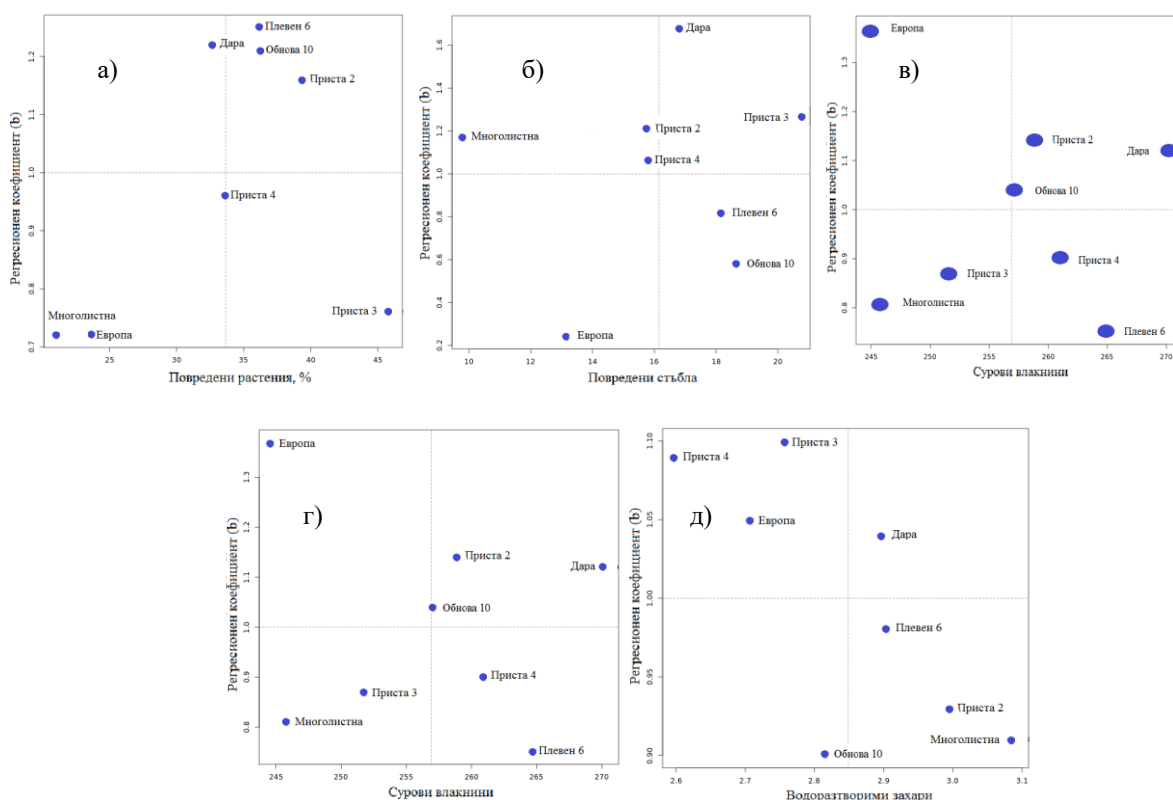


Figure 14. Stability and adaptability of alfalfa varieties concerning the regression coefficient (b_i) and the studied indicators

Crude protein: Application of the methodologies developed by Eberhart, Russell and Tai showed that almost all cultivars exhibited high stability ($b_i \approx 1$) except Prista 3 ($b_i = 1.19$) (Fig. 14 c).

Crude fibre: Varieties had different stability responses to the crude fibre content, with high content being an undesirable varietal trait. Stable according to Eberhart and Russell, Theil, Plaisted Peterson and Wricke, with a regression coefficient slightly exceeding 1.0 (b_i ranges from 1.04 to 1.14) are Obnova 10, Dara and Prista 2. Mnogolistna and Prista 4 had regression coefficients below 1.0, which provides higher resistance to environmental change and thus increases specific adaptability to low-fibre environments (Fig. 14 e). Mnogolistna and Europa had the lowest values for that particular indicator.

Water-soluble sugars: Linear regression on the average water-soluble sugar content of one and all cultivars in each environment was associated with regression coefficients ranging from a minimum of 0.90 to 1.10, indicating similar responses of cultivars to environmental changes. Varieties appeared to be stable but had different adaptability. Mnogolistna and Prista 2 were more responsive to unfavourable conditions and had the highest sugar content (Fig. 14e). Pleven 6 and Dara had better adaptability, and Europa - medium adaptability in a favourable environment.

According to the analysis of the ecological stability of Eberhart and Russel about saponin

content, the studied cultivars were evaluated as unstable, being insensitive to environmental changes and having poor adaptation to adverse environments.

Based on a chemical analysis of the above-ground mass, as well as established adaptability and stability, alfalfa cultivars, slightly preferred by *C. seniculus* for the conditions of biological forage production, can be broadcast.

8. FORAGE QUALITY AND EVALUATION OF ENERGY NUTRITIONAL VALUE OF ALFAFER TREATED WITH BIOLOGICAL PRODUCTS

8.1. Forage quality and assessment of energy nutritive value in alfalfa treated with Nimazal T/C and Pyrethrum FS EC

The biological products Nimazal T/C Pyrethrum FS EC had a positive effect, reducing not only the insect pest density but also increasing the forage quality, increasing the crude protein content by 8.4 and 13.4% and decreasing the fibre content by 6.8 and 8.1%, respectively (Table 22). The reduced concentration of fibre in the cell wall improved feed intake. An optimal combination of increased protein and reduced fibre content was found after alfalfa treatment with Pyrethrum.

It was found that the level of lignification was affected by the type of preparation used, with a noticeable increase in trend.

The significance of NDF (Neutral-detergent fibers), ADF (Acid-detergent fibers), ADL (Acid-detergent lignin) and cellulose for assessing the forage quality was their low content. In the present experiment, they mainly occupied lower values than the control. A reliable reduction of NDF, approaching 10% than the untreated variant (442.7 g kg⁻¹), was found in Nurele D (with 43.9 g kg⁻¹). The bioinsecticide reduction was in the range of 1-3%. ADF and ADL were affected to a lesser extent than NDF, and differences compared to the control were less pronounced. An exception was observed for Pyrethrum, where the values were slightly increased.

Table 22. Main composition, content of fibre components and digestibility of dry alfalfa biomass, the average for 2012-2014

Variants	CP	CF	NDF	ADF	ADL	HEMI	SEL	LIGN	DDM
Control	168.2 a*	309.3 b	442.7 b	349.2 b	78.0 a	93.4 b	271.2 a	17.6 a	59.7 a
Nimazal	182.4 ab	288.2 ab	429.6 b	346.0 b	76.8 a	83.6 ab	269.1 a	17.9 ab	63.2 ab
Pyrethrum	190.8 b	284.3 a	436.8 b	353.8 b	79.1 a	83.0 ab	274.7 a	18.0 b	65.1 b
Nurele D	164.4 a	307.9 b	398.8 a	317.4 a	66.9 a	81.5 a	250.4 a	16.7 a	62.9 a
LSD _{0,05%}	20.715	23.178	17.21	21.313	12.542	10.801	25.678	0.325	5.246

Legend: CP-Crude protein; C-Crude fibers; HEMI – Hemicellulose; SEL–Cellulose; LIGN – Degree of lignification; NDF-Neutral-detergent fibers; ADF – Acid-detergent fibers; ADL – Acid-detergent lignin; DDM – Digestibility of dry matter. *Values in columns with the same letters are not statistically significant, $p < 0.05$

The digestibility of dry matter, as the main criterion for the forage quality, increased under the influence of the products used and varied from 62.9 to 65.1%. A significantly increased digestibility of 9.0% was found after the Pyrethrum application.

The studied products had a positive effect on the relative nutritional forage value. The digestible dry matter content was higher than the untreated variant, the increase being from 0.4 to 4.0% (Table 23). The trend regarding DMI (dry matter intake) was similar, with the increase being more pronounced and varying in the range of 1.8 – 11.4%. The relative

nutritional value of the forage after treatment with the synthetic insecticide Nurele D reliably exceeded the control by 15.7%, while the increase with the bioinsecticides was unproven. The modern assessment of forage quality was based on forage units for milk (UFL, FUM and VEM) and forage units for growth (UFV, FUG, VEVI), which determined energy nutritional value. The studied products had a positive effect on the energy nutritional value, increasing it to varying degrees.

Table 23. Energy and protein nutritional value of dry alfalfa biomass, average for 2012-2014

Variants	DDM	DMI	RFV	UFL	UFV	FUM	FUG	VEM	VEVI	PBD	PDIN	PDIE
Control	61.69a	2.71a	129.64a	0.680a	0.569a	0.564a	0.465a	843a	1796a	124.6a	105.6a	87.8a
Nimazal	61.95a	2.80a	134.30a	0.708a	0.598a	0.586a	0.488a	869a	1836ab	138.6ab	114.5b	92.8b
Pyrethrum	61.34a	2.76a	131.58a	0.732b	0.625b	0.606a	0.511b	892b	1872b	146.8b	119.9b	96.0b
Nurele D	64.18a	3.02a	150.05b	0.698a	0.588a	0.578a	0.481a	854a	1814a	121.4a	103.2a	89.0a
LSD _{0,05%}	5.201	2.054	19.432	0.051	0.054	0.101	0.044	47.324	61.207	19.302	8.524	5.002

Legend: DDM – Digestible dry matter, %; DMI - Dry matter intake, % of body weight; RFV – Relative nutritional value, %; UFL (Fr) FUM, VEM (Dutch) – Feed units for milk; UFV (Fr) FUG, VEVI (Dutch) – Feed units for growth g kg⁻¹; PBD (TDP) – Total Digestible Protein (Small Intestine Digestible Protein); PDIN, PDIE digestible protein in the small intestine depending on nitrogen (PDIN) and energy (PDIE), g kg⁻¹; *Values in columns with the same letters are not statistically significant, $p < 0.05$

A maximum increase in forage units for milk and growth, with significant differences from the control, was achieved after treatment with Pyrethrum and an increase of 7.6 and 9.8% for UFL and UFV, respectively, and an increase of 7.4 and 9.9% concerning FUM-FUG (evidence was lacking for the FUM value). The increase in the other two variants was less pronounced and was insignificant.

The protein nutritional value, estimated as total digestible protein /PBD/, digestible protein in the small intestine depending on nitrogen /PDIN/ and energy /PDIE/, had the highest values after treatment with Pyrethrum. Total digestible protein (PBD) increased by 11.2% with Nimazal and 17.8% with Pyrethrum and had a significant difference from the control. The increase of digestible protein in the small intestine depending on nitrogen (PDIN) and energy (PDIE) was significant. The increase in protein was more pronounced after the introduction of Pyrethrum. The synthetic insecticide did not significantly affect the protein's nutritional value.

Treating alfalfa with biological insecticides in the flowering stage increased the forage quality, increasing its digestibility, energy and protein nutritional value.

8.2. Forage quality and assessment of energy nutritive value in alfalfa treated with Agricol, Litovit and Nagro

The treatment of plants with the biological products Agricol, Litovix and Nagro (applied alone and in combination) had a favourable impact with a high positive effect on the forage quality with an increased content of crude protein by 10.1% and a reduced content of fibrous components by 6.4% (Table 24).

The content of the fractions of the structural fibre components (polyosides) of the cell walls of alfalfa plants was associated with a predominant tendency of decrease compared to the untreated control.

The content of acid-detergent fibres (ADF) was from 71.5 to 85.2 g kg⁻¹ lower than NDF, and depending on the products used, the trend was in different directions. A decrease in the values of KDF was found after the individual and combined application of Agricol with Litovit. Only the combination was distinguished by a reliable difference from the control variant. The effect of the products on the content of KDL was entirely positive, related to a

decrease in values from 5.1 (Agricol+Nagro) to 12.9% (Litovit) and evidence of differences after treatment with Litovit.

Table 24. Main composition, fibre components and digestibility of alfalfa treated with biological products, the average for 2015-2017

B	CP	CF	NDF	ADF	ADL	HEMI	CEL	CEL	DDM	DOM
1	155.9 a*	344.7 b	469.5 a	410.5bcd	100.6 b	64.1 a	309.9abc	21.3 b	53.94 a	53.44ab
2	171.0 ab	310.5 a	483.4 a	401.1abc	93.6 ab	82.3bc	307.6abc	19.3 ab	60.41 e	59.72 d
3	176.9 b	318.5 a	490.0 a	416.7cd	93.8 ab	73.3abc	323.0c	19.2 ab	53.49 a	52.89ab
4	176.4 b	328.2ab	468.3 a	383.1 a	87.7a	85.2 c	295.5 a	18.7 a	57.95cd	56.98cd
5	175.4 b	315.1 a	487.6 a	416.1cd	95.5 ab	70.6ab	320.7bc	19.6 ab	59.90de	59.40 d
6	174.6 b	318.7 a	478.7 a	393.8ab	91.9 ab	84.9 c	301.9ab	19.2 ab	56.63bc	56.00bc
LSD _{0.05%}	1.579	2.494	27.006	21.779	12.399	13.217	18.998	2.542	2.381	3.391
Average	171.7	322.62	479.6	403.5	93.8	76.7	309.7	19.4	57.05	56.41

Legend: V-variants; CP-Crude protein; CF-Crude fibres; NDF-Neutral-detergent fibres; ADF – Acid-detergent fibres; ADL–Acid-detergent lignin; HEMI – Hemicellulose; CEL–Cellulose; LIGN – Degree of lignification; DDM – Digestibility of dry matter, %; DOM-Digestibility of organic matter, %; CP, CF, NDF, ADF, CHEMICAL, SEL - g kg⁻¹ dry substance; 1- Control; 2-Agricol; 3-Nagro; 4-Lithovite; 5-Agricol + Nagro; 6-Agricol + Litovit; *Values in columns with the same letters are not statistically significant, p<0.05

The tendency was confirmed that the content of the polyoside hemicellulose completely digestible by animals in the dry mass of alfalfa was low - from 64.1 to 85.2 g kg⁻¹, and that of the incompletely digestible cellulose - high - from 295.5 to 323.0 g kg⁻¹. Regarding the decrease in the cellulose content, the most significant was the influence of Litovit (- 4.7%) and its combination with Agricol (- 2.6%). The degree of lignification was not significantly affected by the action of the products except Litovit and the demonstrable lower content of lignin compared to the control.

Biological products increased the energy nutritional value of alfalfa, with the forage units for milk UFL (FUM, VEM) and growth UFV (FUG, VEVI) occupying reliably the highest values after treatment with the Agricol and Nagro combination. The increase was 20.7 (UFL) and 29.0% (UFV) respectively. A comparative increase in energy nutrition was also observed after the Agricol application of (by 6.1 for UFL and 8.8% for UFV) and Agricol with Litovit (by 4.4 for UFL and 5.6% for UFV). The products significantly increased the protein's nutritional value, and the effect was more pronounced when Agricol and Litovit were added together.

Applying them in insect pest control, bioproducts guarantee the realization of not only high and stable yields but also high-quality forage.

CONCLUSIONS

1. In the alfalfa agrocenosis, for the region of Pleven, 160 plant species have been established, which belong to 118 genera, 46 families and six orders.
In the alfalfa forage agrocenosis, it was found:
 - 1.1. The order Coleoptera included 56 species from 43 genera and 13 families. The main and permanent insect pests were the dominant species *Hypera postica* Hrbst. (69.6%), the subdominant - species of the genus *Sitona* (5.7%) and the secondary species *Catapion seniculus* Kirby (3.4%). Pests were most numerous in the stem formation and budding stages of the first and second regrowth;
 - 1.2. The order Hemiptera - Cicadomorpha and Fulgoromorpha included 28 species from 24 genera and 8 families. The dominant species were *Empoasca pteridis* Dahlbom (22.0%) and *Anaceratagallia laevis* Ribaut (19.4%), and the subdominant ones - *Austroagallia sinuata* Mulsant & Rey (13.6%), *Philaenus spumarius* Linnaeus (11.2%), and *Streptanus (Streptanulus) josifovi* Dlabola (10.4%). The species were constant insect pests during the growing season, with high numbers from April to August;
 - 1.3. Spatial structure of distribution and gender dynamics of *Empoasca pteridis* Dahlbom was studied. It was established that the index between the sexes decreases with the development of the plants in the individual regrowths and with an increase in the number of vegetation days. The study was important for forecasting and signalling when the cicada appears in large numbers;
 - 1.4. The species of the order Hemiptera, suborder Heteroptera were 39, belonging to 25 genera and 12 families. Serious and dominant species were *Adelphocoris lineolatus* Goeze (29.7%) and *Lygus rugulipennis* Poppius (19.1%), subdominant - *Piezodorus lituratus* F. (5.2%), and they were most numerous in the flowering stage of the second and third regrowth. The numerical dynamics of the beneficial species of the genus *Nabis* (10.7%) and *Orius horvathi* Reuter (4.8%) follow or coincide with that of harmful Heteroptera, which emphasises their important role as bioagents;
 - 1.5. Five species of four genera and one family belong to Hemiptera, suborder Sternorrhyncha. The main insect pests were the dominant species *Therioaphis trifolii* Monell (63.3%) and *Acyrtosiphon pisum* Harris & M. (28.8%), with the first species in the highest numbers in the flowering stage of the second and fourth regrowth, and the second - in the flowering stage of the second and third regrowth;
 - 1.6. From the Thysanoptera order, 13 species of 8 genera and three families were identified. Dominant species were *Thrips tabaci* Lindeman (37.4%) and *Aeolothrips intermedius* Bagnall (43.8%), with the highest density in the budding stage - the beginning of flowering of the second regrowth. A subdominant species was *Thrips atratus* Halyday (9.7%), with a maximum number at the beginning of budding and flowering of the first regrowth, and budding of the second ones. The predator *Aeolothrips intermedius* Bagnall can be an effective agent in the biological control of harmful thrips;
 - 1.7. From the Hymenoptera order, in alfalfa seed production, one honey bee and seven species of wild bees were found, belonging to five genera and four families. Dominant species were *Eucera longicornis* Linnaeus and *Halictus maculatus* Smith, and subdominant - *Halictus quadricinctus*, *Andrena falsifica* Perkins and *Andrena dorsata* Kirby, which reached maximum numbers in the mass flowering stage of the second regrowth. It was found that the species *Eucera longicornis* Linnaeus predominated in the pre-afternoon hours, and its abundance was strongly positively correlated with relative air humidity ($r = 0.873$);

2. *Catapion seniculus* Kirby (Coleoptera: Apionidae), as an insect pest of alfalfa, was reported for the first time in the Bulgarian entomological literature. Morphological peculiarities of the adult individuals, numerical dynamics and damage were established, which was expressed in the gnawing of a narrow course by the larvae, in the basal part of the stem, and subsequent breaking and laying of the plants. The species caused damage in the first regrowth of alfalfa;
3. Eleven new alfalfa leafhopper species were reported: *Anaceratagallia laevis* Ribaut, *Streptanus* (*Streptanulus*) *josifovi* Dlabola, *Allygidius* (*Allygidius*) *commutatus* Fieber, *Psammotettix striatus* Linnaeus, *Reptalus panzeri* Low, *Tettigometra sulphurea* Mulsant & Rey, *Tettigometra* (*Mitricephalus*) *obliqua* Panzer, *Eupteryx atropunctata* Goeze, *Agalmatium bilobum* Fieber, *Zygina flammigera* Fourcroy and *Neotalitrus fenestratus* Herrich-Schäffer (Hemiptera, Fulgoromorpha and Cicadomorpha);
4. It was established that the formation process of the entomofauna in an alfalfa crop followed certain regularities, with the insect diversity increasing proportionally from 90 species in the first to 119 species in the fourth year of cultivation. The total number of classes represented had remained relatively constant over the years;
5. The part distribution of the different orders and sub-orders among the four regrowths of alfalfa was determined, which was closely dependent on the development of the plants and followed a characteristic trend over the years. Depending on the food specialization of the predators, their part of subspecies corresponded to that of their prey;
6. An original methodology was developed for determining the damage degree by the larvae of *Otiorhynchus ligustici* Linnaeus, on the roots of eight alfalfa varieties, depending on the root area and the damage area. It was found that:
 - 6.1. In the year of alfalfa crop establishment, *Otiorhynchus ligustici* Linnaeus caused single damages of no economic importance. The root damage area increased proportionally from the second to the fourth year eightfold (from 5.6 to 42.1 cm²) and from spring to autumn regrowth by 130.6%. The percentage of gnawed moves was at its highest intensity during the summer regrowth and the third year of alfalfa cultivation;
 - 6.2. Root damage correlated negatively with the root weight and positively - with the diameter of the root neck and crop filling;
 - 6.3. To assess the resistance of alfalfa cultivars to *Otiorhynchus ligustici* Linnaeus, a six-point scale based on the number of root strokes was developed, as well as a modified resistance scale based on a damage index, according to which Europa, Prista 2 and Mnogolistna were defined as medium resistant varieties;
7. The structure of the aphid-aphidophagous community was studied, and it was found that the latter belong to three groups: 1) Polyphagous predatory bugs; 2) Oligophagous and polyphagous predators and 3) Monophagous and oligophagous parasitoids. Aphidophages were the dominant biological control agent from mid-May to June, while predatory ladybirds from the Coccinellidae family regulated the situation only in September;
8. The bioinsecticides Nimazal T/S, in a dose of 500 ml/day (1% azadirachtin A+0.5% azadirachtin B, B, D, D and 2.5% nim substance) and Pyrethrum FS/EC – 50 ml/day (32% pyrethrum extract /25% pyrethrin/; 36% sesame oil and 36% adhesives), provide good control against *Acyrtosiphon pisum* Harr. and *Adelphocoris lineolatus* Goeze. Pyrethrum exhibited significantly higher efficacy and provided a higher and stable yield of seeds;
9. The biological insecticides Nimazal T/S, in a dose of 500 ml/day and Pyrethrum FS/EC – 50 ml/day, were harmless to adult individuals of *Coccinella septempunctata* Linnaeus and in 70% of cases to *Aeolothrips intermedius* Bagnall. That allowed their application in the organic cultivation of alfalfa and the protection of biological diversity in the

ecosystem;

10. The biological product with insecticidal action Agricol, in a dose of 300 ml/day (natural polysaccharides), had an effective action against the important insect pests in alfalfa seed production - *Bruchophagus roddi* Guss., *Acyrtosiphon pisum* Harr., and *Adelphocoris lineolatus* Goeze. It was found that Agricol exhibited a more efficient and long-lasting effect when combined with the organic nano fertilizer Nagro, at a dose of 50 ml/da (contains micro and macro elements, meso elements, microhumates, vitamins, fulvic acids, etc.) and provided significantly more high productivity. Nagro applied alone, exhibited insecticidal properties and protected crops from pests;
11. The biological insecticide Pyrethro-Natura, at a dose of 75 ml/da (pyrethrins 40 g/l), was efficient and had significantly the highest toxic effect against the larvae and adults of *Hypera postica* Gyllenhal 72 hours after treatment, compared to Nimazal T/C – 500 ml/day and Rapax – 200 ml/day (*Bacillus thuringensis*, subsp. *kurstaki* strain EG) in laboratory conditions;
12. It was found that among the alfalfa cultivars studied, Europa and Mnogolistna showed middle tolerance to *Catapion seniculus* Kirby and were distinguished by the smallest stem height and slow growth rate in spring;
13. The varieties Obnova 10 and Mnogolistna, which were characterized by the lowest percentage of damaged tubers and gnawed pits, were tolerant to *Sitona* species;
14. It was found that when growing the forage crops sainfoins, birdsfoot trefoil and alfalfa, in mixtures with cocksfoot, damage by *Otiorrhynchus ligustici* Linnaeus was reduced by 25.4%, 65.6% and 10.5%, respectively, compared to stand-alone legume crops. A decrease in the amount of gnawed by *O. ligustici* larvae was found by 30.7; 44.4, and 33.3%, as well as their length - by 20.0; 14.3 and 13.3%. The mixed cultivation of forage crops significantly increased the productivity of above-ground and root biomass, and had a positive effect on the chemical composition of plants;
15. Intercropping of alfalfa with cereal components (smooth brome, birdsfoot trefoil and a combination of both components) showed reduced root damage by *Otiorrhynchus ligustici* Linnaeus, then alfalfa monoculture. The least preferred, with the lowest degree of damage and the highest productivity of dry above-ground and root biomass, was a mixture of alfalfa with birdsfoot trefoil, followed by a mixture with smooth brome. Mixed cultivation with cereal components had a positive effect on the chemical composition of the above-ground biomass of the crops;
16. It was established that in roots of alfalfa cultivars damaged by *Otiorrhynchus ligustici* Linnaeus: 1) the content of crude protein and crude fibre decreased, while in medium-resistant cultivars Europa and Mnogolistna it increased; 2) the content of calcium, phosphorus, water-soluble sugars and saponins increased, but the intensity of increase was different for Europa and Mnogolistna;
17. The chemical composition of the above-ground mass of alfalfa varieties was determined, and the tolerant to *Catapion seniculus* Kirby Europa and Mnogolistna were distinguished by the highest content of crude proteins and saponins and the lowest – of crude fibre and calcium. The varieties were characterized by high ecological stability and adaptability;
18. The application of biological products (Nimazal, Pyrethrum, Agricol, Nagro and Litovit), alone and in combinations, had a positive effect on the composition, digestibility, energy and protein nutritional value of the forage, increasing the quality and productivity.

VII. CONTRIBUTIONS

Original scientific contributions

1. An inventory of the entomofauna in alfalfa agrocenosis for the region of Pleven was carried out. One hundred and sixty insect species belonging to 118 genera, 46 families and six orders were identified, and numerical dynamics of important harmful and useful species were presented.
2. New species for *Medicago sativa* L. in Bulgaria were reported: *Catapion seniculus* Kirby and 11 species of cicadas (*Anaceratagallia laevis* Ribaut, *Streptanus* (*Streptanulus*) *josifovi* Dlabola, *Allygidius* (*Allygidius*) *commutatus* Fieber, *Psammotettix striatus* Linnaeus, *Reptalus panzeri* Low, *Tettigometra sulphurea* Mulsant & Rey, *Tettigometra* (*Mitricephalus*) *obliqua* Panzer, *Eupteryx atropunctata* Goeze, *Agalmatium bilobum* Fieber, *Zygina flammigera* Fourcroy and *Neoaliturus fenestratus* Herrich-Schäffer). The results of the faunal studies were a significant contribution to Bulgarian entomological science;
3. The spatial structure of distribution and gender dynamics of *Empoasca pteridis* Dhlb was established with the development of the plants during the individual regrowths and years of cultivation;
4. For the first time in our country, studies were carried out on alfalfa damage caused by *Catapion seniculus* Kirby. Morphological peculiarities of the adult individuals, numerical dynamics and damage were established;
5. The process and regularities in the formation of the entomofauna in the alfalfa crop and the proportion of different orders and sub-orders in the individual regrowths were established;
6. The structure of the aphid-aphidophagous community and the potential for biological control was established

Scientific - methodical contributions

1. An original methodology was developed for determining the damage caused by *Otiorhynchus ligustici* Linnaeus (Coleoptera, Curculionidae) in alfalfa, depending on the root area and the damaged area;
2. To evaluate the resistance of alfalfa cultivars to *Otiorhynchus ligustici* Linnaeus, a six-point scale was developed, depending on the number of furrows, and a modified resistance scale based on a damage index.

Scientific-applied contributions

1. In practical terms, the results provided an answer to the quantitative and qualitative question changes in alfalfa resulting from the damage caused by the larvae of *Otiorhynchus ligustici* Linnaeus. The studies were original for our country and are of interest to alfalfa production;
2. Dominant species of wild bees in alfalfa seed production crops, the dynamics of the pollinator bee population and the factors that affect it have been established;
3. The influence of the system of cultivation of perennial legume crops (sainfoins, birdsfoot trefoil and alfalfa), alone and in a mixture with cocksfoot, on the degree of damage by

main soil-dwelling insect pests (*Sitona* spp. and *Otiorhynchus ligustici* Linnaeus), productivity and chemical composition was studied of above-ground and root biomass;

4. The influence of the alfalfa cultivation system was studied - in a separate and a mixed crop with wheat components (smooth brome, birdsfoot trefoil and a combination of both components), on the degree of damage by *Otiorhynchus ligustici* Linnaeus, productivity of dry above-ground and root biomass and plant chemical composition;
5. The toxic effect of biological insecticides, as well as biological products with insecticidal action, applied alone and in combination with organic fertilizers, against important insect pests in alfalfa seed production (*Tychius flavus* Beck, *Bruchophagus roddi* Gussakovskiy, *Acyrtosiphon pisum* Harris and *Adelphocoris lineolatus* Goeze) was established), and the toxic response of biological insecticides to some predatory species (*Coccinella septempunctata* Linnaeus and *Aeolothrips intermedius* Bagnall). The bioinsecticidal toxic effect against larvae and adults of *Hypera postica* Gyllenhal in laboratory conditions was established;
6. Alfalfa varieties with middle tolerance to *Catapion seniculus* Kirby, genus *Sitona* and *Otiorhynchus ligustici* Linnaeus were released;
7. The impact of applied biological products with different effects on forage quality, energy and protein nutritional value was determined.

DISSERTATION RELATED PUBLICATIONS

1. N. Georgieva, **I. Nikolova**, A. Ilieva. 2012. Biochemical composition of root mass in alfalfa cultivars. *Plant Sciences*, 49: 20-25.
2. Georgieva N. and **I. Nikolova**. 2012. Studying the formation of root mass and root collar in alfalfa varieties (*Medicago sativa* L.). *Journal of Mountain Agriculture on the Balkans*, 15, 1: 139-152.
3. **Nikolova I.** 2012. Study on the qualitative composition and population dynamics of aphids (Homoptera: Aphidinea) on alfalfa (*Medicago sativa* L.). *Plant Sciences*, 49: 55-61.
4. Georgieva N. and **I. Nikolova**. 2015. Stem formation at alfalfa varieties and correlative dependences with some main parameters. *J of Central European Agriculture*, 16 (2): 89-98.
5. **Nikolova I.** and N. Georgieva. 2010. Tolerance of lucerne varieties to *Apion seniculus* Kirby (Coleoptera: Curculionidae). *Agricultural science and technology*, vol.2, 1, 44-47.
6. **Nikolova I.** and I. Lecheva. 2010. Study of harmful species from Coleoptera order in alfalfa biocenosis for forage. Jubilee Scientific Conference with International Participation "Traditions and Challenges of Agricultural Education, Science and Business, Scientific Works, vol. LV, book 2, 23-30, AU-Plovdiv
7. **Nikolova I.** and N. Georgieva. 2011. Study on the tolerance of alfalfa varieties (*Medicago sativa* L) to *Sitona* species (Coleoptera: Curculionidae). *Agricultural science and technology*, 3, 3: 249 – 253.
8. **Nikolova I.** and N. Georgieva. 2012. *Otiorrhynchus ligustici* L. (Coleoptera: Curculionidae) I. Comparative study of the resistance of alfalfa varieties. *Banat's Journal of Biotechnology*, III (2), 68-74. SJIF Impact Factor Evaluation = 3.904
9. **Nikolova I.**, N. Georgieva and A. Ilieva. 2012. *Otiorrhynchus ligustici* L. (Coleoptera: Curculionidae) II. Method for determination of the degree of damage by larva and biochemical composition of the root system in different alfalfa varieties. *Banat's Journal of Biotechnology*, III (2), 75-80. SJIF Impact Factor Evaluation = 3.904
10. **Nikolova I.** and N. Georgieva. 2012. *Otiorrhynchus ligustici* L. (Coleoptera: Curculionidae) III. Modeling scale for resistance in alfalfa varieties. *Banat's Journal of Biotechnology*, III (2), 81-85. DOI: 10.7904/2068 – 4738 – 3(2) – 81. SJIF = 3.904
11. **Nikolova I.** 2012. Entomofauna of Heteroptera order in alfalfa forage. *Journal of Mountain Agriculture on the Balkans*, 15, 1, 124-138.
12. **Nikolova I.** 2012. Study on cicada entomofauna (Homoptera, Auchenorrhyncha) in alfalfa forage. *Journal of Mountain Agriculture on the Balkans*, vol.15, 1: 153-167.
13. Georgieva N. and **I. Nikolova**. 2012. Density and reduction of the stand at alfalfa varieties (*Medicago sativa* L.). *Banat's Journal of Biotechnology*, III (2), 18-23. DOI:10.7904/2068 – 4738 – 3 (2) – 18. SJIF Impact Factor Evaluation = 3.904
14. **Nikolova I.** 2015. Order Thysanoptera in alfalfa grown for forage. *Journal of Global Agriculture and Ecology*, Vol. 3, Issue 4: 195-202. ISSN No.: 2454-4205.
15. **Nikolova I.**, N. Georgieva and A. Ilieva. 2015. Correlative relationship between the degree of damage by *Otiorrhynchus ligustici* L. and some parameters related with the attack in alfalfa varieties. *Proceeding of the Union of Scientists – Ruse*, book 3, Agrarian & Veterinary sciences, vol. 7: 216-222.
16. **Nikolova I.**, N. Georgieva and A. Ilieva. 2015. Chemical composition of above-ground dry mass in alfalfa varieties and correlations to the preference of *Apion seniculus* Kirby (Coleoptera: Curculionidae). *Proceeding of the Union of Scientists – Ruse*, book 3, Agrarian & Veterinary sciences, vol. 7: 223-228.
17. **Nikolova I.** 2016. Effects of organic products with insecticidal action on key insect pests in alfalfa seed production. *The Emirates Journal of Food and Agriculture [EJFA]* Online First: 14 May, 2016. doi:10.9755/ejfa.2016-04-381, Impact Factor Thomson Reuters: 0.623
18. **Nikolova I.**, Georgieva N. and Naydenova Y. 2016. Forage quality and energy feeding value estimation of alfalfa (*Medicago sativa* L.), treated by biological active compounds.

Journal of mountain agriculture on the Balkans, 19, 1: 78-95.

19. Nikolova I. 2016. Side effects of neem and pyrethrum products on natural enemies of insects in alfalfa seed production. *Acta entomologica Serbica*, 21: 133-142. DOI: 10.5281/zenodo.198388; SJR – 0.530

20. Nikolova I. and E. Zhekova. 2017. Entomofauna of heteroptera in alfalfa agrocenoses. *Journal of mountain agriculture on the Balkans* 20 (6): 113-135.

21. Младенова Р., А. Кирилов, И. Николова, Н. Георгиева, П. Маринов-Серафимов, Я. Димитров, П. Зоровски, С. Георгиев, Н. Палагачева. 2016. Операция опрашители - инициатива за устойчиво земеделие и биоразнообразие в България. http://focalpointbg.com/files/8Konferenciq/Day1/RositsaMladenova_et_al._Operation_Pollinator.pdf

22. Nikolova I. and N. Georgieva. 2017. Efficacy of organic products against insect pests in alfalfa grown for seeds. *Journal of Bioscience and Biotechnology* 6(2): 91-98.

23. Nikolova I. 2019. Sex Ratio of *Empoasca pteridis* Dhlb. (Hemiptera: Cicadellidae) and Its Seasonal and Annual Variation in Alfalfa Field. *Asian Journal of Research and Review in Agriculture*, 1(1), 45-52. SJR – 0.18

24. Nikolova I., N. Georgieva and V. Vasileva. 2018. Chemical composition in perennial forage crops, depending on the farming system and correlative dependencies with damage of root insect pests. *Journal of mountain agriculture on the Balkans* 21 (4): 93-105.

25. Nikolova I., N. Georgieva and V. Vasileva. 2018. A system of forage crops cultivation, productivity and damage level caused by *Sitona* spp. and *Otiorrhynchus ligustici* L. (Coleoptera: Curculionidae). *Polish Journal of Entomology*, 87 (4): 302–322 (2018). RG Journal Impact: 0.61

26. Николова И., Н. Георгиева и Г. Делчев. 2018. Оценка стабилността на инсектицидни продукти прилагани за контрол на основни неприятели при люцерна. *Field Crop Studies* (2018) XI(1): 17-24.

27. Николова И. 2018. Основни представители от разред Hemiptera (Sternorrhyncha), подразред Aphididae в люцерновата агроценоза (Литературен обзор). *Field Crop Studies* (2018) XI(1): 5-16.

28. Nikolova I. 2018. Important insect pest in *Medicago sativa* L. in Bulgaria. *Asian Journal of Research and Review in Agriculture* 1(1): 8-24., Article no.AJRRRA.59.

29. Nikolova I. 2018. Insect Pests in Forage Crops and Integrated Plant Protection. *Agricultural Research & Technology: Open Access Journal (ARTOAJ)*. 2018; 17(5): 556038. DOI: 10.19080/ARTOAJ.2018.17.556038. Impact Factor Thomson Reuters – 1.191

30. Nikolova I. and N. Georgieva. 2018. Effect of biological products on the population of aphids and chemical components in alfalfa. *Banat's Journal of Biotechnology*, IX(19), 43-50.

31. Nikolova I. 2018. Coleoptera etomofauna in alfalfa agrocenoses. *Bulgarian Journal of Crop Science*, 2019, 56(1): 36-46.

32. Nikolova I. 2019. Stability and adaptivity of alfalfa cultivars concerning *Apion seniculus* Kirby (Coleoptera: Curculionidae) tolerance and some biochemical traits. *Journal of Mountain Agriculture on the Balkans* 22 (1), 80-95.

33. Nikolova I. 2019. Order Thysanoptera in alfalfa agrocenosis. Literature review. *Journal of Mountain Agriculture on the Balkans*. *Journal of Mountain Agriculture on the Balkans*, 2019, 22 (6) 200-215

34. Nikolova I. 2019. Order Hemiptera, suborder Fulgoromorpha and Cicadomorpha in alfalfa agrocenosis. Literature review. *Journal of Mountain Agriculture on the Balkans* 2019, 22 (6), 216-229.

35. Nikolova I., N. Georgieva, V. Vasileva. 2020. Effect of growing legumes as pure and mixed crops on *Otiorrhynchus ligustici* L. (Coleoptera: Curculionidae) damage. *Bulgarian Journal of Agricultural Science*, 26 (4): 835-840.

ENTOMOFAUNA IN ALFALFA (*MEDICAGO SATIVA* L.) IN THE PLEVEN REGION AND METHODS FOR INSECT PEST CONTROL IN THE ORGANIC FORAGE PRODUCTION

SUMMARY

An inventory of the entomofauna in alfalfa agroecosystem for the region of Pleven has been conducted. As a result, 160 insect species have been identified, which belong to 118 genus, 46 families and six orders. Furthermore, a numerical dynamic of important harmful and beneficial species is presented.

As new species in Bulgaria for *Medicago sativa* L. are reported the following: *Catantop seniculus* Kirby and 11 species of cicadas (*Anaceratagallia laevis* Ribaut, *Streptanus* (*Streptanulus*) *josifovi* Dlabola, *Allygidius* (*Allygidius*) *commutatus* Fieber, *Psammotettix striatus* Linnaeus, *Reptalus panzeri* Low, *Tettigometra sulphurea* Mulsant & Rey, *Tettigometra* (*Mitricephalus*) *obliqua* Panzer, *Eupteryx atropunctata* Goeze, *Agalmatium bilobum* Fieber, *Zygina flammigera* Fourcroy и *Neoliturus fenestratus* Herrich-Schäffer). The findings of the faunal studies are a significant contribution to the Bulgarian entomological academic literature.

A spatial structure of distribution and sex dynamics of *Empoasca pteridis* Dhlb was established with the development of plants during the individual regrowth and the years of cultivation.

For the first time in Bulgaria, a research has been carried out on alfalfa damage caused by *Catantop seniculus* Kirby. Morphological features of the elderly individuals, numerical dynamics and a picture of damages have been established. Further, the process and regularities concerning the formation of the entomofauna in alfalfa crops and the share ratio of different orders and suborders in the individual regrowth have been established. The structure of the aphid community has been studied and as a result a potential for the biological control has been found to be significant.

For determining the damage caused by *Otiorrhynchus ligustici* L. on the root system of alfalfa, an original methodology was developed depending on the root area and the damage area. For estimation of the resistance of alfalfa varieties to *O. ligustici*, six-point scale was developed based on the number of furrows on the root system as well as a modified scale for resistance based on the index of attack.

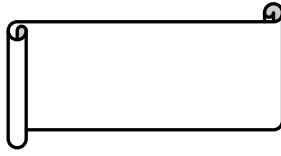
In practical plan, the results of the research give an answer to the question about the quantitative and qualitative changes in alfalfa due to plant damage caused by the larvae of *O. ligustici*. The studies are original to our country and are of interest to alfalfa production.

The dominant species of wild bees in alfalfa crops, their dynamics and factors affecting the bee pollinator population were found. The impact of alone-seeded perennial (alfalfa, birdsfoot trefoil and sainfoin) and grass-legume mixtures with cocksfoot on the degree of damage of basic soil pathogens (*Sitona* spp. and *O. ligustici*) was studied. The influence of the alfalfa cultivation system - alone and in mixed, with cereal components sowing (with *Bromus* sp., *Dactylis glomerata* L. and in combination with both components) on the damage degree from *Otiorrhynchus ligustici* L., the productivity of dry above and root biomass, and chemical composition of plants was studied.

The toxic effect of organic insecticides, as well as organic products with insecticidal action, applied alone and in combination with organic fertilizers, against basic pests in alfalfa production (*Tychius flavus* Beck, *Bruchophagus roddi* Guss, *Acyrtosiphon pisum* Harris and *Adelphocoris lineolatus* Goeze) was studied. The toxic response of organic insecticides to some predatory species (*Coccinella septempunctata* L. and *Aeolothrips intermedius* Bg) was established. The toxic effect of biological insecticides against larvae and adults of *Hypera postica* Gyllenhal under laboratory conditions was studied.

Alfalfa cultivars with good tolerance to *Catantop seniculus* Kirby, *Sitona* spp. and *Otiorrhynchus ligustici* Linnaeus was determined.

The impact of applied organic products with different effects on the forage quality, energy and protein nutritional value were determined.



A series of horizontal lines for writing, consisting of a solid top line, a dashed midline, and a solid bottom line, repeated multiple times.