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Evaluation of the effect of locally produced biological pesticide (AқKөbelek ™) on biodiversity and abundance of beneficial insects in four forage crops in the Almaty region of Kazakhstan

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***Abstract*—** *Using a non-replicated plot design, we experimentally assessed the effects of a locally produced biological pesticide on the abundance, species richness and Shannon diversity of beneficial insects in four forage crops (alfalfa, soybeans, corn, and triticale) in southeastern Kazakhstan. 2-way ANOV tests detected no effect of the biological pesticide treatment on the abundance (N) of either predators or pollinators. However, there were significant differences in pollinator and predator abundances among crops. Pairwise t-tests between the experiment and control plots for each crop detected no significant differences in predator or pollinator Shannon diversity index values (H). Paired t-tests revealed significant differences in diversity index values for both predator and pollinator functional groups among crops within each treatment (experiment, control). Corn and triticale plots had notably similar predator abundance (N), species richness (S) and Shannon diversity index (H) values. Corn, alfalfa and soy-triticale differed in pollinator Shannon H, N and S values, suggesting each contained a distinct pollinator assemblage. A trial rapid assessment for differences using a point-based system for indicator species showed only small difference among crops and between treatment and control plots. This method may be more applicable in situations sampling disturbance needs to be minimized and a rapid but less thorough assessment is required.*

Keywords*— *Bacillus thuringiensis, beneficial insects, pollinators, biodiversity, forage crops.

I. INTRODUCTION

Anthropogenic impact on the environment leads to a sharp disruption of the existing equilibrium in ecosystems of different levels, including in agricultural systems. Broadly speaking, more biologically diverse communities appear to be more stable in the face of perturbations [1]. In undisturbed communities, abiotic and biotic factors control the number and diversity of organisms. Agricultural systems, with extensive monocultures, disrupt the processes of natural regulation of abundance and diversity of species. As a result, crop systems experience periodic outbreaks of one or more crop pests. As these pest populations grow, they create opportunities for additional opportunistic pest species and pathogens to become established and further destabilize the agricultural system. The common response to pest outbreaks in Kazakhstan and neighboring countries has been to use chemical insecticides of various types. Use of chemical pesticides for pest control has many negative consequences, among the more important are including the loss of critically important but non-target beneficial species (pollinators and pest predators), dramatic declines in agricultural biodiversity and the rise of pesticide-resistant pest populations. In addition, the toxic and teratogenic products of the chemical pesticide decomposition accumulate in the soil, vegetation, and eventually in the tissues and organs of other organisms, including humans and domestic animals. One of the alternatives to the chemical method of control is the use of biological preparations based on entomo-pathogenic viruses, bacteria, fungi, protozoa and nematodes. However, since many biological preparations are polytrophic, i.e. they can affect beneficial and non-target species, it is critical to assess such effects prior to broader use of biological preparations in agricultural systems. As an example, the impacts of widely used *Bacillus thuringiensis* (Bt) based biological preparations on beneficial insects have been broadly assessed in the work of researchers from around the world [2-21].

The list of pesticides approved for use in the Republic of Kazakhstan includes biological preparations on the bacterium *Bacillus thuringiensis* (Bt) All of these preparations are rated as non-hazardous to bees (known toxicity to honey bees *Apis*) by Kazakh regulatory agencies. Recognizing the potential risk to beneficial insects, the application of these products is closely regulated (similar to Category II restrictions in the University of California IPM Bee precaution pesticide ratings [22]): application only when wind speed <5-6 m/s, a mandatory minimum 1-2 km вorder-protection zone, and restrictions of 6-12 hour periods on daytime application in the summer months.

With the increasing use of IPM pest control in Kazakhstan, including use of Bt based biological preparations, it is important to better understand their effects on the critically important pollinator and beneficial predator species. This research focused on a preliminary assessment of the effect of the locally produced Bt-based biological preparation AкKəbelek™ on the broad suite of beneficial insect species (predator and pollinator) in four forage crops commonly grown in southeast Kazakhstan.

II. MATERIALS AND METHODS

The study was conducted at the research farm LLP "Bayserke Agro" (Panfilov district, Almaty region of Kazakhstan). An organic farm research facility, the agricultural complexes support a very diverse and well-studied insect and arachnid fauna [23-35], including several insect species listed in the Red Book of the Republic of Kazakhstan and the Red Book of the Almaty region. These are: the dragonfly *Calopteryx virgo*, the mantids *Hierodula tenuidentata*, and *Bolivaria brachyptera*, the heteropterans *Zicrona caerulea* and *Coranus subapterus*, and the lady beetle *Coccinella sedakovi* (Figures 1-6).

This study was part of a larger 2015-2017 program to assess the environmental effects of a number of IPM practices in forage crop production, specifically looking at how the abundance, species composition and diversity of pest species, their predators and pollinators responded to various practices. One of the 2016 objectives of the project was to evaluate the effect of the locally produced Bt-based biological pesticide on four forage crops, soybeans, alfalfa, corn and triticale.

Two 4-hectare plots were selected in each of the four crop fields, one randomly assigned for the experimental treatment and one for the control treatment. The biological pesticide preparation used a culture of *Bacillus thuringiensis* var. *kurstaki* strain 2123-3k produced by the Kazakh Research Institute for Plant Protection and Quarantine named after Z. Zhiembaev. The experimental application used a concentration of 150 billion life-capable Bt spores/g and a flow rate 2.5 L/Ha, as per national regulatory guidelines[1]. The control solution was an equal amount of distilled water. We used SPC-25 knapsack sprayers (Figure 7-8) to apply the experimental and control treatments. We applied the treatment and control sprays every 14 days May-September 2016 for a total of 10 applications.

We collected insects and other arthropods using a methods previously described for work at the research farm LLP "Bayserke Agro" [24-36], methods developed to standardize entomological research in former Soviet states [37-40]. We used regular transect collection methods to sample foliage dwelling arthropods in treatment and control plots, including vegetation sweeps along randomly placed 1 m wide x 10 m long within-plot transects, beating 10 randomly selected 1 row-meter sections of each crop, and netting visible specimens along established 100 m transects. We collected soil-surface and subsurface arthropods manually along vegetation transects, by beating at selected collection points (ten 1 m crop row sections per plot per sampling period), and by trapping with dry Barber pitfall traps (10 traps/plot) baited with moistened dry pet food. We also collected ground nesting Hymenoptera using artificial nesting sites [28]. We used a novel variation of the traditional Barber trap [41], made from .5 L plastic bottles, for the collection of ground fauna.

Indicator species can be useful in defining distinct communities and have been used successfully to assess community change. We used a point system of relative abundances of indicator species [42, 43] as a relative measure of plot biodiversity. Previous research [44] suggested that changes in such a point system could be useful in identifying potential treatment effects. We counted the individuals of each species captured manually and/or visually noted in each 100 m transect walk, scoring these as follows: 1 point - 1-2 individuals, 2 points - up to 5 individuals, 3 points - 5 -10 individuals, 4 points - 11-20 individuals, 5 points - more than 20 individuals. We confirmed the identity of species from experts and standard references and used published life history information to identify predator and pollinator species [45-70].

Methods – Statistical analysis

The experiment was a randomized block design with only one datum for each combination of factors (crop type, treatment). With only a single treatment and control plot within each crop type, we utilized a 2-factor Analysis of Variance (ANOV) without replication [71] to test the null hypothesis that the abundance of predator or pollinator

[1] List of pesticides against Lepidoptera caterpillars from the family of Noctuidae.

species was the same in all plots. Factor A (rows) were treatments (experiment, control) and Factor B (columns) was crop type (soy, alfalfa, corn, triticale). The results of the ANOV tests allowed us to test hypotheses about each of the two factors, crop type and experimental treatment. We assumed that the effects of the experimental treatment did not vary by crop type and that there was no significant interaction between factors [71].

We used PAST [72] to calculate the Shannon diversity index (entropy, H), which takes into account the number of individuals as well as number of taxa in compared units. While the ANOV tests for differences in total abundance in all blocks, showing overall block and treatment effects, we can also test for differences in the Shannon diversity index among any pair of samples. We used a post hoc Hutchinson t-test in Excel [73] to make pairwise comparisons between plot pairs. To test for a crop effect on H we compared pairs of crop plots within each treatment (experiment, control) to each other. Comparing Shannon diversity index values within treatments across crop types allowed us to detect underlying differences in diversity index values among crops types, unrelated to treatment effects.

III. RESULTS

We sorted all of the collected specimens into predators and pollinators, based on previously cited published life history information. Any taxa not falling into one of these two groups were discarded as not relevant to the study. Specimens are archived at the Kazakh Research Institute of Plant Protection and Quarantine, Almaty, Kazakhstan.

We recorded 4795 individuals in 84 taxa that we classified as predators (Table 1a). The most species rich taxonomic groups of predators were in the Insecta: Coleoptera, with 25 species, and Hymenoptera, with 20 species. The latter included 4 species of Formicidae. The next most species rich taxon were the spiders (Aranei), with 13 species. 7 families of other insect predators, each with between 1 and 5 species, accounted for the remaining individuals.

The number of individuals (N) in predator taxa ranged from low in the soy plots (447-488) to a high of 702 in the triticale control plot (Figure 7a). Predator abundances for alfalfa, corn and triticale plots were broadly similar (range of approx. 600-700 individuals). The number of predator taxa (S) varied fairly widely among crops for both treatments (Figure 7b). The lowest number of predator species occurred in the soy plots (55, 57 taxa), and the highest number in the corn and triticale plots (63-64 taxa). Predator N values for the alfalfa plot fell between the soy and corn-triticale plots.

We also collected 3075 pollinator individuals, in 58 taxa belonging to four orders of Insecta: 15 species of Lepidoptera, 4 Coleoptera, 29 Hymenoptera (including 1 Formicidae) and 10 Diptera. (Table 1b). Species in several families (Hymenoptera, Coleoptera and Diptera) were listed as both predators and pollinators because they exhibited functional characteristics of both groups.

Patterns in the number of pollinator individuals (N) and in pollinator species (S) among crops (Figure 8a and b) were similar. In general, fewest pollinator individuals and species were reported in the corn plots, and highest reported for alfalfa, with N and S values for soy and triticale falling between these values.

Results of 2-way ANOV without replication (1-tailed, α=.05, Figure 9) indicated that the insecticide preparation (rows) had no effect on either predator abundances ($F=1.49$, $F_{critical}=10.13$) or on pollinator abundances ($F=1.26$, $F_{critical}=10.13$) across crop types. Total abundances of either predators or pollinators did not differ significantly between insecticide treatment and controls for any of the 4 crop types tested. However, we did detect significant column (crop) effects for predator abundances ($F=15.54$, $F_{critical}=9.28$) and pollinators abundances ($F=82.59$, $F_{critical}=9.28$), indicating significant differences in among-crop abundances of both predator and pollinator assemblages.

The numerical data (Tables 1 and 2, Figures 1 and 2) and the ANOV results (Figure 9) suggested crop effects (and perhaps some treatment effects as well) on N and S values of both predators and pollinators. In a without replication design, parametric tests for differences in N values were not possible, so we tested for differences in diversity index values between plots. The Shannon diversity index (H) takes account the number of individuals (N) as well as number of taxa (S) in compared units. We calculated Shannon H values for all crop blocks (Table 2). We then made three sets of pairwise comparisons (2-tailed Hutchinson's t-test, $p<.05$). The first, between experiment and control for each crop, tested for significant experiment effects on H diversity index values. The second and third, between all crop pairs within the Bt experiment and within the control, tested for crop effects on predator and pollinator H values.

Results of these tests are in Table 3, and presented visually in Figure 9. For predators: we detected no significant differences in predator H index values (* = significance) in biological pesticide experiment to control comparisons in any of the four crops tested. Three of four comparisons of pollinator H values detected no significant differences. We detect one significant difference in pollinator H index values, in the triticale plots, but not in soy, alfalfa or corn comparisons. While the t-test test resulted in a significant difference in pollinator H index values in the triticale plots, we do not believe this is a significant result. Examination of the triticale pollinator H index values (Figure 10, Table 2) show very similar experiment and control H values. Based on the closeness of the triticale pollinator H index values and on the large estimated variance in H for these

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plots, we concluded that the detected difference was in error, an artifact of the high variance. This suggests predator and pollinator diversity, as estimated by the H index value, was not affected by the biological pesticide treatment in any crop type.

Within-treatment (experiment, control) comparisons between crops indicated predator H values were generally not significantly different. We detected significant differences in experimental plots between soy and the other three crop plots. Comparisons within control plots showed that predator diversity H values differed significantly between soy and both corn and triticale plots. Predator H values were not different for the soy-alfalfa comparison. Triticale predator H values, for both experiment and control, were significantly greater than predator H values in any of the other crop types.

For pollinators: biological pesticide treatment had no effect on pollinator assemblages in any of the tested crops. Overall pollinator N, S and H index values followed similar patterns across the tested crops: low values for corn, high values for alfalfa and lower values for soy and triticale that were very similar. Pollinator diversity index values did not differ significantly in soy, alfalfa or corn plots in experiment to control comparisons (Table 3). Significant differences in pollinator H values were detected between triticale experiment and control plots. While the test results indicate a treatment effect on pollinator diversity, closer inspection of triticale experiment and control results for pollinator N, S (Table 2, Figure 8) and Shannon H index (Table 2, Figure 10) showed little differences in these values, less than other pair-wise comparisons. We concluded the result was a product of high variances, and treated this result as an artifact.

Tests for crop effects (pair-wise comparisons within treatments) on pollinator diversity index values found significant differences in all but one of the between-crop comparisons (Table 3). The absence of significant differences in pollinator H values for soy and triticale, in both experiment and control plots, suggests that these two crops contained pollinator assemblages of similar diversity. All other pair-wise comparisons showed significant differences in pollinator diversity index values, suggesting that corn and alfalfa contain pollinator assemblages of differing diversity, different from each other and from the soy-triticale pollinator assemblage.

Point-based indicator species data are summarized in Table 4. Indicator species point scores differ by crop type, but not by much. Similarly, differences in scores between the experimental Bt treatment and the control were very small (soya - 230 and 232 points, alfalfa - 320 and 318 points, maize - 246 and 252 points, triticale - 282 and 283 points on the test and control areas respectively).

Most Lepidoptera and Diptera scored highest in the legume plots (soybeans and alfalfa) compared to cereals (corn and triticale). Hymenoptera, combining pollinators and predators, scored slightly lower in the cereals, but did not show clear preferences for crop types. This was particularly true for Hymenoptera known to prefer artificial nest sites. Some predatory beetles, especially moisture loving species of ground beetles, and species found primarily on plant stems and leaves, scored highest in corn plots. Stands of corn provide the most favorable moisture and shade conditions for these species among the four crop types. Some spiders, such as *Argiope bruennichi*, by contrast, scored higher in soybean crops, where favorable light conditions, structure for web construction and higher pollinator insect abundances exist. A related species *Argiope lobata*, a xerophile common in dry in steppes and semi-deserts, scored high in the relatively arid triticale plots, where it had more optimal conditions for existence.

IV. DISCUSSION

Several families (Hymenoptera, Coleoptera and Diptera) exhibited functional characteristics of both predators and pollinators and were included in both groups. Larvae of the syrphid flies (Diptera) are predators, but the adults are recognized pollinators [74]. Many adult forms of Hymenoptera are both predators and pollinators. Large hunting wasps (Sphecidae, Vespidae) prey on various arthropods to feed themselves or to provision their nests. Other Hymenoptera are parasitoids (e.g. Ichneumonidae, Braconidae, Scoliidae some Sphecidae), with adults serving as pollen vectors but with predatory or parasitic larvae. Larvae of the soldier beetles (Cantharidae) are predators, but the adults are pollinators that primarily feed on nectar, pollen and honeydew [75]. Of the ant species we collected (Formicidae), four were identified as predators [76, 77]. Only one ant species, *Lasius niger*, classified as a predator, was also listed as a pollen vector [78].

Evidence for biological pesticide treatment effects on predator abundance (N) or S was inconsistent or not evident (no significant ANOV result). While predator abundance (N) in the experiment plots was lower than in the controls for alfalfa, corn and triticale, the opposite occurred in the soy plots. Predator S values were lower in the soy experimental plot than the control, but the opposite in the alfalfa plots, while predator S values in the corn and triticale plots remained nearly identical. There did not appear to a treatment effect on pollinators, with pollinator N and S values nearly identical in experiment and control plots for all four crops, but a potential crop effect was suggested by the wide differences in N and S values among crops, for both treatment and control plots.

Defined by differences in Shannon diversity, N and S, there appear to be three predator assemblages in the test plots (experiment and control): a similarly diverse predator assemblage in the alfalfa and corn crops, with a less diverse predator assemblage in the soy plots and higher diversity predator assemblage in the triticale plots. Predator N, S and H values in all corn and triticale plots are notably similar. Corn and triticale can have significant pest populations. Research in Eastern Europe showed 24 species of insects from three orders, Hemiptera, Coleoptera and Diptera, were commonly found as pests on triticale [79]. There are 9 principal pets of corn in Eurasia, 4 Lepidoptera, 2 Coleoptera, 2 Heteroptera and 1 Diptera [80] and multiple minor pests. These prey populations may successfully support diverse and numerous predator populations.

Corn, alfalfa and soy-triticale (Figure 16) each contained apparently distinct pollinator assemblages, defined by their differences in Shannon diversity index values (species composition and relative abundance). The corn pollinator assemblage was notably different, with much lower species number (N), abundances (S) and Shannon diversity (H) index values, than any of the other crop types. This may be a reflection of the wind pollinated nature of corn and the lack of flowers that would attract pollinators.

The points based system using indicator species was of limited use in detecting effects on diversity. As a proxy measure of diversity it showed only small differences (a weak trend) in point scores among crops. It also showed small differences in point scores between experiment and control, which indicated that the biological pesticide treatment had no effect on diversity. However, it was very difficult to determine how much of a difference in point scores should be considered a significant change. In general, this approach may be of greater use as a rapid assessment tool than for experimental studies, which demand more detailed numerical information.

In previous research, we used point score system to evaluate other ecosystems, including protected national parks in Kazakhstan, where the biodiversity assessment was constrained by the need for a rapid methodology and for a method that did the least damage to the environment during the survey. The technique has been used in reserves in the Russian Federation [42]. The use of the points based system was a first trial of its usefulness in an agricultural setting. Broader adoption of this approach must consider the trade-off between the benefits of speed of assessment and minimal damage to the biota and environment against cost of lost information, due to the under-sampling of rarer and less numerous species that are important contributors to overall biodiversity.

Biological preparations based on entomo-pathogenic viruses, bacteria, fungi, protozoa and nematodes are an attractive alternative to chemical pesticides for pest control in agriculture. However, because these preparations do have effects on certain species of arthropods, preliminary assessments of their overall effect are needed. For example, preparations based on the bacterium *Bacillus thuringiensis* are widely used worldwide, and their impact on the non-target fauna of agricultural ecosystems have been evaluated [2-21]. The list of pesticides approved for use in the Republic of Kazakhstan [22] includes 7 biological products based on the bacterium *Bacillus thuringiensis* including locally developed AқKөbelek™. As a local preparation, it holds significant potential for widespread adoption in agriculture in Kazakhstan because it will be a widely available at low cost, providing a viable alternative to both imported Bt preparations and to current chemical pesticides. However, it has not been evaluated previously for its effect on pollinators and predators.

Based on this preliminary assessment, we found AқKөbelek™ to not have significant effects on resident pollinator or predator populations in forage crops and can thus can provide a good biological alternative to chemical control of a variety of lepidopteran pests of forage crops. These results support the use of this Bt preparation for use in both agrarian and forestry applications. We believe AқKөbelek™ can be used in combination with artificial nest sites (used to increase populations of a suite of important solitary bee pollinators) to increase crop yields throughout Kazakhstan.

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Table.1a: Predator taxa and number of individuals recorded from each plot.

Taxon	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticlae Control	
Aranei									
Agelena orientalis	2	2	2	2	0	0	0	0	general predator
Araniella cucurbitina	4	5	10	11	10	11	21	23	general predator
Araneus diadematus	0	0	0	0	0	3	6	7	general predator
Aculepeira armida	0	0	4	4	9	12	14	15	general predator
Heliophanus potanini	0	0	0	0	0	11	7	9	general predator
Argiope bruennichi	11	5	1	2	1	0	0	5	general predator
Argiope lobata	1	3	0	0	0	0	0	1	general predator
Pardosa agrestis	8	10	11	10	4	4	15	16	general predator
Pardosa paludicola	2	2	4	2	12	13	8	10	general predator
Pisaura mirabilis	9	10	20	22	4	4	23	25	general predator
Steatoda paykulliana	0	1	0	0	0	0	3	4	general predator
Thomisus albus	10	11	11	14	3	2	4	11	general predator
Thomisus onustus	3	7	7	8	2	3	3	5	general predator
Xysticus striatipes	12	0	20	21	6	7	11	10	general predator
Insecta: Odonata									
Anax parthenope	1	1	2	2	2	2	5	4	
Calopteryx virgo	0	0	0	0	4	2	0	0	aerial predator
Sympetrum vulgatum	14	13	21	23	7	8	14	16	aerial predator
Platycnemis pennipes	9	10	12	10	20	21	22	23	aerial predator
Enallagma cyathigerum	12	12	6	7	15	17	15	17	aerial predator
Insecta: Mantodea									
Hyerodula tenuidentata	0	0	0	0	3	4	0	0	general predator
Iris polystictica	4	5	2	2	2		4	4	general predator
Mantis religiosa	1	2	2	2	1	1	2	2	general predator
Insecta: Orthoptera									
Decticus verrucivorus	0	0	0	0	0	0	9	10	opportunistic predator
Platycleis intermedia	3	5	10	11	4	5	13	12	opportunistic predator
Tettigonia viridissima	14	16	21	20	8	4	18	21	general predator
Tettigonia caudata	4	11	4	5	2	2	9	11	general predator
Insecta: Dermaptera									
Anechura bipunctata	0	0	9	11	21	16	4	17	opportunistic predator
Labidura riparia	7	2	0	0	13	16	4	4	general predator
Insecta: Heteroptera									
Coranus subapterus	4	6	3	4	2	3	3	4	general predator
Nabis ferus	11	5	10	12	10	22	14	15	general predator
Orius minutus	7	5	4	4	22	20	8	10	general predator

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Rhynocoris annulatus	1	2	2	2	1	1	2	2		general predator
Insecta: Coleoptera										
Anchomenus dorsalis	10	15	14	15	23	25	17	25		general predator
Brachinus crepitans	12	10	4	5	11	13	4	6		general predator
Calathus halensis	19	21	22	20	24	20	17			general predator
Callistus lunatus	1	2	0	0	0	0	0	0		general predator
Calosoma denticolle	2	2	2	2	0	0	0	0		general predator
Calosoma auropunctatum	0	0	1		1	1	1	0		general predator
Carabus cumanus	0	0	1		0	0	0	0		general predator
Carabus cicatricosus	0	0	0	4	1	1	0	0		general predator
Carabus nemoralis	0	0	1		0	0	0	0		general predator
Chlaenius spoliatus	0	0	0	0	2	2	0	0		general predator
Elaphrus cupreus	0	0	0	0	23	27	0	0		general predator
Lebia cruxminor	2	2	4	2	2	2	2			general predator
Lebia chlorocephala	1	2	6	4	0	0	0	0		general predator
Nebria aenea splendida	25	27	10	14	21	19	10	12		general predator
Scarites terricola	0	0	0	0	2	2	2	2		general predator
Paederus riparius	15	17	2	2	25	28	4	4		general predator
Pachylister inaequalis	0	0	0	0	1	0	0	0	0	general predator
Cantharis fusca	7	2	5	4	2	4	7	9		larva general predator
Adalia bipunctata	17	2	19	22	22	25	24	26		specialized predator
Coccinella sedakovi	2	2	2	2	7	9	3	2		specialized predator
Coccinella septempunctata	24	26	24	25	27	28	25	25		specialized predator
Coccinula quatuordecimpustulata	14		12	5	18	21	27	21		specialized predator
Harmonia axyridis	0	2	0	0	0	0	0	0		specialized predator
Hippodamia variegata	21	20	25	27	23	10	18	22		specialized predator
Hippodamia tredecimpunctata	2	2	2	2	0	0	0	1		specialized predator
Propilaea quatuordecimpunctata	0	0	13	14	17	22	9	11		specialized predator
Insecta: Neuroptera										
Chrysopa carnea Chrysopidae	11	10	12	10	19	20	11	10		larva specialized predator
Insecta: Hymenoptera										
Ophion sp.	0	3		0	3	4	6	0	0	specialized predator on noctuid larva
Netelia sp.	0	0	0	0	0	2	8	7		parasitoid on Lepidoptera
Ammophila heydeni	0	3	0	0	0	0	0	0		general predator
Eremochares dives	0	11	0	0	0	0	0	0		general predator
Apanteles sp.	2	0	0	0	0	0	0	4		parasitoid on Lepidoptera
Leucospis intermedia	0	0	0	0	0	0	0	2		parasitoid on Lepidoptera
Scolia schrencki	0	0	3	9	1	1	4	4		specialized predator on soil grubs
Pemphredon inornata	0	0	9	10	21	19	21	23		larva specialized predator on aphids
Pemphredon lethifer	0	0	12	14	18	21	17	16		larva specialized predator on aphids
Sceliphron destillatorium	22	10	4	6	0	0	0	0		specialized predator on Aranei
Sceliphron deforme	0	0	2	5	0	0	0	0		
Sphex funerarius	0	2	2	2	3	4	5	6		specialized predator on Orthoptera
Paravespula germanica	8	2	3	4	7	8	4	4		specialized predator Lepidoptera larvae

Polistes dominula	5	2	13	14	21	18	21	23		general predator
Polistes gallicus	17	18	20	25	23	25	23	22		general predator
Polistes nimpha	2	0	0	0	0	0	0	0		general predator
Chrysis ignita	0	0	0	0	1	3	3	4		parasitoid on Hymenoptera larvae
Insecta: Hymenoptera: Formicidae										
Cataglyphis aenescens	2	2	23	25	3	4	23	20		general predator/scavanger
Formica pratensis	9	4	25	29	4	2	10	10		general predator/scavanger
Lasius niger	17	19	17	23	14	16	4	6		general predator/scavanger
Tetramorium caespitum	22	10	20	25	13	17	23	19		general predator/scavanger
Diptera										
Dasisyrphus sp.	7	9	22	23	2	4	9	10		larva specialized predator on aphids
Syrphus ribesii	10	12	21	23	4	4	10	9		larva specialized predator on aphids
Sphaerophoria sp.	26	25	25	27	4	6	16	19		larva specialized predator on aphids
Promachus leontochlaenus	0	0	0	0	0	0	2	4		general predator
Selidopogon diadema	0	0	0	0	0	0	1	1		general predator
total number	488	447	600	654	601	651	652	702	4795	

Table 1b. Pollinator taxa and number of individuals recorded from each plot.

Taxon	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticlae Control
Lepidoptera								
Chazara briseis	4	11	11	10	1	1	8	11
Chazara enervata	7	13	14	15	1	3	15	14
Macroglossum stellatarum	4	6	11	11	1	1	4	5
Melanargia russiae	12	14	21	23	5	6	10	12
Papilio machaon	1	1	1	2				
Colias hyale	11		22	24	3	2	9	10
Colias erate	8	10	20	21	1	1	10	14
Pieris brassicae	10	11	21	10				
Pieris rapae	19	21	23	25			14	15
Pontia daplidice	21	23	21	20	4	4	13	14
Polyommatus icarus	23						19	
Thersamonia thersamon	11	10	16	19			4	6
Nymphalis urticae	12			10			4	4
Vanessa cardui	6	7	11	10			8	
Inachis io			2	2				
Argynnis pandora			5	4				
Coleoptera								
Trichodes hauseri Cleridae	4	2	10	11	2	2	6	
Trichodes spectabilis Cleridae	6	7	3	2	0	0	0	0
Malachius aeneus Cleridae	2	5	11	10	4	4	5	4
Cantharis fusca Cantharidae	7	2	5	4	2	4	7	9

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Hymenoptera								
Ophion sp.		3			3	4	6	8
Netelia sp.						2	8	7
Apanteles sp.	2							4
Leucospis intermedia								2
Scolia schrencki			6	9	1	1	4	4
Ammophila heydeni		3						
Eremochares dives		11						
Pemphredon inornata			9	10	21	19	21	23
Pemphredon lethifer			12	14	18	21	17	16
Sceliphron destillatorium	22	10	4	6				
Sceliphron deforme			2	5				
Sphex funerarius		2	2	2	3	4	5	6
Polistes dominula	5	2	13	14	21	18	21	23
Polistes gallicus	17	18	20	25	23	25	22	22
Polistes nimpha	2							
Paravespula germanica	8	2	3	4				
Chrysis ignita					1	3	3	4
Hylaeus arenarius	3	2	10	21	4			
Andrena cineraria	1	2	4	5	5	6	4	5
Halictus quadricinctus	5	7	20	22	2	4	7	7
Anthidium cingulatum	10	11	11	13	2	3	21	23
Megachile rotundata	11	10	22	25	10	11	19	18
Osmia coerulescens	5	4	19	21	4	4	6	7
Anthophora borealis	2	5	14	14	1	1	4	4
Apis mellifera	2	3	8	9				
Bombus lucorum	2	3	11	12	1	1	3	4
Bombus laesus	1	2	7	8				
Xylocopa valga	2	5	5	7	1	1	4	4
Hymenoptera, Formicidae								
Lasius niger	17	19	17	23	14	16	4	6
Diptera								
Eristalis tenax	19	21	14	15	18	21	12	14
Dasisyrphus sp.	7	9	22	23	2	4	9	10
Syrphus ribesii	10	12	21	23	4	4	10	9
Spirophora sp.	26	25	25	27	4	6	16	16
Lucilia caesar	11	10	11	14	2	3	8	9
Calliphora vicina					6	7	4	4
Sarcophaga haemorrhoidalis			6	6	5	4	7	8
Promachus leontochlaenus							2	4
Selidopogon diadema							1	1
Stratyomis sp.							12	10
Total	358	344	546	610	200	221	396	400

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Table 2. Shannon Diversity (H) Index values, number of taxa and number of individuals of predator and pollinator groups in all plots.

Predator taxa	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticale Control
Shannon H	3.67	3.67	3.77	3.76	3.74	37.8	3.87	3.9
No. taxa S	54	56	60	58	62	62	62	63
Individuals N	488	447	600	654	601	651	652	702
Pollinator taxa	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt	Triticale Control
Shannon H	3.42	3.41	3.62	3.65	3.56	3.11	3.56	3.44
No. taxa S	41	40	45	46	35	35	43	42
Individuals N	358	344	546	610	200	221	396	400

Table 3. Results of pair-wise t-tests testing for differences in Shannon H diversity index values a) between treatment vs control pairs within crops, b) between Bt experiment plots for all crop pairs, and c) between control plots for all crop pairs. (*) indicates significant differences in Shannon-Weiner Diversity Index values (2-tailed Hutchinson's t-test, α=.05, tcritical =1.96); (–) indicates plot pairs were not tested (results not useful).

	Soy Expt.	Soy Control	Alfalfa Expt.	Alfalfa Control	Corn Expt.	Corn Control	Triticale Expt.	Triticale Control
Predator	Values of t							
Soy Expt.	x	0.01	2.20*	-	1.46	-	4.73*	-
Soy Control		x	-	1.81	-	2.25*	-	5.06*
Alfalfa Expt.			x	0.31	0.72	-	2.57*	-
Alfalfa Control				x	-	0.55	-	3.87*
Corn Expt.					x	0.95	4.06*	-
Corn Control						x	-	3.19*
Triticale Expt.							x	0.80
Triticale Control								x
Pollinator	Values of t							
Soy Expt.	x	0.11	4.54*	-	4.76*	-	0.39	-
Soy Control		x	-	5.42*	-	4.19*	-	0.46
Alfalfa Expt.			x	0.92	7.91*	-	3.57*	-
Alfalfa Control				x		8.25*	-	4.48*
Corn Expt.					x	0.68	6.83*	-
Corn Control						x	-	4.42*
Triticale Expt.							x	2.35*

Table 4 - Number of pollinator and predator species and total population points in experiment and control plots of forage crops.

Plot №	Crop type	The number of indicator species	The population in points
1	Soybean (experiment)	85	230
2	Soybean (control)	86	232
3	Alfalfa (experiment)	97	320
4	Alfalfa (control)	95	318
5	Corn (experiment)	85	246
6	Corn (control)	84	252
7	Triticale (experiment)	91	282
8	Triticale (control)	92	283

Fig.1: Dragonfly Beautiful Demoiselle Calopteryx virgo Linnaeus, 1758, male and female (Photo by I.I. Temreshev).

Fig.2: Larva of wood mantis Hierodula tenuidentata Saussure, 1869, consuming a moth at a light trap (Photo by I.I. Temreshev).

Fig.3: Short-winged Bolivaria Bolivaria brachyptera (Pallas, 1773). (Photo by I.I. Temreshev).

Fig.4: Blue Zikrona Zicrona caerulea (Linnaeus, 1758) consuming a leaf beetle larva (Photo by P.A. Esenbekova).

Fig.5: Short-winged Coranus Coranus subapterus (De Geer, 1773). (Photo by P.A. Esenbekova).

Fig.6: Tien Shan ladybird Coccinella sedakovi Mulsant, 1850 (tianschanica Dobrzh, 1927.) (Photo I.I. Temreshev).

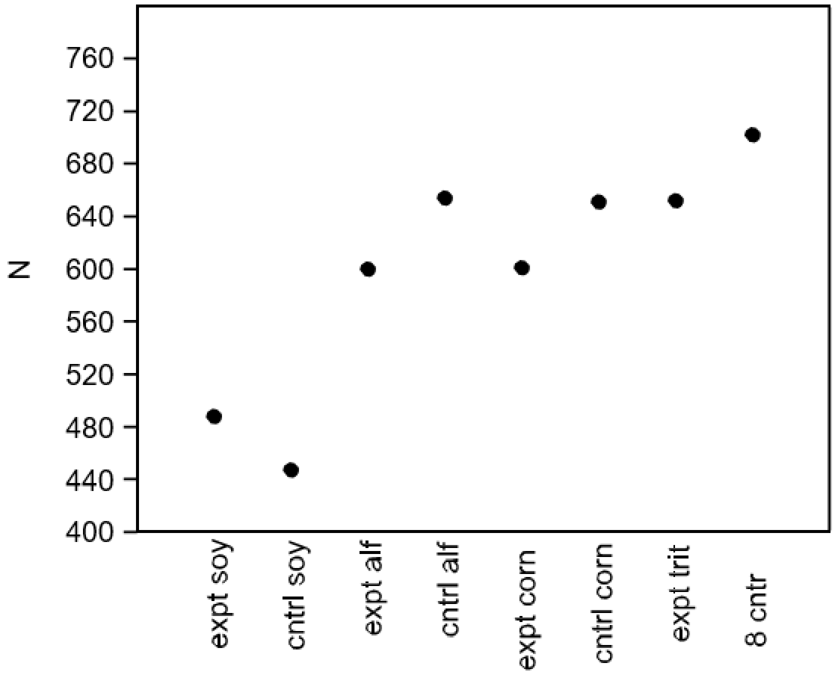

Fig.7a: Number of predator individuals (N) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

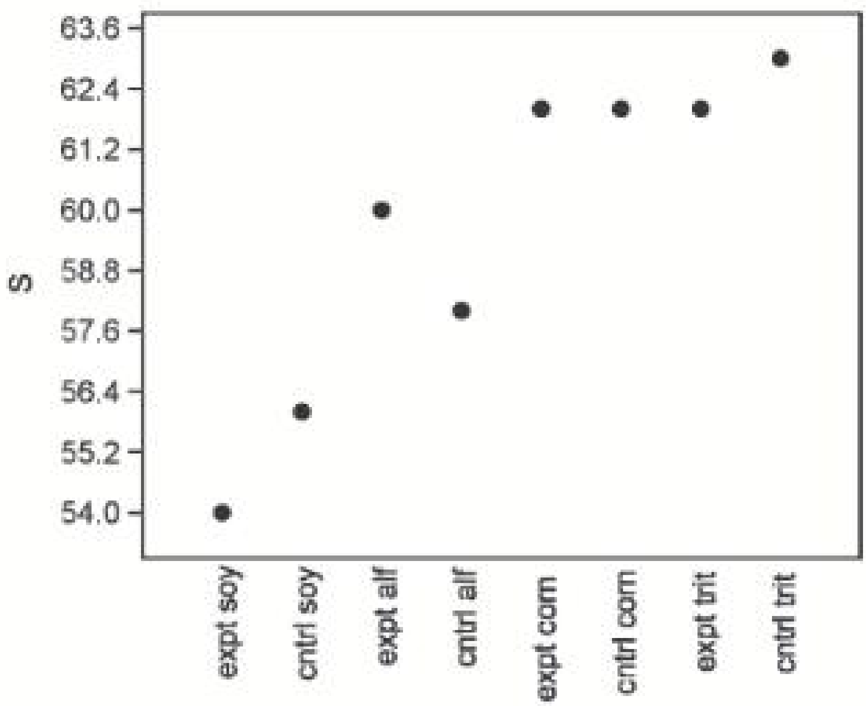

Fig.7b: Number of predator taxa (S) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

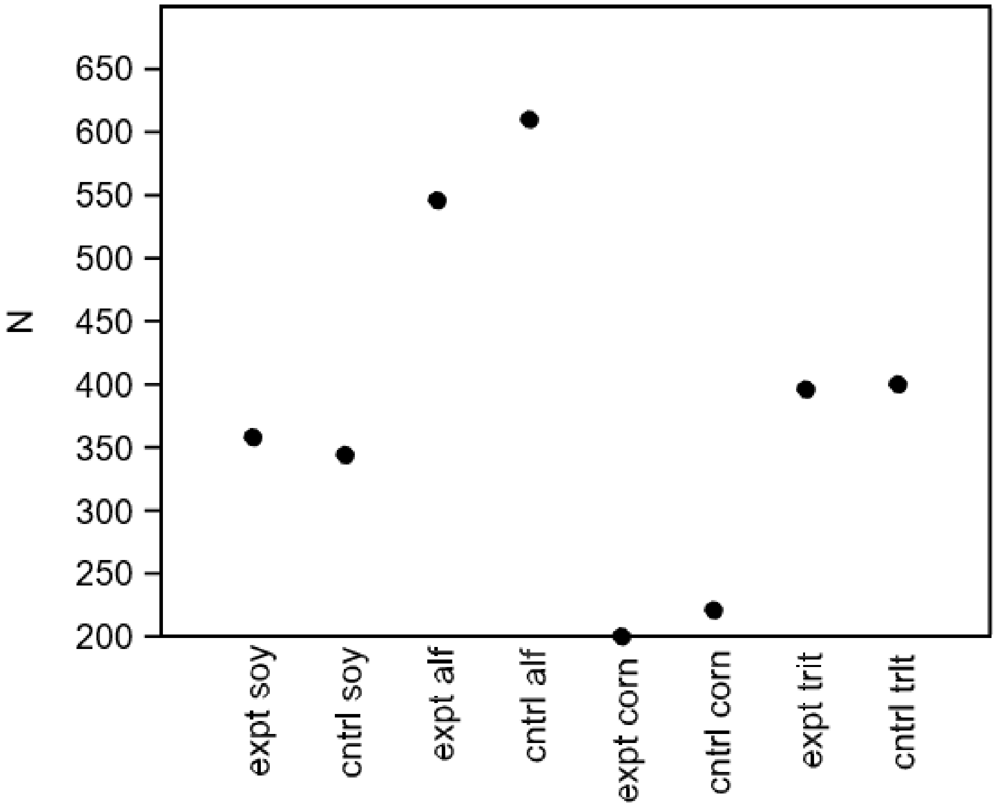

Fig.8a: Number of pollinator individuals (N) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

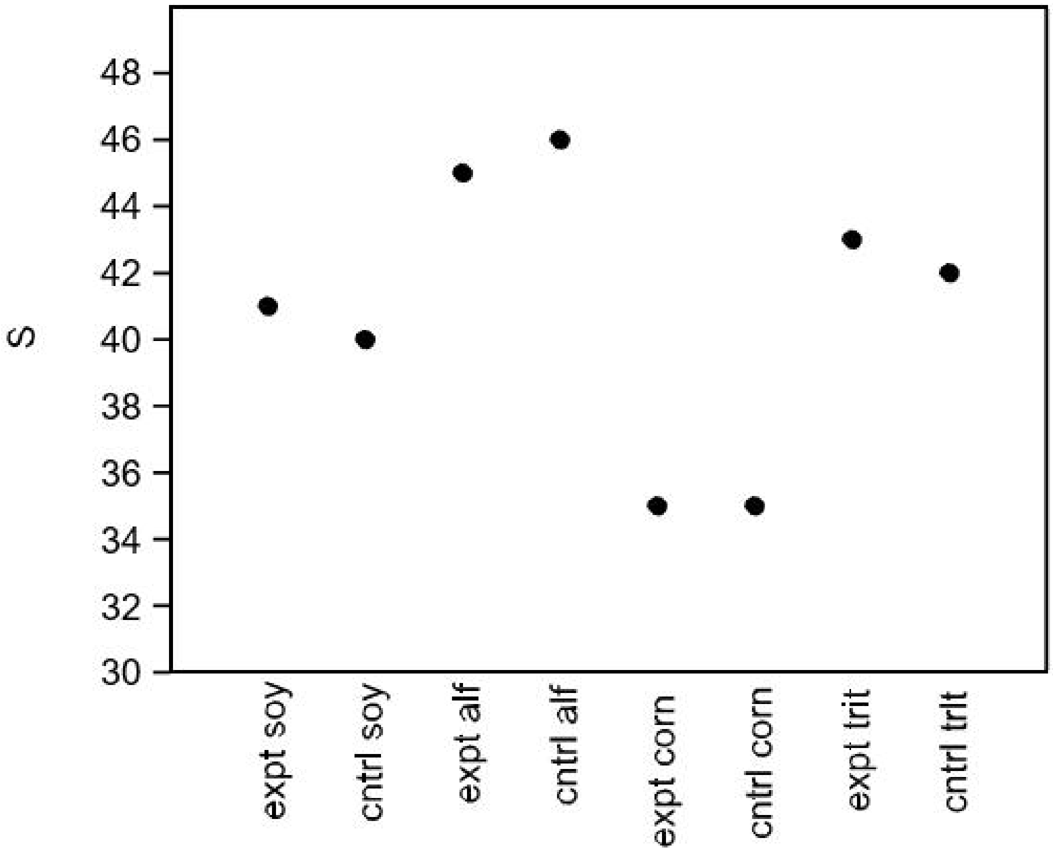

Fig.8b: Number of pollinator species (S) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

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A. Result for predator species.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
tmt	4	2341	585.25	4792.917
control	4	2454	613.5	12867
soy	2	935	467.5	840.5
alfalfa	2	1254	627	1458
corn	2	1252	626	1250
triticale	2	1354	677	1250

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	1596.125	1	1596.125	1.495257	0.308702	10.12796
Columns	49777.38	3	16592.46	15.54389	0.024766	9.276628
Error	3202.375	3	1067.458			
Total	54575.88	7				

B. Result for pollinator species.

SUMMARY	*Count*	*Sum*	*Average*	*Variance*
tmt	4	1500	375	20198.67
control	4	1575	393.75	26373.58
soy	2	702	351	98
alfalfa	2	1156	578	2048
corn	2	421	210.5	220.5
triticale	2	796	398	8

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Rows	703.125	1	703.125	1.26206	0.34305	10.12796
Columns	138045.4	3	46015.13	82.5939	0.002213	9.276628
Error	1671.375	3	557.125			
Total	140419.9	7				

Fig.9: ANOV without replication. F values with astersiks (*) indicate significant differences in abundances.

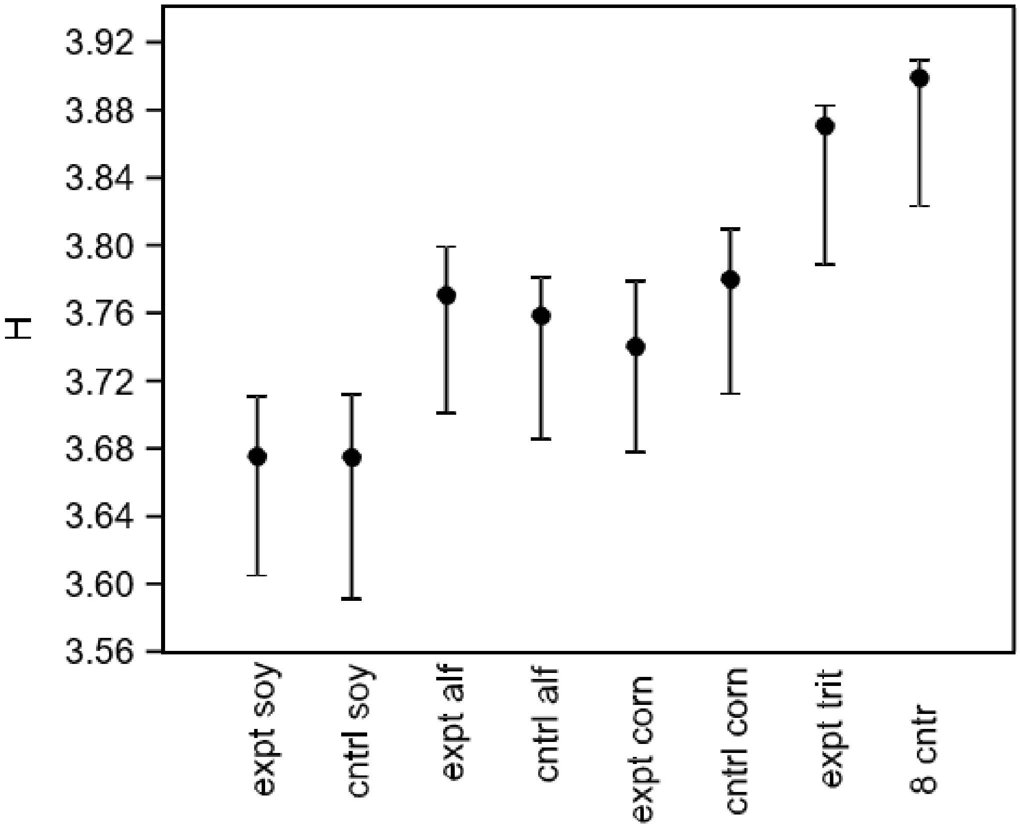

Fig.10a: Predator Shannon diversity index values (H) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.

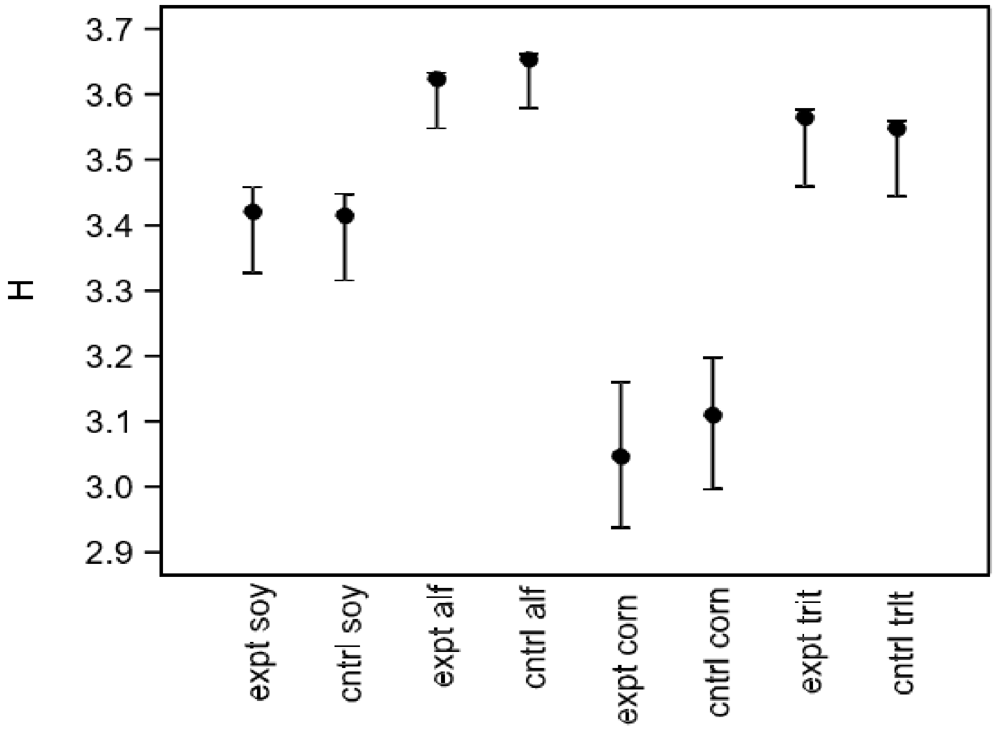

Fig.10b: Pollinator Shannon diversity index values (H) among crop plots. X axis: 1-2 Soy, 3-4 Alfalfa, 5-6 Corn, 7-8 Triticale.