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ORIGINAL ARTICLE

# The effect of thioredoxin-gene-expressed transgenic soybean on associated non-target insects and arachnids

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## Abstract

The evaluation of genetically modified (GM) crops regarding their effect on non-target organisms is necessary to safeguard ecosystem components. In this study, we assessed the effects of transgenic soybean events TRX (expressing the human thioredoxin gene under the control of the β-conglycinin promoter with tolerance to the herbicide glufosinate, PPT) on plant-dwelling non-target insects and arachnids compared with those of a non-GM parental cultivar, Gwangan soybean. Field surveys of soybean fields were carried out at Ochang and Jeonju, Korea, in 2016 and 2017. We found that the number of captured individuals was higher at Ochang in 2017 on both TRX and Gwangan soybean plants. From 2016 to 2017, the average population density of the insect pests and natural enemies decreased; however, in the case of other insects, the population density remained unchanged. The dominance index of the captured non-target insects and arachnids decreased, whereas the diversity and richness indices increased over time on the genotypes at both regions. The evenness index of non-target insects and arachnids decreased at Jeonju but showed no change at Ochang, regardless of the genotype. Hemiptera, Hymenoptera, and Diptera were the most dominant orders of the insect pests, natural enemies, and other insects, respectively. The score from PROXSCAL multidimensional scaling using combined data showed that insects and arachnids in different natural environments differed due to their cultivation regions and years, irrespective of soybean cultivars. Overall, the results indicated that the GM soybean TRX did not negatively affect the community of plant-dwelling non-target insects and arachnids.

**Keywords** Non-target arthropods · Thioredoxin gene · Transgenic soybean

## Introduction

Soybean is one of the most prevalent legume crops and is cultivated as both a food crop and soil nutrient enhancer. It is known for its content of good quality dietary protein (40%) as well as high oil content (20%) (Saha and Mandal 2019). The introduction of herbicide-tolerant transgenic soybean has greatly increased the commercial cultivation of soybean (Pratap et al. 2012). Because of the high demand for this crop, new transgenic soybean plants are being developed in South Korea (Nam et al. 2019). The cultivation of genetically modified (GM) crops has increased globally since 1996 (Grossi-de-Sa et al. 2011). James (2007) reported that ~ 21% of all biotech crops developed worldwide are transgenic soybean plants with different traits, particularly herbicide tolerance and insect resistance or a combination of both. GM cotton and corn also represent a significant portion of all GM cultivars planted worldwide (James 2009; ISAAA 2020).

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Kim et al. (2020) reported that three transgenic soybean events expressing human epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), or thioredoxin (TRX) have been developed to obtain desired proteins for use in the skin care industry. In these events, β-conglycinin promoter controls the transgenes for seed-specific expression. TRX protein has antioxidant, anti-inflammatory, and anti-allergic properties and is involved in many redox reactions (Zhou et al. 2020). However, the cultivation of transgenic crops has become a major concern regarding the potential ecological effects on the abundance and diversity of non-target organisms in the field (Torres and Ruberson 2006; Rose and Dively 2007; Dhillon and Sharma 2013).

Zukoff et al. (2019) reported a number of insect and arachnid species of various orders that are associated with non-GM soybean cultivation, including bean leaf beetles, blister beetles, grasshoppers, potato leafhoppers, wooly bear caterpillars, soybean aphids, stink bugs, thrips, and two-spotted spider mites. One of the goals of introduction of GM crops is to control target insect pests via insecticidal activity through specific modes of action. Thus, GM crops are considered to have value in integrated pest management because they can minimize the use of chemicals applied in insect pest management, which will conserve the beneficial natural enemies of soybean pests, including the diversity of generalist parasitoids and predators (Naranjo 2005).

Not only the herbivores but also some of the predatory insects, such as those in the order Heteroptera, consume plant tissues, including pollen grains and young leaves, as supplementary food when their prey become scarce (Rutledge and O'Neil 2005; Desneux et al. 2006). Lundgren et al. (2008) and Pilcher et al. (1997) stated that as these predators are likely to be orally exposed to Cry proteins expressed in *Bacillus thuringiensis* (Bt) plants, they may face the same consequence as do the insect pests. It is well known that natural enemies can play an important role in regulating populations of insect pests. Thus, understanding the potential impact of pest management tools on non-target arthropod populations in the field is an important consideration when introducing any transgenic crops (Marques et al. 2018). Before wide-spread cultivation of transgenic soybean events, their potential risks to human health, non-target invertebrates, and the whole ecosystem should be evaluated (Kim et al. 2020). Therefore, in the present study, we explored the effects of transgenic soybean—as compared with non-GM soybean—on the abundance and diversity of plant-dwelling non-target insects and arachnids.

## Materials and methods

### Experimental site and duration

Two soybean genotypes, TRX and Gwangan soybean, were used in this study. The study was conducted during late August to mid-October over 2 consecutive years, 2016 and 2017, at Ochang (Korea Research Institute of Bioscience and Biotechnology, Facility registration number RDA-2016-052) and Jeonju (National Institute of Agricultural Sciences, Facility registration number RDA-2013-041). TRX is a transgenic soybean TRX expressing the human thioredoxin gene under the control of the β-conglycinin promoter with tolerance to the herbicide glufosinate (PPT). The effects of the transgenic soybean TRX over the non-GM parental cultivar Gwangan soybean on the biodiversity of non-target insects and arachnids in agroecosystems were evaluated. The crops were grown according to the standard cultivation method of the National Institute of Agricultural Sciences. The size of each plot was 12 $m^2$ (3 m × 4 m), with corridors of 1 m between them. The layout of the experimental field was a randomized complete plot design with four replicates. Proper irrigation and fertilization were performed, and no insecticides were used. This study used the results of the arthropods investigation on Gwangan soybean conducted by Amin et al. (2020b). Also, Arthropod investigation analysis on each genetically modified soybean was conducted at the same place and period.

### Sampling strategy

Non-target insects and arachnids were collected using vacuum insect aspirators (Agricultural Backpack 2-Cycle Aspirator, M1612, John W. Hock Company, Gainesville, FL, USA) from both the TRX and Gwangan soybean cultivated fields. Sampling was performed five times every 2 weeks. This process was repeated four times for each soybean genotype with a randomized block design. The collected specimens were kept in polyethylene bags and immediately frozen using dry ice to avoid metabolism and/or release of toxins.

### Identification and categorization

The collected arthropods were brought to the Laboratory of Systematic Entomology, School of Applied Biology, Kyungpook National University, Daegu, Korea, where they were preserved in a refrigerator. The preserved specimens were placed in Petri dishes over dry ice and examined using an Olympus SZX16 stereomicroscope (Olympus Corporation, Tokyo, Japan) for taxonomic identification and categorization. Collected specimens were categorized into three functional groups, namely insect pests, natural enemies, and other insects (i.e., insects other than insect pests and natural enemies; Choi et al. 2015). All specimens were counted and identified down to the species level for the insect pests and to the family level for the natural enemies and other insects.

### Data analysis

ANOVA was employed to compare changes in the average densities of the captured three groups of insects and arachnids belong to different orders. The dominance, diversity, evenness, and richness indices were calculated following the formulas of McNaugton's dominance index, the Shannon–Weaver diversity index, Pielou index, and Margalef species richness index, respectively (Choi et al. 2015). Multidimensional scaling (MDS) was performed to analyze the similarity of the species level for the insect pests and the family level for the natural enemies and other insects. The data presented in Table 1 were standardized for analysis, and the perceptual map was prepared using Euclidean similarity using PROXSCAL. The resulting Kruskal's stress-1 value was 0.0570 and Turcker's coefficient of congruence was 0.99837; both of these results indicate high explanatory power of the model. All the analyses were conducted using IBM SPSS 25.0. Principal component analysis (PCA) was performed using SIMCA-P (version 12.0; Umetrics, Umeå, Sweden) to analyze the variation of community structure of the identified arthropods. All the data were normalized with unit variance (UV) scaling before conducting the multivariate analysis.

## Results

In total, 13,073 individuals of non-target insects and arachnids were found, representing 63 families and 15 orders from the surveyed soybean fields of Ochang and Jeonju in 2016 and 2017. After categorizing into functional groups, a total of 5391 individuals (22 families and 7 orders) were found to be insect pests, 3997 individuals (19 families and 6 orders) were found to be natural enemies, and 3685 individuals (22 families and 7 orders) were categorized as other insects (Table 1).

Altogether, 1,999 individuals were collected from the TRX soybean field at Ochang in 2016. Among these, 1,157 individuals were in the category of insect pests, 369 natural enemies, and 473 other insects. Among the orders of insect pests, Hemiptera comprised the major portion which entailed *Yemma exilis* (Berytidae) as the most abundant species (823 individuals) followed by *Riptortus pedestris* (Alydidae), with 118 individuals. Among the orders of natural enemies, Hymenoptera comprised the major portion, with 230 and 109 individuals from the families Eulophidae and Braconidae, respectively. Chloropidae (Diptera), with 367 individuals, represented the largest taxon in the other insects group, followed by Cicadellidae (Homoptera) with 56 individuals. The total number of individuals collected from Gwangan soybean at Ochang in 2016 was 1978, of which 1140 were insect pests, 308 were natural enemies, and 530 were other insects. Among the orders of insect pests, similar to the survey of TRX soybean, Hemiptera comprised the major portion, dominated by *Y. exilis* (Berytidae) and *R. pedestris* (Alydidae), with 778 and 127 individuals, respectively. Hymenopteran insects were the largest portion of natural enemies, particularly Eulophidae (171 individuals) and Braconidae (108 individuals). Among the other insects, Chloropidae (Diptera), with 418 individuals, was the most dominant group, followed by Cicadellidae (Homoptera) with 70 individuals (Table 1).

A total of 3215 individuals were collected from the TRX soybean field at Ochang in 2017, among which were 1045 insect pests, 1362 natural enemies, and 808 other insects. Among the captured insect pests, *Y. exilis* (Hemiptera: Berytidae) was the most abundant species, with 601 individuals, followed by *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae) with 274 individuals. Hymenoptera comprised the major portion of natural enemies, primarily with specimens of Eulophidae (1081 individuals), followed by Braconidae (187 individuals). Diptera comprised the largest portion of other insects, particularly Chloropidae and Dolichopodidae (288 and 187 individuals, respectively). The number of individuals collected from Gwangan soybean at Ochang in 2017 was 3218, among which were 1161 insect pests, 1327 natural enemies, and 730 other insects. Among the orders of insect pests, Hemiptera comprised the major portion, with 655 individuals of *Y. exilis* (Berytidae), followed by Homoptera, with 354 individuals of *T. vaporariorum* (Aleyrodidae). Hymenoptera comprised the major portion of natural enemies, with 1058 individuals in the family Eulophidae and 188 in Braconidae. Diptera comprised the largest portion of other insects, with 294 individuals in the family Chloropidae and 158 in Dolichopodidae (Table 1).

A total of 246 individuals were collected from the TRX soybean field at Jeonju in 2016, 71 of which were insect pests, 83 were natural enemies, and 92 were other insects. Hemiptera comprised the major portion of the captured insect pests, with 23 individuals of *Y. exilis* (Berytidae), followed by Coleoptera with 10 individuals of *Medythianigro bilineata* (Chrysomelidae). Hymenoptera comprised the major portion of natural enemies, with 43 individuals of the family Eulophidae. Among the other insects, Chloropidae (Diptera) comprised the major portion with 56 individuals, followed by Cicadellidae (Homoptera) with 27 individuals. The number of individuals collected from Gwangan soybean at Jeonju in 2016 was 202, among which were 69 insect pests, 77 natural enemies, and 56 other insects. Among the

**Table 1** Total number of common plant-dwelling non-target insects and arachnids captured using vacuum suction at fields planted with two different genotypes of soybeans at Ochang and Jeonju, Korea, in 2016 and 2017

| Category | Order | Family | Species | 2016 | | | | 2017 | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ochang | | Jeonju | | Ochang | | Jeonju | | |
| | | | | TRX[a] | GW[b] | TRX | GW | TRX | GW | TRX | GW | |
| Insect pests | Orthoptera | Tettigoniidae | *Phaneroptera falcata* | 1 | 1 | – | – | – | – | – | – | 2 |
| | | Pyrgomorphidae | *Atractomorpha lata* | – | – | – | – | 1 | – | 1 | – | 2 |
| | | Acrididae | *Acrida cinerea* | – | 1 | – | – | – | – | – | – | 1 |
| | Thysanoptera | Thripidae | *Frankliniella occidentalisi* | – | – | – | – | 44 | 34 | 93 | 94 | 265 |
| | | | *Frankliniella intonsa* | – | – | – | – | 11 | 9 | 28 | 39 | 87 |
| | Hemiptera | Miridae | *Adelphocoris suturalis* | 10 | 6 | 3 | 2 | 2 | 2 | 4 | 3 | 32 |
| | | | *Apolygus lucorum* | 1 | – | 1 | – | 1 | 1 | – | – | 4 |
| | | Berytidae | *Yemma exilis* | 823 | 778 | 23 | 35 | 601 | 655 | 53 | 52 | 3020 |
| | | Lygaeidae | *Panaorus albomaculatus* | – | – | – | – | 21 | 20 | – | – | 41 |
| | | | *Nysius plebejus* | 18 | 12 | 8 | 3 | 3 | 2 | 7 | 6 | 59 |
| | | Coreidae | *Cletus punctiger* | 1 | – | – | – | – | – | – | – | 1 |
| | | | *Coreus marginatus* | – | – | 1 | – | – | – | – | – | 1 |
| | | Alydidae | *Riptortus pedestris* | 118 | 127 | 9 | 12 | 11 | 13 | 5 | 2 | 297 |
| | | Rhopalidae | *Rhopalus maculatus* | 4 | 5 | – | – | – | – | 3 | – | 12 |
| | | Pentatomidae | *Homalogonia obtusa* | 1 | – | – | – | – | – | – | – | 1 |
| | | | *Nezara antennata* | 3 | 1 | – | – | – | – | – | – | 4 |
| | | | *Piezodorus hybneri* | – | 1 | – | 2 | – | – | 1 | – | 4 |
| | | | *Dolycoris baccarum* | 1 | 4 | – | 1 | 1 | 2 | 1 | – | 10 |
| | Homoptera | Cicadellidae | *Cicadellaviridis* | – | – | – | 1 | 1 | – | 1 | – | 3 |
| | | | *Empoascarybiogon* | – | – | – | – | 43 | 29 | 53 | 43 | 168 |
| | | Ricaniidae | *Ricania shantungensis* | 2 | 5 | – | – | 1 | – | – | – | 8 |
| | | | *Ricaniataeniata* | – | – | – | – | – | 1 | – | – | 1 |
| | | Flatidae | *Geishadistinctissima* | 43 | 41 | – | – | – | – | – | – | 84 |
| | | Aleyrodidae | *Trialeurodes vaporariorum* | – | – | – | – | 274 | 354 | 97 | 124 | 849 |
| | Coleoptera | Chrysomelidae | *Pagriasignata* | 7 | 3 | 2 | 1 | – | – | – | 2 | 15 |
| | | | *Medythianigrobilineata* | 76 | 110 | 10 | 2 | 6 | 12 | 7 | 13 | 236 |
| | | | *Monoleptaquadriguttata* | 3 | 1 | 1 | – | – | – | – | – | 5 |
| | | Apionidae | *Pseudopiezotracheluscollare* | 1 | – | – | – | 2 | 1 | – | – | 4 |
| | | Curculionidae | *Callosobruchus chinensis* | – | – | – | – | 1 | 2 | – | – | 3 |
| | Diptera | Platystomatidae | *Rivellia flaviventris* | 2 | 2 | 1 | 1 | 2 | – | – | – | 8 |
| | | | *Rivellia alini* | – | – | – | – | – | 1 | – | – | 1 |
| | | | *Rivellia apicalis* | 2 | 5 | – | – | 2 | – | 2 | – | 11 |

**Table 1** (continued)

| Category | Order | Family | Species | 2016 | | | | 2017 | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ochang | | Jeonju | | Ochang | | Jeonju | | |
| | | | | TRX[a] | GW[b] | TRX | GW | TRX | GW | TRX | GW | |
| | | Agromyzidae | *Liriomyzacongesta* | 13 | 12 | 5 | 1 | 3 | 7 | – | 2 | 43 |
| | | | *Liriomyza trifolii* | – | – | – | – | 2 | – | – | – | 2 |
| | Lepidoptera | Crambidae | *Omiodesindicata* | 7 | 8 | 3 | 4 | – | – | – | – | 22 |
| | | | spp. | 20 | 15 | 3 | 4 | 12 | 16 | 5 | 7 | 82 |
| | | Nolidae | *Celamataeniata* | – | 2 | 1 | – | – | – | – | – | 3 |
| | Subtotal | | | 1157 | 1140 | 71 | 69 | 1045 | 1161 | 361 | 387 | 5391 |
| Natural enemies | Odonata | Coenagrionidae | | 1 | – | – | – | – | – | – | – | 1 |
| | Hemiptera | Nabidae | | – | 6 | – | – | – | – | – | – | 6 |
| | | Anthocoridae | | – | – | – | – | 17 | 10 | 4 | 1 | 32 |
| | Neuroptera | Chrysopidae | | 8 | 7 | 14 | 8 | 4 | 1 | – | – | 42 |
| | Coleoptera | Coccinellidae | | 1 | – | – | – | 1 | 1 | 1 | 1 | 5 |
| | | Staphylinidae | | – | – | – | – | – | 1 | – | – | 1 |
| | Hymenoptera | Braconidae | | 109 | 108 | 14 | 12 | 187 | 188 | 15 | 16 | 649 |
| | | Ichneumonidae | | 2 | 3 | – | 1 | 4 | – | – | 1 | 11 |
| | | Chalcididae | | – | – | – | – | – | 1 | – | – | 1 |
| | | Scelionidae | | – | – | – | – | 30 | 19 | 12 | 20 | 81 |
| | | Eulophidae | | 230 | 171 | 43 | 34 | 1081 | 1058 | 168 | 171 | 2956 |
| | Araneae | Tetragnathidae | | 11 | 8 | 10 | 13 | 12 | 20 | 8 | 12 | 94 |
| | | Thomisoidea | | 1 | 2 | – | 2 | 8 | 11 | 4 | 2 | 30 |
| | | Theridiidae | | 1 | – | 1 | 3 | 10 | 9 | 11 | 13 | 48 |
| | | Salticidae | | 2 | – | – | 1 | 3 | 4 | 2 | 1 | 13 |
| | | Clubionidae | | 1 | – | – | – | – | – | – | – | 1 |
| | | Araneidae | | – | 1 | 1 | 3 | 1 | 2 | – | 2 | 10 |
| | | Linyphiidae | | – | 2 | – | – | 4 | 2 | 3 | 3 | 14 |
| | | Oxyopidae | | 2 | – | – | – | – | – | – | – | 2 |
| | Subtotal | | | 369 | 308 | 83 | 77 | 1362 | 1327 | 228 | 243 | 3997 |
| Other insects | Psocoptera | Psocidae | | – | – | – | – | 56 | 35 | 1 | 2 | 94 |
| | Hemiptera | Miridae | | 1 | – | 1 | – | 5 | 2 | – | 1 | 10 |
| | | Tingidae | | 3 | 1 | – | 1 | 7 | 3 | – | – | 15 |
| | Homoptera | Membracidae | | – | – | – | – | 23 | 14 | – | – | 37 |
| | | Delphacidae | | – | – | – | – | 55 | 66 | 7 | 4 | 132 |
| | | Cicadellidae | | 56 | 70 | 27 | 16 | 23 | 28 | 176 | 171 | 567 |
| | Neuroptera | Hemerobiidae | | 1 | – | – | – | – | – | 1 | – | 2 |

**Table 1** (continued)

| Category | Order | Family | Species | 2016 | | | | 2017 | | | | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Ochang | | Jeonju | | Ochang | | Jeonju | | |
| | | | | TRX[a] | GW[b] | TRX | GW | TRX | GW | TRX | GW | |
| | Coleoptera | Elateridae | | – | – | – | – | – | – | 2 | – | 2 |
| | | Latridiidae | | – | – | – | – | 17 | 19 | 6 | 6 | 48 |
| | | Phylliidae | | – | – | – | – | 2 | 4 | 79 | 97 | 182 |
| | Diptera | Chironomidae | | – | – | – | – | – | – | 43 | 47 | 90 |
| | | Stratiomyidae | | 3 | 1 | – | – | 7 | 8 | – | – | 19 |
| | | Empididae | | – | – | – | – | 10 | 8 | 4 | 5 | 27 |
| | | Dolichopodidae | | 25 | 34 | 5 | – | 187 | 158 | 38 | 34 | 481 |
| | | Syrphidae | | 5 | – | – | – | 1 | 2 | – | – | 8 |
| | | Sciomyzidae | | 2 | – | 1 | 1 | 1 | 2 | – | – | 7 |
| | | Lauxaniidae | | 9 | 5 | – | 1 | 14 | 15 | 1 | 5 | 50 |
| | | Chloropidae | | 367 | 418 | 56 | 37 | 288 | 294 | 78 | 105 | 1643 |
| | | Ephydridae | | – | – | – | – | 101 | 62 | 57 | 26 | 246 |
| | | Anthomyiidae | | – | – | 1 | – | – | – | – | – | 1 |
| | | Muscidae | | 1 | 1 | – | – | 11 | 10 | – | – | 23 |
| | Hymenoptera | Vespidae | | – | – | 1 | – | – | – | – | – | 1 |
| | Subtotal | | | 473 | 530 | 92 | 56 | 808 | 730 | 493 | 503 | 3685 |
| | Total | | | 1999 | 1978 | 246 | 202 | 3215 | 3218 | 1082 | 1133 | 13,073 |

[a]Genetically modified soybean

[b]Non-genetically modified soybean; Gwangan soybean data were used the data from a paper published in 2020 (Amin et al. 2020b)

orders of insect pests, Hemiptera comprised the major portion, with 35 individuals of *Y. exilis* (Berytidae) and 12 individuals of *R. pedestris* (Alydidae). Hymenoptera comprised the major portion of natural enemies, with 34 individuals of the family Eulophidae, and Araneae was the second most prevalent taxon, with 13 individuals of the family Tetragnathidae. Chloropidae (Diptera), with 37 individuals, was the largest portion of the other insects, followed by Cicadellidae (Homoptera) with 16 individuals (Table 1).

A total of 1082 individuals were collected from TRX soybean field at Jeonju in 2017, among which were 361 insect pests, 228 natural enemies, and 493 other insects. Among the orders of insect pests, Hemiptera comprised the major portion, with 97 individuals of *T. vaporariorum* (Aleyrodidae) and 93 individuals of *Frankliniella occidentalisi* (Thysanoptera: Thripidae). Among the captured natural enemies, Hymenoptera comprised the major portion, with the major family being Eulophidae (168 individuals), followed by Braconidae (15 individuals). Cicadellidae (Diptera), with 176 individuals, represented the dominant taxon in the other insects group, followed by Phylliidae (Coleoptera) with 79 individuals. The number of individuals collected from Gwangan soybean at Jeonju in 2017 was 1,133, among which were 387 insect pests, 243 natural enemies, and 503 other insects. The greatest number of insect pests was in the order Homoptera, with 124 individuals of *T. vaporariorum* (Aleyrodidae) and 94 individuals of *F. occidentalisi* (Thysanoptera: Thripidae). Hymenoptera comprised the major portion of natural enemies, with Eulophidae (171 individuals) and Braconidae (16 individuals). Among other insects, Cicadellidae (Diptera) comprised the major portion with 171 individuals, followed by Chloropidae (Homoptera) with 105 individuals (Table 1).

The average population densities of the non-target insect pests of Hemiptera significantly decreased from 2016 to 2017 at both Ochang and Jeonju, irrespective of the genotype. The average population densities of the insect pests of the orders Orthoptera and Lepidoptera remained unchanged (no statistically significant difference) from 2016 to 2017 on both soybean genotypes at Ochang as well as Jeonju (Table 2). The densities of the insect pests of Thysanoptera and Homoptera increased on both the genotypes from 2016 to 2017 at Jeonju but showed no change at Ochang. On the other hand, the densities of the insect pests of Coleoptera increased on both the genotypes from 2016 to 2017 at Ochang but showed no change at Jeonju (Table 2).

The average population densities of the natural enemies of the taxa Odonata and Coleoptera statistically remained unchanged from 2016 to 2017 on both the soybean genotypes at Ochang and Jeonju (Table 3). The population densities of natural enemies in the order Hemiptera slightly decreased both at Ochang and Jeonju from 2016 to 2017 on both the genotypes. The population of Neuroptera

**Table 2** Average population densities of non-target insect pests in common plant-dwelling non-target insect groups and arachnid captured using vacuum suction at fields planted with two different genotypes of soybeans at Ochang and Jeonju, Korea in 2016 and 2017

| Order | 2016 | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|
| | Ochang | | Jeonju | | Ochang | | Jeonju | |
| | TRX[a] | Gwangan[b] | TRX | Gwangan | TRX | Gwangan | TRX | Gwangan |
| Orthoptera | 0.3 ± 0.5a | 0.5 ± 0.6a | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.3 ± 0.6a | 0.0 ± 0.0a |
| Thysanoptera | 0.0 ± 0.0a | 0.0 ± 0.0a | 13.8 ± 9.7b | 10.8 ± 5.2b | 0.0 ± 0.0a | 0.0 ± 0.0a | 40.3 ± 10.5c | 44.3 ± 4.7c |
| Hemiptera | 245.0 ± 25.0d | 233.5 ± 45.5cd | 160.0 ± 28.2b | 173.8 ± 98.1bc | 11.3 ± 3.4a | 13.8 ± 7.3a | 24.7 ± 12.3a | 21.0 ± 8.2a |
| Homoptera | 11.3 ± 6.1a | 11.5 ± 4.4a | 79.8 ± 35.4b | 96.0 ± 80.5b | 0.0 ± 0.0a | 0.3 ± 0.5a | 50.3 ± 8.5ab | 55.7 ± 17.9ab |
| Coleoptera | 21.8 ± 12.6b | 28.5 ± 13.5b | 2.3 ± 1.0a | 3.8 ± 2.8a | 3.3 ± 3.3a | 0.8 ± 1.0a | 2.3 ± 2.3a | 5.0 ± 1.7a |
| Diptera | 4.3 ± 2.9bc | 4.8 ± 3.1c | 2.3 ± 0.5abc | 2.0 ± 1.4abc | 1.5 ± 2.4ab | 0.5 ± 0.6a | 0.7 ± 0.6a | 0.7 ± 0.6a |
| Lepidoptera | 6.8 ± 5.9a | 6.3 ± 4.6a | 3.0 ± 2.2a | 4.0 ± 0.8a | 1.8 ± 1.3a | 2.0 ± 2.4a | 1.7 ± 1.5a | 2.3 ± 0.6a |
| Total | 289.3 ± 31.6b | 285.0 ± 49.7b | 261.3 ± 73.5b | 290.3 ± 183.4b | 17.8 ± 4.6a | 17.3 ± 8.5a | 120.3 ± 24.8a | 129.0 ± 30.4a |

The results shown are the mean ± SD, $n = 3$ or 4 replicates for each group, and least significant difference (LSD) at $p < 0.05$ within row

[a]Genetically modified soybean

[b]Non-genetically modified soybean; Gwangan soybean data were used the data from a paper published in 2020 (Amin et al. 2020b)

**Table 3** Average population densities of natural enemies in common plant-dwelling non-target insect groups and arachnids captured using vacuum suction at fields planted with two different genotypes of soybeans at Ochang and Jeonju, Korea, in 2016 and 2017

| Order | 2016 | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|
| | Ochang | | Jeonju | | Ochang | | Jeonju | |
| | TRX[a] | Gwangan[b] | TRX | Gwangan | TRX | Gwangan | TRX | Gwangan |
| Odonata | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a |
| Hemiptera | 0.0 ± 0.0a | 1.5 ± 0.6ab | 4.3 ± 1.9c | 2.5 ± 2.1b | 0.0 ± 0.0a | 0.0 ± 0.0a | 1.3 ± 0.6ab | 0.3 ± 0.6a |
| Neuroptera | 2.0 ± 1.4ab | 1.8 ± 2.4ab | 1.0 ± 0.8ab | 0.3 ± 0.5a | 3.5 ± 2.6b | 2.0 ± 2.2ab | 0.0 ± 0.0a | 0.0 ± 0.0a |
| Coleoptera | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.3 ± 0.5a | 0.5 ± 1.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.3 ± 0.6a | 0.3 ± 0.6a |
| Hymenoptera | 85.3 ± 30.7a | 70.5 ± 19.4a | 325.5 ± 140.0b | 316.5 ± 222.1b | 14.3 ± 1.3a | 11.8 ± 7.3a | 65.0 ± 15.7a | 69.3 ± 34.0a |
| Araneae | 4.5 ± 4.0ab | 3.3 ± 2.6a | 9.5 ± 4.0bcd | 12.0 ± 4.5d | 3.0 ± 1.4a | 5.5 ± 2.6abc | 9.3 ± 4.5bcd | 11.0 ± 4.6cd |
| Total | 92.3 ± 34.8a | 77.0 ± 21.6a | 340.5 ± 144.0b | 331.8 ± 219.9b | 20.8 ± 3.1a | 19.3 ± 7.4a | 76.0 ± 10.8a | 81.0 ± 38.7a |

The results shown are the mean ± SD, $n = 3$ or 4 replicates for each group, and Least significant difference (LSD) at $p < 0.05$ within row

[a]Genetically modified soybean

[b]Non-genetically modified soybean; Gwangan soybean data were used the data from a paper published in 2020 (Amin et al. 2020b)

showed a slight decrease from 2016 to 2017 at Jeonju on both the genotypes, whereas there was no change in population density on Gwangan soybean but a slight increase on TRX from 2016 to 2017 at Ochang. The average population densities of the insect pests in the order Hymenoptera decreased from 2016 to 2017 at Jeonju on both the genotypes but showed no change at Ochang from 2016 to 2017 on either genotype. The population of Araneae fluctuated on both the genotypes at both the locations but did not show any major change over the period (Table 3).

The average population densities of other insects from all the orders (Psocoptera, Hemiptera, Homoptera, Neuroptera, Coleoptera, Diptera, and Hymenoptera) did not show any statistically significant difference based on soybean genotype, from 2016 to 2017, at either location. Thus, the total population was statistically static over the period (Table 4).

The analysis of diversity of the captured non-target insect groups and arachnids revealed dominance indices ranging from 0.29 ± 0.06 to 0.60 ± 0.06 on both the soybean genotypes at Ochang and Jeonju from 2016 to 2017 (Table 5). The dominance indices of non-target insects and arachnids decreased from 2016 to 2017 at both Ochang and Jeonju on both the genotypes. The diversity index of the insects and arachnids was the lowest during 2016 on both the genotypes at Ochang but increased in 2017. The diversity index of the insects and arachnids at Jeonju also showed a similar trend but was higher than that at Ochang. Both TRX and Gwangan soybean plants at Jeonju during 2017 showed the highest and statistically similar diversity indices of the captured arthropods (2.70 ± 0.04 and 2.67 ± 0.10, respectively). The evenness index of the insects and arachnids did not show any change over the study periods at Ochang on either genotype. In contrast, evenness indices of the non-target insect groups and arachnids at Jeonju decreased from 2016 to 2017 on both the genotypes. The richness index of the insects and arachnids increased over time, from 2016 to 2017, at both the locations irrespective of the soybean genotype (Table 5).

The occurrence rate of insect pests, natural enemies, and other insects altogether on TRX and Gwangan soybean cultivars at Ochang and Jeonju in 2016 and 2017 was 41.23%, 30.58%, and 28.19%, respectively (Fig. 1). Hemiptera accounted for the highest (26.66%) occurrence among the identified orders of insect pests, followed by Homoptera (8.51%), and the lowest occurrence was Diptera (0.50%). Of the natural enemies, Hymenoptera (28.29%) was most abundant. In the other insect category, Diptera (19.85%) occurred most often.

Average population densities in common plant-dwelling non-target insects and arachnids were highest at Ochang in 2017 on the TRX soybean genotype. Conversely, the average population density of these arthropods was lowest on both the cultivars at Jeonju in 2016 (Fig. 2).

**Table 4** Average population densities of other insects in common plant-dwelling non-target insect groups captured using vacuum suction at fields planted with two different genotypes of soybean at Ochang and Jeonju, Korea, in 2016 and 2017

| Order | 2016 | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|
| | Ochang | | Jeonju | | Ochang | | Jeonju | |
| | TRX[a] | Gwangan[b] | TRX | Gwangan | TRX | Gwangan | TRX | Gwangan |
| Pscoptera | 0.0 ± 0.0a | 0.0 ± 0.0a | 14.0 ± 5.1a | 8.8 ± 9.3a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.3 ± 0.6a | 0.7 ± 1.2a |
| Hemiptera | 1.0 ± 2.0a | 0.3 ± 0.5a | 3.0 ± 3.2a | 1.3 ± 0.5a | 0.3 ± 0.5a | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.3 ± 0.6a |
| Homoptera | 14.0 ± 7.0a | 17.5 ± 10.0a | 25.3 ± 7.0a | 27.0 ± 8.0a | 6.8 ± 4.3a | 4.0 ± 1.4a | 61.0 ± 22.3a | 58.3 ± 8.1a |
| Neuroptera | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.3 ± 0.6a | 0.0 ± 0.0a |
| Coleoptera | 0.0 ± 0.0a | 0.0 ± 0.0a | 4.8 ± 2.8a | 5.8 ± 4.9a | 0.0 ± 0.0a | 0.0 ± 0.0a | 29.0 ± 3.5a | 34.3 ± 4.2a |
| Diptera | 103.0 ± 20.3a | 114.8 ± 27.0a | 155.0 ± 82.4a | 139.8 ± 22.6a | 15.8 ± 5.0a | 9.8 ± 5.7a | 73.7 ± 13.0a | 74.0 ± 13.0a |
| Hymenoptera | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a |
| Total | 118.3 ± 28.3a | 132.5 ± 23.4a | 202.0 ± 87.7a | 182.5 ± 32.3a | 23.0 ± 8.0a | 14.0 ± 5.9a | 164.3 ± 35.4a | 167.7 ± 16.7a |

The results shown are the mean ± SD, $n = 3$ or 4 replicates for each group, and least significant difference (LSD) at $p < 0.05$ within row

[a]Genetically modified soybean

[b]Non-genetically modified soybean; Gwangan soybean data were used the data from a paper published in 2020 (Amin et al. 2020b)

The score from PROXSCAL multidimensional scaling showed that the number of insects collected in 2016 and 2017 from TRX and Gwangan soybean fields did not differ within the same cultivation year at Jeonju and Ochang. Analysis of the combined data showed that insects and arachnids in different natural environments were isolated according to the cultivation region and year of study (Fig. 3). The arthropods composition obtained from Ochang fields in 2017 was more varied compared to those in other fields. For exsample, *F. occidentalisi* was not captured at the fields in 2016 while the insect was collected at the Ochang fileds in 2017 with very different levels for each sampling time. These data resulted in the wider cluster in the 2017 Ochang samples compare to those in other samples (Fig. 3A). To confirm the impact of natural environments on arthropods composition, PCA was conducted using the arthropods profile data from two different GM soybean, IGF (expressing the human insulin-like growth factor) and vitamin A-enhanced soybean which previously reported by our groups (Amin et al. 2020b; Oh et al. 2020), and TRX. The PCA results showed differences of arthropods composition according to soybean plant's growing years and regions, which demonstrated the absence of significant variance within the same variety and no noticeable differences between the transgenic soybean and non-transgenic counterparts (Fig. 4).

## Discussion

According to the findings of current study, the largest number of insect pests, natural enemies, and other insects were recorded from Hemiptera, Hymenoptera, and Diptera, respectively, and the result was evidently observed on both of the genotypes of soybean in either region or study period. However, it was also noted that the number of individual insects of different taxa varied according to the regions and study periods, with Ochang corresponding to a far higher insect abundance than did Jeonju. The environmental conditions of Ochang might be more suitable for the reproduction and development of the insects, resulting in the large numbers collected at this site. The findings also revealed that the number of insect pests did not vary according to soybean genotypes. Oh et al. (2020) obtained similar results in the case of vitamin A-enhanced transgenic soybean and non-GM Gwangan soybean. The results indicate that there is no detectable effect of GM events on the abundance of insect pests, natural enemies, and other insects.

Considering the average population densities of non-target insect pests represented in the survey, the average population densities of the insect pests significantly decreased from 2016 to 2017 at both Jeonju and Ochang on both genotypes. Therefore, the occurrence of insect pests varied significantly based on region and survey year, but there was no

**Table 5** Analysis of diversity in common plant-dwelling non-target insect groups and arachnid captured using vacuum suction at fields planted with two different genotypes of soybeans at Ochang and Jeonju, Korea in 2016 and 2017

| Order | 2016 | | | | 2017 | | | |
|---|---|---|---|---|---|---|---|---|
| | Ochang | | Jeonju | | Ochang | | Jeonju | |
| | TRX[a] | Gwangan[b] | TRX | Gwangan | TRX | Gwangan | TRX | Gwangan |
| Dominance (DI) | 0.58 ± 0.06a | 0.60 ± 0.06a | 0.36 ± 0.05cd | 0.42 ± 0.09c | 0.55 ± 0.05ab | 0.50 ± 0.10bc | 0.34 ± 0.03cd | 0.29 ± 0.06d |
| Diversity (*H′*) | 2.02 ± 0.16cd | 1.96 ± 0.09d | 2.38 ± 0.08b | 2.22 ± 0.09bc | 2.17 ± 0.09bc | 2.28 ± 0.28b | 2.70 ± 0.04a | 2.67 ± 0.10a |
| Evenness (EI) | 0.62 ± 0.02c | 0.62 ± 0.03c | 0.89 ± 0.03a | 0.85 ± 0.05ab | 0.62 ± 0.02c | 0.65 ± 0.09c | 0.80 ± 0.02b | 0.80 ± 0.03b |
| Richness (RI) | 4.02 ± 0.69bc | 3.63 ± 0.12cd | 3.50 ± 0.25cd | 3.27 ± 0.53d | 4.88 ± 0.58a | 5.07 ± 0.39a | 4.94 ± 0.22a | 4.62 ± 0.24ab |

The results shown are the mean ± SD, $n = 3$ or 4 replicates for each group, and least significant difference (LSD) at $p < 0.05$ within row

[a]Genetically modified soybean

[b]Non-genetically modified soybean; Gwangan soybean data were used the data from a paper published in 2020 (Amin et al. 2020b)

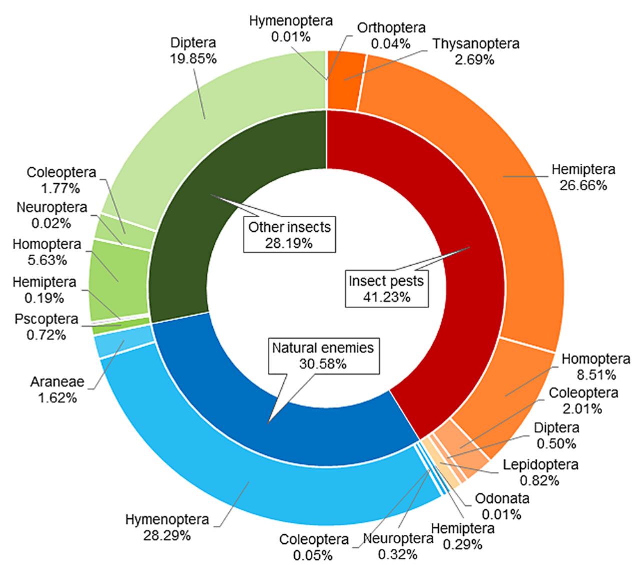


**Fig. 1** Occurrences of common plant-dwelling non-target insects and arachnids captured using vacuum suction on two different genotypes of soybean at Ochang and Jeonju, Korea, in 2016 and 2017

difference between the soybean genotypes. In case of natural enemies, the average population densities showed drastic decrease from 2016 to 2017 at Jeonju on both the genotypes, but showed only a small decrease at Ochang, from 2016 to 2017, regardless of the genotype. Moreover, the average population density of natural enemies was always significantly higher at Jeonju than at Ochang but did not differ due to the variation of the genotypes. The population of other insects indicated that there was not any significant effect of soybean genotype, region, or study period on their average population densities. In contrast, the average population densities of the insect pests and natural enemies markedly decreased from 2016 to 2017 at both the regions, irrespective of the genotypes.

The diversity indices of the non-target insects and arachnids increased from 2016 to 2017 at both the study sites on either genotype. Although these results are from two consecutive years only, the findings did not reflect the general trend of global decline of insect biodiversity reported by Sanchez-Bayo and Wyckhuys (2019) who stated an

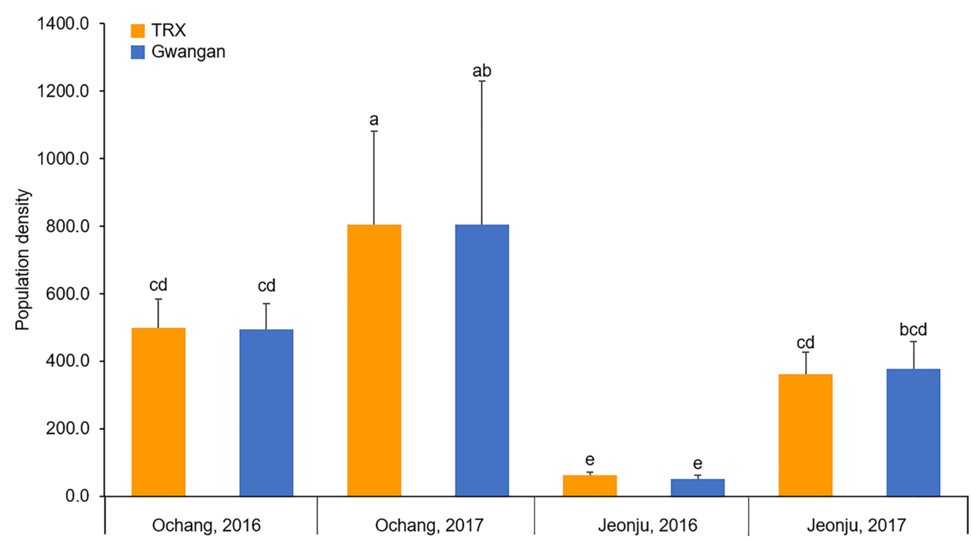


**Fig. 2** Average population densities in common plant-dwelling non-target insects and arachnid captured using vacuum suction at fields planted with two different genotypes of soybean at Ochang and Jeonju, Korea, in 2016 and 2017. The results shown are the mean ± SD, $n = 3$ or 4 replicates for each group. TRX: genetically modified soybean. Different letters represent significant ($p < 0.05$) differences between means according to least significant difference test

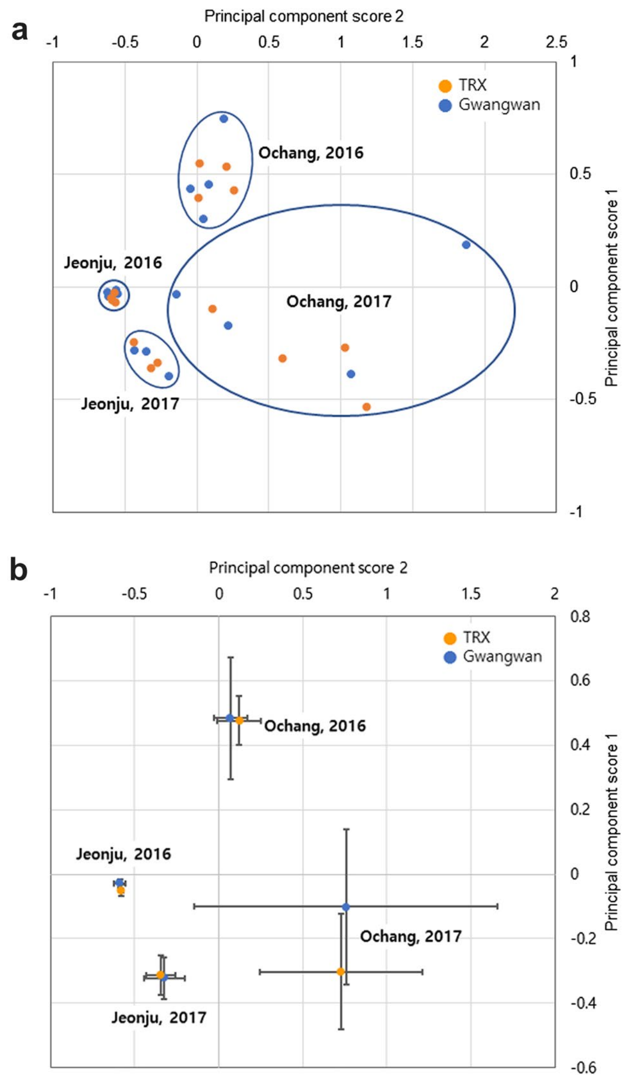


**Fig. 3** PROXSCAL multidimensional scaling ordination with common plant-dwelling non-target insects and arachnids captured using vacuum suction at fields planted with two different genotypes of soybean at Ochang and Jeonju, Korea, in 2016 and 2017 (stress-1 = 0.0570, Tucker's coefficient of congruence = 0.99837). TRX: Genetically modified soybean. **A** Perceptual map; **B** means and standard deviations of dimensional values

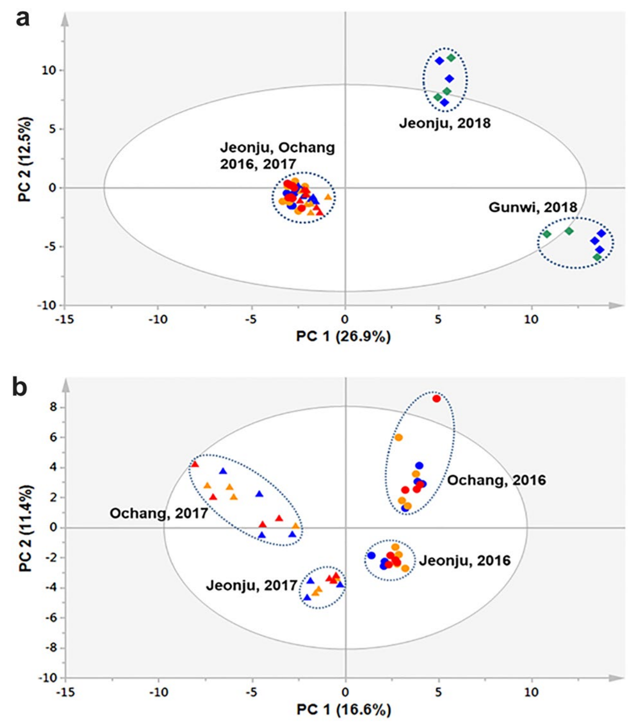


**Fig. 4** **A** Score plots of principal components 1 and 2 of the PCA results obtained from data on the insects collected at fields planted with three genetically modified soybean plants (orange, TRX; red, IGF; green, vitamin A-enhanced soybean) and a non-GM cultivar (blue, Gwangan) at three different regions (Jeonju, Ochang, and Gunwi) for 3 years (2016, circle; 2017, triangle; 2018, diamond). **B** Score plots of the PCA results obtained from data on the insects collected at fields planted with two genetically modified soybean plants, TRX and IGF

unexpected worldwide decline in insect species belonging to different taxa.

Overall, the average population densities of the non-target insects and arachnids decreased from 2016 to 2017 on both the genotypes at both sites. Therefore, the average population densities of captured insects and arachnids were significantly different according to survey year rather than varietal difference. The results of the present study are similar to those of Amin et al. (2020a, b), who found no adverse effect of transgenic events on plant-dwelling non-target arthropods. Furthermore, it was confirmed that the arthropods profiles of three different GM soybean plants (TRX, IGF, and vitamin-enhanced soybean) were classified based on the captured field's environment conditions such as years or regions irrespective of their arresting methods and with or without a genetic modification in which the vitamin-enhanced soybean's arthropods were captured using a yellow sticky trap at fields (Oh et al. 2020). Lee (2021), Svobodova et al. (2015), and Resende et al. (2016) also reported no significant differences in the community structure of non-target arthropods between GM and non-GM crops. In contrast, some authors stated that Bt toxins affect arthropod communities (Cunha et al. 2012; Hansen et al. 2012; Pessoa et al. 2016). It should be noted, however, that field conditions, such as temperature, humidity, rainfall, sunshine, and the presence of natural enemies, can influence the effect of Bt and non-Bt crops on arthropod populations.

In conclusion, the findings of the current study indicated that the transgenic soybean TRX did not affect the non-target arthropod community, but the temporal pattern of non-target insects and arachnids varied for both of the soybean genotypes. In addition, the different conditions of the two study sites where the soybean genotypes were grown might have affected the abundance and diversity of insect and arachnid communities.

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