Influence of Genetically Modified Soybean Expressing Epidermal Growth Factor on Arthropod Biodiversity

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ABSTRACT

The field study was undertaken to examine the potential for adverse effects of transgenic soybean expressing bioactive human epidermal growth factor (with tolerance to the herbicide glufosinate, PPT) on the abundance and diversity of plant-dwelling arthropods by comparing with those of a non-GM parental cultivar, Gwangan soybean. Field surveys of soybean fields were carried out over two consecutive years, 2016 and 2017 at Ochang and Jeonju, Korea. The number of captured individuals associated with either of EGF and Gwangan soybean plants increased in 2017 compared with 2016 in both Ochang and Jeonju. During the survey period, the diversity and richness of the occurred insects and arachnids increased, dominance decreased, and the evenness of the insects remained static. The insects of Hymenoptera Order occurred most often comprised 25.4% of total captured insect pests. On the contrary, natural enemy from Hymenoptera Order and other insects from Diptera Order occurred more frequently (29.9% and 19.0%, respectively) in both the survey regions during the study periods. The score from PROXSCAL multidimensional scaling using combined data showed that the occurrence of insects and arachnids were separated due to their cultivation regions and years, irrespective of soybean cultivars. Consequently, the results indicated that there happened no notable change in the composition of arthropod communities in soybean agroecosystem due to GM event in soybean expressing EGF.

Introduction

The genetically modified organisms are produced for the particular advantages they provide such as increased crop yield, resistance to insect herbivores and tolerance to herbicides. Corn, canola, soybean, and cotton are the most common crops using which GMOs have been developed. The cultivation of soybean is popular because of serving particular agronomic, nutritional and industrial interests such as improved yield by enhanced tolerance to stresses, improved nutritional traits, the capability to produce therapeutic products and the capability of being feedstock for biodiesel. The adoption of transgenic soybean has greatly increased worldwide after introducing herbicide tolerant traits (glyphosate-resistance) and cultivation of this soybean genotype remains dominant in the recent occupying almost half of the global biotech area. The transgenic soybean developed with the expression of Bt proteins has insect-resistance capacity. Another three transgenic soybean events expressing human epidermal growth factor (EGF), insulin-like growth factor 1 (IGF-1), or thioredoxin (TRX) have been developed from industrial interest to obtain recombinant proteins for skin care. EGF is a small polypeptide that after binding to the EGF receptor on the cell surface of the human epidermis promotes cell growth. It can prevent cellular damage caused due to aging and stimulates the growth of skin and corneal epithelium. It is also associated with cell proliferation, migration, and differentiation and acts as a potent mitogenic factor.
The biosafety of transgenic crop considering the risk of outcrossing (genes from GM crops pass into wild plants and other crops) and a negative impact on non-target insects and other species has always been a concern for releasing them into the environment. The countries, like United States, Argentina, Brazil, Canada, Mexico, Romania, Uruguay, and South Africa where the cultivation of transgenic soybean is concentrated, have already started to pay increased focus on the potential risk of the crop to the environment.7

The effects of some transgenic events on arthropod communities were evaluated recently. According to research reports, GM soybean IGF and TRX did not affect arthropod diversity over non-GM soybean.8,9 Several other studies investigated the safety of the non-target arthropod populations in the presence of transgenic Bt proteins in cotton, maize and soybean, and reported their negligible short-term impact on them.10–12

The development and cultivation of biotech crops have never been free of controversies, particularly when the concern is to ensure the safety of non-target organisms. Before commercializing transgenic soybean events, an evaluation of their potential risks to human health and the environment should be done. Thus, understanding the non-target effects of transgenic crops on associated arthropods should also get a concern.12 Therefore, in the present study, the effects of transgenic soybean EGF on the abundance and diversity of plant-dwelling insects and arachnids were assessed through field surveys.

Materials and Methods

Experimental Site and Duration

The study was conducted with GM soybean, EGF and non-GM soybean Gwangan during mid-August to early October over the years of 2016 and 2017. The field surveys were done in the soybean fields of Ochang (Korea Research Institute of Bioscience & Biotechnology, Facility registration number RDA-2016-052 (36.7° N, 127.4° E)) and Jeonju (National Institute of Agricultural Sciences, Facility registration number RDA-2013-041 (35.7° N, 128.7° E)). EGF is a transgenic soybean expressing bioactive human epidermal growth factor (EGF) with tolerance to the herbicide glufosinate (PPT). The study used the non-GM parental cultivar, Gwangan soybean as the control to compare the influence of transgenic soybean event on the above ground arthropods. The crops were grown according to the standard cultivation method of the National Institute of Agricultural Sciences. The size of each plot was 4.0 m × 4.0 m with distance of 1.0 m between the plots. There were four replications for each of the varieties, i.e. a total of eight plots which were investigated each year in both locations, except at Jeonju in 2017, where there were six plots in total with three replications for the genotypes. Proper irrigation and fertilization were done, and no insect management approach was taken.

Sampling Strategy

The arthropods including the 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 EGF and Gwangan soybean cultivated fields. In total, four samplings were performed at 2-week intervals, where one sampling was done at the vegetative stage and the rest three were done at the reproductive stage of the plant. All samplings were done during the first part of the day, i.e. between 10:30 and 12:00. The collected specimens were kept in polyethylene bags and immediately frozen using dry ice for preservation.

Identification and Categorization

The collected arthropods (both immature and adult) were brought to the Laboratory of Systematic Entomology, School of Applied Biology, Kyungpook National University, Daegu, Korea for identification. 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), and identified down to the family level. Since many insect pest species have
already been identified and well documented, it is convenient to identify any collected insect pests down to the species level; hence, we identified the species of the insect pests as well in the present study.

**Statistical Analysis**

ANOVA employing Tukey HSD (\( P < .05 \)) was used to compare changes in insect abundance on soybean genotypes in the survey regions during the study years. The dominance, diversity, evenness, and richness indices were calculated following McNaughton’s dominance index, the Shannon–Weaver diversity index, Pielou index, and Margalef species richness index, respectively. Multidimensional scaling (MDS) was performed to analyze the similarity of insect occurrence. The data in Table 1 were standardized for analysis, and the perceptual map was prepared using Euclidean similarity using PROXSCAL. As a result of analyzing, Kruskal’s stress-1 value was 0.05073, which was good, and Tucker’s coefficient of congruence was 0.99871, which showed a very high explanatory power of the model. All the analyses were conducted using IBM SPSS version 26.0.

**Results**

**Total Arthropod Communities Occurred in GM and Non-GM Soybean Fields**

Altogether 13,161 individuals of insects and arachnids representing 61 families and 12 orders were found in the EGF and Gwangan soybean fields of Ochang and Jeonju during the survey periods of 2016 and 2017. The functional group of insect pests was comprised of a total of 5,375 individuals of 36 species from 22 families. On the other hand, the functional groups of natural enemies and other insects were comprised of 19 families with 4,234 individuals and 22 families with 3,553 individuals, respectively (Table 1).

In total, 1990 individuals from EGF soybean field of Ochang were found in 2016 which reached 3309 in 2017. A total of 1978 individuals were collected from Gwangan soybean agroecosystem from Ochang in 2016 which was lower than the insects found in 2017 (3218 individuals). A total of 267 individuals were collected from EGF soybean of Jeonju in 2016 which showed a swift increase in 2017 and became 1064. A total of 202 individuals were collected from Gwangan soybean agroecosystem from Jeonju in 2016 which dramatically increased in 2017 showing 1133 individuals (Table 1).

**Average Population Densities of Non-target Insect Pests Captured from GM and Non-GM Soybean Fields**

The average population densities of the non-target insect pests of Orthoptera and Lepidoptera remained unchanged (no statistically significant difference) from 2016 to 2017 at both Ochang and Jeonju, irrespective of the genotype. The average population densities of the insect pests of the Order Thysanoptera increased from 2016 to 2017 on both soybean genotypes at Jeonju but the individuals of Thysanoptera were completely absent at Ochang in entire survey periods. The densities of the insect pests of Hemiptera and Homoptera decreased on both genotypes from 2016 to 2017 at Jeonju and Ochang. On the contrary, the densities of the insect pests of Coleoptera and Diptera showed no change on both genotypes from 2016 to 2017 at Jeonju but decreased at Ochang (Table 2).

**Average Population Densities of Natural Enemies Captured from GM and Non-GM Soybean Fields**

The average population densities of the natural enemies of the taxa Odonata, Hemiptera, Neuroptera and Coleoptera statistically remained unchanged from 2016 to 2017 on both the soybean genotypes at Ochang and Jeonju. The average population densities of the natural enemies in the order Hymenoptera decreased from 2016 to 2017 at Ochang and Jeonju on both the genotypes. The average population densities of the natural enemies in the order Araneae increased from 2016 to 2017 at Ochang on both the genotypes but showed no change at Jeonju from 2016 to 2017 on either of the genotypes (Table 3).

**Average Population Densities of Other Insects Captured from GM and Non-GM Soybean Fields**

The average population densities of the other insects of order Hemiptera did not show any
Table 1. Total number of common plant-dwelling insects and arachnids captured using vacuum suction in fields planted with two different genotypes of soybeans at Ochang and Jeonju, Korea, in 2016 and 2017 (EGF: Genetically modified soybean; GW: non-GM Gwangan).

| Category          | Order                  | Family               | Species                          | Ochang EGF | Ochang GW | Jeonju EGF | Jeonju GW | Total |
|-------------------|------------------------|----------------------|----------------------------------|------------|-----------|------------|-----------|-------|
| Insect pests      | Orthoptera             | Tettigoniidae        | Phaneroptera falcata             | -          | -         | -          | -         | 1     |
|                   |                        | Pygromorphidae       | Atractomorpha lata               | 1          | -         | -          | -         | 1     |
|                   |                        | Acrididae            | Acrida cinerea                   | -          | 1         | -          | -         | 2     |
|                   | Thysanoptera           | Thripidae            | Frankliniella occidentalis       | -          | -         | -          | 47        | 34    |
|                   |                        |                     | Frankliniella intonsa            | -          | -         | -          | 7         | 32    |
|                   | Hemiptera              | Miridae              | Adelphocoris suturalis           | 13         | 6         | 3          | 2         | 33    |
|                   |                        | Apolagus lucorum     | 3                                 | -          | -         | 1          | 1         | 5     |
|                   | Berytidae              | Yemna exilis         | 773                               | 778        | 32        | 35         | 510       | 655   |
|                   | Lygaeidae              | Panaorius albomaculatus | -                       | -          | -         | 8          | 20        | -     |
|                   |                        | Nysius plebeius      | 19                                 | 12         | 5         | 3          | 2         | 2     |
|                   |                        | Alydidae             | Riportus pedestris               | 100        | 127       | 8          | 12        | 10    |
|                   |                        | Rhopalidae           | Rhopalus maculatus               | 5          | 5         | 2          | -         | -     |
|                   |                        | Pentatomomidae       | Homalogonia obtusa               | 2          | -         | -          | -         | 2     |
|                   |                        | Nezara antennata     | -                                 | 1          | -         | -          | -         | 1     |
|                   |                        | Piezodorus hybneri   | -                                 | 1          | 2         | 2          | -         | 5     |
|                   |                        | Dolycoris baccarum   | 1                                 | 4          | 1         | 1          | 2         | -     |
| Homoptera         | Cacalidellida          | Cicadellaviridis     | -                                 | -          | -         | 1          | -         | -     |
|                   |                        | Empoascaerybiogen    | -                                 | -          | -         | 34         | 29        | 43    |
|                   |                        | R eBookanna           | -                                 | 5          | 5         | -          | -         | -     |
|                   |                        | Ricianidae           | Riciania shantungensis           | 2          | -         | -          | 1         | 12    |
|                   |                        | Ricianiataeniata     | -                                 | -          | -         | 1          | -         | 1     |
|                   | Flatidae               | Geishadistinctissima | 47                                 | 41         | -         | -          | -         | 88    |
|                   | Aleyrodidae            | Triealeurodes vaporariorum | -                       | -          | -         | 304        | 354       | 108  |
| Coleoptera        | Chrysomelidae          | Pagriagignata        | 150                               | 110        | 10        | 2          | 9         | 12    |
|                   |                        | Medythianigrilineata | 5                                 | 10         | 2         | 9          | 12        | 10    |
|                   |                        | Mono.Seekragniprignutata | 1                                 | 1          | -         | 1          | -         | -     |
|                   | Apionidae              | Pseudopiezotricheluscollare | -                                 | -          | -         | 1          | 1         | -     |
|                   | Curculionidae         | Calosbrus bruchis chinensis | -                                 | -          | -         | 2          | 1         | 4     |
| Coleoptera        | Diptera                | Platystomatidae      | Rivellia flaviwetris             | 1          | 2         | 1          | 1         | 2     |
|                   |                        | Rivellia alini       | -                                 | -          | -         | 1          | -         | 1     |
|                   |                        | Rivellia apicalis    | -                                 | 5          | -         | 3          | -         | 8     |
| Coleoptera        | Lepidoptera            | Crambididae          | Omodesinicalata                  | 7          | 8         | 13         | -         | 30    |
|                   |                       | spp                  | 16                                 | 3          | 4         | 13         | 6         | 7     |
| Coleoptera        | Coleoptera             | Nolidae              | Celatomaenatix                   | -          | 2         | -          | -         | 2     |
| Subtotal          |                        |                      |                                   | 1193       | 1140      | 90         | 69        | 961   |
| Natural enemies   | Odonata                | Coenagrionidae       | -                                 | 1          | -         | -          | -         | -     |
|                   | Hemiptera              | Nabidae              | 2                                 | 6          | -         | -          | -         | 8     |
|                   | Anthocoridae           | -                    | 2                                 | 7          | 8         | 4          | 1         | 28    |
| Lepidoptera       | Neuropeuta             | Chrysopidae          | 2                                 | 7          | 8         | 1          | 1         | 28    |
| Coleoptera        | Coccidellida           | Coccinellida          | 1                                 | -          | 2         | -          | 1         | 2     |
|                   | Staphylinidae         | -                    | 1                                 | -          | 1         | -          | 1         | 1     |
| Coleoptera        | Hymenoptera            | Braconidae           | 87                                 | 108        | 21        | 12         | 231       | 188   |
|                   | Ichneumonidae          | 2                    | 3                                 | 3          | 1         | 1          | 2         | 1     |
|                   | Chalcidae              | -                    | 1                                 | -          | -         | 1          | -         | 1     |
| Coleoptera        | Scelionidae           | -                    | 1                                 | 33         | 19        | 26         | 20        | 98    |
| Coleoptera        | Eulophidae            | -                    | 214                               | 171        | 39        | 34         | 1295      | 1058  |
| Coleoptera        | Araneae                | Tetragenathidae      | 4                                 | 8          | 10        | 13         | 19        | 20    |
|                   | Therididae            | -                    | 2                                 | 2          | 3         | 2          | 7         | 11    |
|                   | Salticidae            | -                    | 8                                 | 6          | 3         | 8          | 9         | 18    |
|                   | Clubionidae           | -                    | 2                                 | 1          | 2         | 4          | 4         | 1     |
| Coleoptera        | Araneidae             | 1                    | 1                                 | -          | 3         | 2          | 2         | 1     |
|                   | Linyphiidae           | -                    | 2                                 | -          | 3         | 2          | 1         | 3     |
| Subtotal          |                        |                      | 316                               | 308        | 95        | 77         | 1611      | 1327  |
| Other insects     | Psocoptera             | Psocidae             | -                                 | -          | -         | 75         | 35        | -     |
|                   | Hemiptera              | Miridae              | -                                 | -          | -         | 2          | 2         | 1     |
|                   | Tingidae              | 3                    | 1                                 | 1          | 1         | 6          | -         | -     |
|                   | Homoptera              | Membracidae          | -                                 | -          | -         | 10         | 14        | -     |
|                   | Delphacidae           | -                    | 38                                 | 66         | 11        | 4          | 119       |       |
|                   | Cacalidellida         | 63                   | 70                                 | 33         | 16        | 27         | 28        | 149   |
| Coleoptera        | Elateridae            | -                    | 1                                 | -          | -         | 1          | 1         | -     |
|                   | Latrididae            | -                    | 19                                 | 19         | 4         | 6          | 48        |       |
|                   | Phylidiidae           | -                    | 2                                 | 4          | 70        | 97         | 173       |       |
| Coleoptera        | Diridae               | Chironomidae         | -                                 | -          | -         | 16         | 47        | 63    |
| Coleoptera        | Stratiomyidae         | -                    | 1                                 | 1          | 8         | 8          | -         | 18    |
| Coleoptera        | Empididae             | -                    | 9                                 | 29         | 8         | 5          | 50        |       |

(Continued)
Table 1. (Continued).

| Category | Order | Family | Species |
|----------|-------|--------|---------|
|          | Dolichopodidae |       |         |
|          | Cyrtophoridae |       |         |
|          | Syrphidae |       |         |
|          | Sciaridae |       |         |
|          | Sepsidae |       |         |
|          | Anthomyiidae |       |         |
|          | Muscidae |       |         |

| 2016 | Ochang | Jeonju | Ochang | Jeonju |
|------|--------|--------|--------|--------|
|       | GEF | GW | GEF | GW | GEF | GW | GEF | GW | Total |
|-------|-----|----|-----|----|-----|----|-----|----|-------|
| Dolichopodidae | 42 | 34 | 2 | - | 112 | 158 | 41 | 34 | 423 |
| Cyrtophoridae | 3 | - | - | - | - | 1 | 2 | 1 | 7 |
| Sepsidae | 1 | - | - | 1 | - | 2 | - | - | 4 |
| Muscidae | 7 | 5 | - | 1 | 22 | 15 | 1 | 5 | 56 |
| Syrphidae | 358 | 418 | 45 | 37 | 319 | 294 | 81 | 105 | 1657 |
| Sciaridae | 5 | - | - | 1 | 52 | 62 | 47 | 26 | 187 |
| Anthomyiidae | 1 | - | - | - | - | 1 | - | - | 1 |
| Subtotal | 482 | 530 | 82 | 56 | 737 | 730 | 433 | 503 | 3553 |
| Total | 1990 | 1978 | 267 | 202 | 3309 | 3218 | 1064 | 1133 | 13,161 |

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

| Order | Ochang | Jeonju | Ochang | Jeonju |
|-------|--------|--------|--------|--------|
|       | GEF    | Gwangan | GEF    | Gwangan | GEF    | Gwangan | GEF    | Gwangan |
| Orthopteran | 0.3 ± 0.5a | 0.5 ± 0.6a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.0 ± 0.0a |
| Thysanoptera | 0.0 ± 0.0a | 0.0 ± 0.0a | 13.5 ± 10.5a | 10.8 ± 5.2a | 0.0 ± 0.0a | 0.0 ± 0.0a | 38.7 ± 11.2b | 44.3 ± 4.7b |
| Hemiptera | 229.0 ± 63.7c | 233.5 ± 45.5c | 133.3 ± 31.1bc | 173.8 ± 98.1c | 3.5 ± 6.2a | 13.8 ± 7.3a | 29.0 ± 6.2ab | 21.0 ± 8.2ab |
| Homoptera | 13.0 ± 6.4ab | 11.5 ± 4.4ab | 85.0 ± 56.0ab | 96.0 ± 80.5b | 0.0 ± 0.0a | 0.3 ± 0.5a | 50.3 ± 8.5ab | 55.7 ± 17.9ab |
| Coleoptera | 38.0 ± 16.9b | 28.5 ± 13.5b | 3.0 ± 2.2a | 3.8 ± 2.8a | 3.3 ± 2.1a | 0.8 ± 1.0a | 4.0 ± 3.6a | 5.0 ± 1.7a |
| Diptera | 9.5 ± 6.2b | 4.8 ± 3.1ab | 2.3 ± 1.7a | 2.0 ± 1.4a | 1.5 ± 1.0a | 0.5 ± 0.6a | 0.7 ± 0.6a | 0.7 ± 0.6a |
| Lepidoptera | 8.5 ± 4.5a | 6.3 ± 4.6a | 3.3 ± 1.0a | 4.0 ± 0.8a | 4.0 ± 1.8a | 2.0 ± 2.4a | 2.0 ± 1.0a | 2.3 ± 0.6a |
| Total | 298.3 ± 71.7b | 285.0 ± 49.7b | 240.3 ± 95.6b | 290.3 ± 183.4b | 22.5 ± 9.7a | 17.3 ± 8.5a | 124.7 ± 25.4ab | 129.0 ± 30.4ab |

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

| Order | Ochang | Jeonju | Ochang | Jeonju |
|-------|--------|--------|--------|--------|
|       | GEF    | Gwangan | GEF    | Gwangan | GEF    | Gwangan | GEF    | 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 | 0.5 ± 0.6a | 1.5 ± 0.6a | 2.3 ± 2.1a | 2.5 ± 2.1a | 0.0 ± 0.0a | 0.0 ± 0.0a | 1.7 ± 1.2a | 0.3 ± 0.6a |
| Neuroptera | 0.5 ± 0.6a | 1.8 ± 2.4a | 0.3 ± 0.5a | 0.3 ± 0.5a | 2.0 ± 2.2a | 2.0 ± 2.2a | 0.3 ± 0.6a | 0.0 ± 0.0a |
| Coleoptera | 0.3 ± 0.5a | 0.0 ± 0.0a | 0.0 ± 0.0a | 0.5 ± 1.0a | 0.5 ± 1.0a | 0.0 ± 0.0a | 0.7 ± 0.6a | 0.3 ± 0.6a |
| Hymenoptera | 75.8 ± 42.4ab | 70.5 ± 19.4ab | 390.0 ± 177.9c | 316.5 ± 222.1bc | 15.8 ± 10.5a | 11.8 ± 7.3a | 70.3 ± 9.5ab | 69.3 ± 34.0ab |
| Araneae | 1.8 ± 1.7a | 3.3 ± 2.6ab | 10.3 ± 2.2bc | 12.0 ± 4.5c | 5.5 ± 2.4abc | 5.5 ± 2.6abc | 12.7 ± 3.9c | 11.0 ± 4.6c |
| Total | 79.0 ± 43.2ab | 77.0 ± 21.6ab | 402.8 ± 176.4c | 331.8 ± 219.9bc | 23.8 ± 9.0a | 19.3 ± 7.4a | 85.7 ± 9.5ab | 81.0 ± 38.7ab |

*Genetically modified soybean. The results shown are the mean ± standard deviation, n = 3 or 4 replicates for each group, and Tukey’s honestly significant difference at p < 0.05 within row.*

statistically significant difference based on soybean genotype, from 2016 to 2017, at either location. Psocoptera, Homoptera and Coleoptera showed no change at Ochang from 2016 to 2017 on both of the soybean genotypes. On the contrary, at Jeonju the average population densities of the other insects of Psocoptera decreased; whereas, the insects of Homoptera and Coleoptera increased over time from 2016 to 2017 on both the genotypes. The average population densities of the order Diptera showed drastic decline on soybean genotypes, from 2016 to 2017, at either of the locations (Table 4).

**Diversity of Common Plant Dwelling Non-target Insects and Arachnids in GM and Non-GM Soybean Fields**

The dominance of non-target insects and arachnids did not change at Ochang but decreased
at Jeonju on both the genotypes from 2016 to 2017. On the contrary, the diversity of the insects increased from 2016 to 2017 at Jeonju but remained unchanged on both the genotypes from 2016 to 2017. The evenness of the insects and arachnids did not show any change based on year both at Ochang and Jeonju on either genotypes. The richness of the insects and arachnids increased over time, from 2016 to 2017, at both the locations irrespective of the soybean genotype (Table 5).

### Percent Occurrences of Different Arthropods on GM and Non-GM Soybean Crops

The occurrence of the insect pests, natural enemies, and other insects altogether on EGF and Gwangan soybean cultivars at Ochang and Jeonju in 2016 and 2017 was 40.8%, 32.2%, and 27.0%, respectively (Figure 1). Hemiptera accounted for the highest (25.4%) occurrence among the identified Orders of insect pests, followed by Homoptera (8.7%). Among the natural enemies, individuals of Hymenoptera (29.9%) were most abundant. In the other insect category, Diptera (19.0%) occurred most often.

### Average Population Densities of Common Plant-dwelling Non-target Insects and Arachnids at the Fields of GM and Non-GM Soybean

Average population densities in common plant-dwelling non-target insects and arachnids were the highest on the EGF soybean genotype which was statistically similar with that of on Gwangan soybean, at Ochang in 2017. Conversely, the average population density of these arthropods was the lowest on both the cultivars at Jeonju in 2016 (Figure 2).

### Multidimensional Scaling Ordination with the Captured Non-target Insects and Arachnids of GM and Non-GM Soybean Fields

The score from PROXSCAL multidimensional scaling showed that the number of insects collected in 2016 and 2017 from EGF and Gwangan soybean fields differed within the same cultivation year at Jeonju and Ochang but did not differ based on the genotypes. Analysis of the combined data showed that insects and arachnids in different natural environments were more separated by growing year and cultivation region than by gene modification of the crop. The occurrence

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

| Order     | Ochang EGF | Ochang Gwangan | Ochang EGF | Ochang Gwangan | Jeonju EGF | Jeonju Gwangan | Jeonju EGF | Jeonju Gwangan |
|-----------|------------|----------------|------------|----------------|------------|----------------|------------|----------------|
| Coleoptera| 0.0 ± 0.0a | 0.0 ± 0.0a     | 18.8 ± 10.3b | 8.8 ± 9.3ab    | 0.0 ± 0.0a | 0.0 ± 0.0a     | 0.0 ± 0.0a | 0.0 ± 0.0a     |
| Hemiptera | 0.8 ± 1.5a | 0.3 ± 0.5a     | 2.0 ± 2.2a  | 1.3 ± 0.5a     | 0.3 ± a    | 0.3 ± 0.5a     | 1.0 ± 0.0a | 0.3 ± 0.6a     |
| Homoptera | 15.8 ± 2.4ab| 17.5 ± 10.0ab  | 18.8 ± 7.4ab| 27.0 ± 8.0b    | 8.3 ± 3.5a| 4.0 ± 1.4a     | 53.3 ± 6.8c| 58.3 ± 8.1c    |
| Coleoptera| 0.0 ± 0.0a | 0.0 ± 0.0a     | 5.3 ± 4.6a  | 5.8 ± 4.9a     | 0.3 ± 0.5a| 0.0 ± 0.0a     | 24.7 ± 4.7b| 34.3 ± 4.2c    |
| Diptera   | 103.8 ± 41.7b| 114.8 ± 27.0bc| 139.5 ± 46.7c| 139.8 ± 22.6c  | 11.8 ± 7.8a| 9.8 ± 5.7a     | 65.3 ± 15.9ab| 74.0 ± 13.0abc |

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

| Index    | Ochang EGF | Ochang Gwangan | Ochang EGF | Ochang Gwangan | Jeonju EGF | Jeonju Gwangan | Jeonju EGF | Jeonju Gwangan |
|----------|------------|----------------|------------|----------------|------------|----------------|------------|----------------|
| Dominance (DI) | 0.57 ± 0.04cd | 0.60 ± 0.06d | 0.41 ± 0.06ab | 0.44 ± 0.08abc | 0.55 ± 0.04bcd | 0.50 ± 0.10bcd | 0.29 ± 0.05a | 0.30 ± 0.04a     |
| Diversity (H') | 2.01 ± 0.15ab | 1.96 ± 0.09a | 2.40 ± 0.07bc | 2.22 ± 0.10ab | 2.15 ± 0.12ab | 2.29 ± 0.28ab | 2.77 ± 0.09d | 2.67 ± 0.10cd   |
| Evenness (E)  | 0.65 ± 0.03a | 0.62 ± 0.03a | 0.84 ± 0.04b  | 0.85 ± 0.05b  | 0.61 ± 0.04a | 0.65 ± 0.09a | 0.80 ± 0.03b | 0.80 ± 0.03b    |
| Richness (Ri) | 3.47 ± 0.66a | 3.63 ± 0.12ab | 4.01 ± 0.52abc| 3.27 ± 0.53a | 4.85 ± 0.35 cd| 5.07 ± 0.39 cd| 5.16 ± 0.54d | 4.62 ± 0.24bcd  |

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1Genetically modified soybean. 2The results shown are the mean ± standard deviation, n = 3 or 4 replicates for each group, and Tukey’s honestly significant difference at p < 0.05 within row.
of insects within the same growing region got separated by the time of the surveys. The averages and deviations of the principal component scores showed a clearer scenario (Figure 3).

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

Figure 2. Average population densities in common plant-dwelling non-target insects and arachnids captured using vacuum suction in fields planted with two different genotypes of soybeans at Ochang and Jeonju, Korea, in 2016 and 2017. The results shown are the mean ± standard deviation, n = 3 or 4 replicates for each group. EGF: Genetically modified soybean. *: Tukey’s honestly significant difference at p < .05.
Discussion

The study investigated the effects of GM events expressed EGF on the occurrence and diversity of arthropod communities associated with soybean crops. Overall, there was a prominent increase in average population densities of insects from 2016 to 2017 on either of the genotypes both at Ochang and Jeonju. Here, the fact to be considered that the change in the diversity of arthropod community in soybean agroecosystems at the survey regions was found based on survey years but not between GM and non-GM events. In 2016, the growth of soybeans decreased due to high temperature and drought during the growing season from late July to mid-August (Figure A1); as a result, the density of insects associated with soybean crops was lower during this period. The findings corresponded to the results of the research where the effect of thioredoxin-gene-expressed transgenic soybean in comparison to non-GM soybean on associated insects and arachnids was examined, and an increase of insect populations over the period from 2016 to 2017 was marked irrespective of the genotypes of the crop.9

Ochang served as the site where the occurrence and diversity of insects were naturally higher than Jeonju. The geographical location of a region acts as an important factor to influence the abundance and diversity of arthropods. Between the current survey regions, Ochang is situated at a comparatively higher altitude, i.e. 85.0 m above sea level whereas Jeonju is at 50.0 m above sea level. It is known that mountains are biodiversity hotspots because of the richness of plants that harbor a lot of terrestrial life. As a hilly area, Ochang serves comparatively higher plant biodiversity than Jeonju. As a result, the occurrence of insects was higher at Ochang than that at Jeonju in both survey years. The current findings supported the results of the previous findings where a higher occurrence of insects was found in the soybean field of Ochang than Jeonju, and also no harmful impact of IGF (Insulin-like Growth Factor) soybean on arthropods over the non-GM soybean was indicated.8

The diversity and richness of the insects at both locations increased over time from 2016 to 2017 irrespective of the soybean genotypes. The unfavorable weather conditions were responsible for the reduced occurrence of insects on soybean crops in 2016. The survey site Jeonju was recorded to have the least population density of insects in 2016 considering both genotypes. On the contrary, the population density of the insects was the maximum at Ochang in 2017 on both EGF and Gwangan soybeans. The two-year survey reports suggested that there was a negligible impact of the biotech crop on the assemblage of arthropods in soybean fields. The variation in the composition of arthropod community of the two regions was detected in isolated years only. Therefore, genetic modification
in soybean crops was unlikely to affect the community of non-target arthropods.

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Figure A1. Representation of weather conditions at Ochang and Jeonju during the survey periods of 2016 and 2017