

Analysis of Arthropod Communities in Sunflower-cultivated Fields to Develop Risk Assessment Guidelines for LMO Used for Environmental Remediation

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ABSTRACT

Living modified organisms (LMOs) are managed by seven government agencies according to their use in South Korea. The Ministry of Environment is responsible for LMOs used for environmental remediation. This study aimed to develop guidelines for assessing potential risks posed by transgenic plants used for remediation to insect ecosystems by investigating arthropod communities in sunflower fields. A total of 2,350 insects and spiders belonging to 134 species of 10 orders and 71 families were collected from sunflower fields over four growth stages ranging from anthesis to seed maturity. At the R3 phase of flower-bud formation, Chironomidae sp. of a decomposer insect guild presented the highest density, while *Apis mellifera* of a pollinator guild was the most abundant in the R5.8 phase of flowering. During the R7 seed-filling phase and the R9 phase of seed maturity, herbivorous *Pochazia shantungensis* predominated. During the R9 phase, richness and diversity indices of arthropod communities were distinctly lower whereas their dominance indices were significantly higher than those at other phases. In addition, the composition of arthropod communities was strongly correlated not only with the sampling date, but also with the sampling method depending on the growth stage of sunflowers. Our results suggest that appropriate sampling timing and methods should be considered in advance and that long-term field trials that cover a variety of environmental conditions should be carried out to evaluate potential risks to insect ecosystems.

Keywords: Arthropod, Diversity, Environmental remediation, Living modified organism, Risk assessment, Transgenic plant

Introduction

The impact of living modified organisms (LMOs) on non-target insects is a major ecological risk associated with the release of LMOs into the environment (Conner *et al.*, 2003). Transgenes or their associated metabolites may directly flow into insects through transgenic plants. Their accumulation in insects may successively influence upper trophic levels of the food chain including birds and mam-

mals. Many studies have reported effects of LMOs on target or non-target insects in the context of risk assessments, particularly effects of insect-resistant transgenic plants (Chen *et al.*, 2006; Duan *et al.*, 2002; Ferry *et al.*, 2007; Sharma & Pampapathy, 2006). In addition, researchers have also explored changes in biodiversity and composition of insect communities under the influence of transgenic plants (Dively, 2005; Whitehouse *et al.*, 2005).

In Korea, LMOs are managed by seven government agencies according to their use. The Ministry of Environment (MOE) oversees the import and export of LMOs used for environmental remediation and reviews the risk to natural ecosystems posed by LMOs that may be released into the environment (LMO Environmental Safety Center (LESC), 2021; Ministry of Trade, Industry and Energy (MOTIE), 2017). The National Institute of Ecology (NIE) supports the

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MOE's LMO safety management efforts by operating an LMO risk review agency and an LMO risk assessment institute (Lee *et al.*, 2015; Nam *et al.*, 2019). Worldwide, LMOs have been commercialized owing to their resistance to biotic and abiotic stresses, altered growth and product quality, and ability to control pollination systems (International Service for the Acquisition of Agri-biotech Applications (ISAAA), 2021). In Korea, most LMOs have been approved for food and agricultural use, with only a small number of LMOs approved for industrial, health, and medical purposes (Korea Biosafety Clearing House (KBCH), 2021). LMOs for environmental remediation have not yet been commercialized worldwide. However, as the development of LMOs for environmental remediation is increasing globally, it is necessary to prepare guidelines for safety management of such LMOs prior to their commercial use (Aken, 2009; Bennett *et al.*, 2003; James & Strand, 2009).

Unlike LMOs intended for use as human or animal food, LMOs used for environmental remediation can negatively impact other trophic levels in the food chain since they can be applied in a variety of contaminated sites. Butt *et al.* (2018) have reported that bioaccumulation of heavy metals along a soil, plant berseem (*Triofoium alexandrinum*), herbivorous insect aphid (*Sitobion avenae*), and predatory insect beetle (*Coccinella septempunctata*) food chain. In a food chain consisting of mustard (*Brassica juncea*), aphid (*Lipaphis erysimi*), and beetle (*Coccinella septempunctata*), Zn is biomagnified from mustard to beetles whereas Cd is bioconcentrated in aphids at the second trophic level, but not in beetles at the third trophic level (Dar *et al.*, 2017). Zhang *et al.* (2009) have demonstrated that herbivorous insects *Eligma narcissus* and *Holotrichia* can accumulate considerable amounts of heavy metals including Hg, Cd, and Pb from their host plants. Furthermore, the mobility of Cd and Cu from leaves in mulberry (*Morus alba* L.) to larva of silkworm (*Bombyx mori* L.) in the food chain is elevated with increasing concentrations of these metals in the soil (Prince *et al.*, 2001). These findings suggest a more significant bioaccumulation of environmental pollutants at upper trophic levels of ecosystems treated with LMOs that accumulate or migrate environmental pollutants. Consequently, careful considerations of these risks are required to assess the effect of LMOs used for environmental remediation on insect ecosystems.

To support commercial application of LMOs for environmental remediation in Korea, the NIE must establish appropriate risk assessment guidelines to facilitate MOE's LMO safety management system (Nam *et al.*, 2019; 2020; Nam & Han, 2020). Toward this end, the main objective of this study was to evaluate potential risks posed by LMOs used for environmental remediation and ultimately establish a standard for preserving the biological diversity of natural ecosystems treated by those LMOs. Sunflowers (*Heli-*

anthus annuus L.) are promising candidates for phytoremediation of inorganic and organic contaminants in water and soil (Prasad, 2007; Rizwan *et al.*, 2016). Here, we investigated the biodiversity and composition of arthropod communities in a sunflower field as a reference. Our findings could support the development of LMO risk assessment guidelines for environmental remediation.

Materials and Methods

Field experiment

Field experiments were conducted in a confined field (36°01'44.81" N, 126°43'20.5" E) located at the NIE, Seocheon-gun, Korea (Nam *et al.*, 2020). The sunflower cultivar used for this study was a standard "Jaeraejongja" type that could grow to a height of 1.6 - 1.8 m (Danong Co., Namyangju, Korea). Three randomized plots were established. Each plot had a size of 11.2 × 10 m. Seeds were sown directly into the soil at 0.6 × 0.8 m intervals in April 2020. A total of 360 plants were set up. Prior to sowing, all plots were fertilized with N, P, and K at rates of 6.1, 19.3, and 13.5 kg per 10 acres, respectively. Sunflowers were cultivated based on crop technology guidelines provided by the Rural Development Administration of Korea (Nongsara, 2020).

Phenotypic characteristics of sunflowers were examined using 10 individuals per plot. Plant height was measured from the place where the stem appeared above the soil to the tallest part of the plant. Stem diameter was determined for the stem that left the soil using a vernier caliper. Total numbers of leaves and flowers were counted. Flower size was measured as the diameter of the largest flower for each plant. Air temperature, precipitation, relative humidity, and daylight-hour data were obtained from the daily weather report provided by the Gunsan Meteorological Station (36°00'19.1" N, 126°45'40.9" E) (Korea Meteorological Administration (KMA), 2020). Meteorological data averaged over 30 years (1991–2020) were also acquired (KMA, 2020).

Arthropod sampling, identification, and classification

Arthropods in each plot of sunflower fields were sampled four times at 2–3 week-intervals, covering the period from sunflower anthesis to seed maturity (Fig. 1). The growth stage of sunflowers was classified according to the method of Grains Research and Development Corporation (GRDC, 2017). The first sampling was performed during the R3 phase at 62 days after sowing when the flower bud was formed. The second sampling was performed during the R5.8 flowering stage on day 82 after sowing. The third sampling was performed during the R7 seed-fill phase at 97 days after sowing. The fourth sampling was performed during the R9 seed physiological maturity stage at 116 days post-sowing.

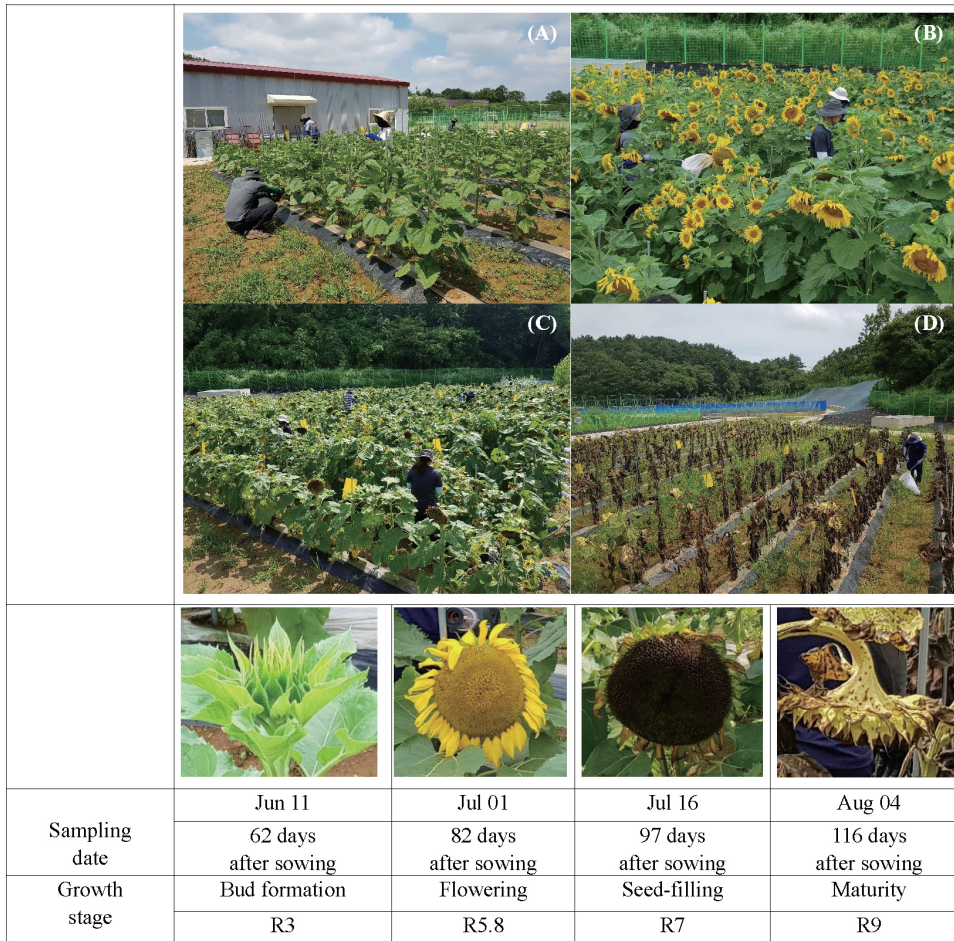


Fig. 1. Representative photographs of sunflowers at each sampling date.

Arthropod samples were collected using an insect net and suction trap. Manned observation was performed using a sticky trap. All species that frequently appeared in sunflower-cultivated fields were targeted. Collection was conducted 20 times per plot using a 40-cm-diameter insect net with a sweeping and beating method. Arthropod suction was performed for sunflower plants from top to bottom using an engine-type aspirator and suction pipe. For flying insects, a total of 24 sticky traps 25 × 15 cm, Iljintype Co., Gimhae, Korea) were installed in the field for each sampling period.

Arthropods collected from each plot were put into a ziplock bag, transferred to the laboratory, and identified through visual observation using a stereoscopic microscope (SZX16; Olympus, Tokyo, Japan) and stored by liquid immersion in 95% ethanol. Identified arthropods were finally classified using the national species database of Korea (National Institute of Biological Resources (NIBR), 2020) and other insect illustrated books.

Statistical analyses

Arthropod biodiversity was analyzed by calculating the

McNaughton's dominance index (McNaughton, 1967), Margalef species richness index (Margalef, 1958), Shannon-Weaver diversity index (Pielou, 1966), and Pielou's species evenness index (Pielou, 1975). The effect of sampling date on arthropod biodiversity was evaluated by one-way analysis of variance (ANOVA) using STATISTICA (StatSoft Inc., Tulsa, OK, USA). Statistically significant differences between means were identified using Duncan's multiple range test ($p < 0.05$). Non-metric multidimensional scaling (NMDS) analysis and permutational multivariate analysis of variance (PERMANOVA) were used to compare changes in arthropod communities according to sampling data and method using Primer 6 version 6.1.13 and Permanova+ version 1.0.3 software (PRIMER-E Ltd., Plymouth, UK).

Results

Phenotypic characteristics of sunflowers

Changes in meteorological conditions at the field site were monitored over the entire experimental period (Fig. 2). Average temperature, precipitation, relative humidity,

and daylight hours from sowing date to sunflower harvest time were 20.8°C, 8.9 mm, 80.5%, and 6.1 h, respectively. Temperature, relative humidity, and daylight hours were similar to mean values over a 30-year period at the field site. However, the average precipitation during the experimental periods increased by 61.8% compared to the 30-year average. In particular, the number of precipitation days in July corresponding to the flowering stage and physi-

ological maturity of seeds was greatly elevated.

Overall, phenotypic characteristics of sunflowers including stem diameter, numbers of leaves and flowers per plant, and flower size did not differ significantly according to the growth stage (Table 1). However, plant heights of sunflowers at R5.8 and R7 phases were 47.5% and 38.6% taller than those at the R3 phase, respectively.

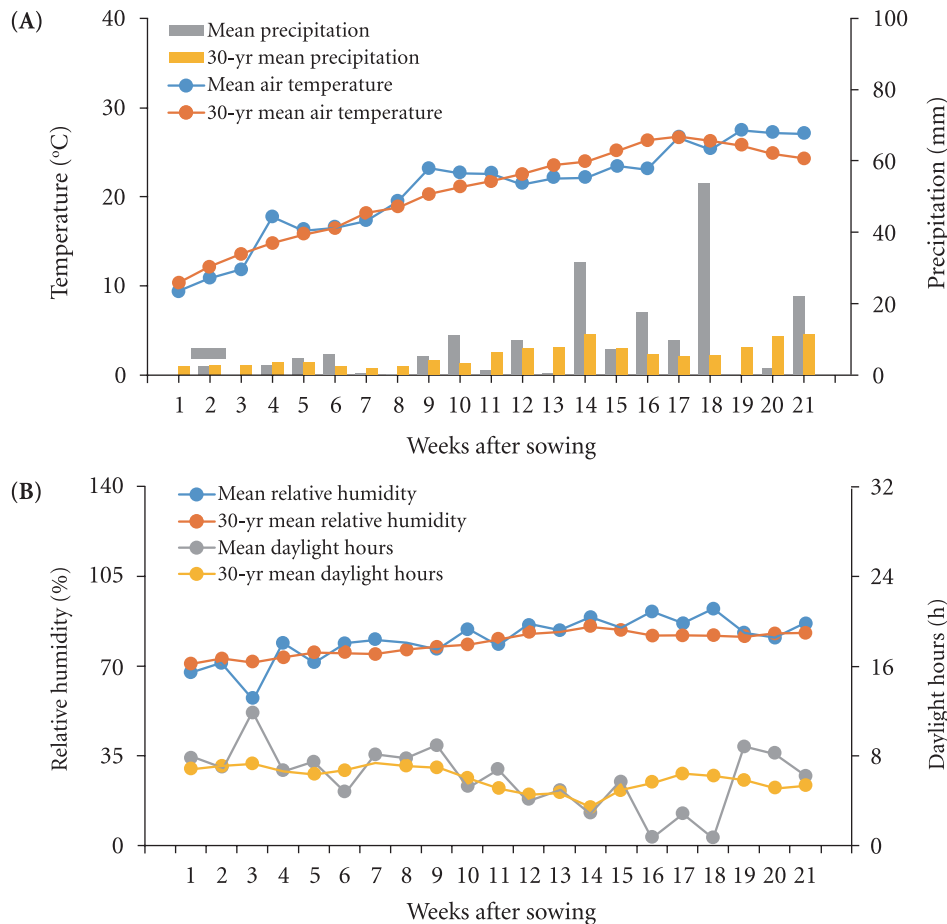


Fig. 2. Meteorological characteristics during experimental periods. Weekly air temperature and precipitation (A) and relative humidity and daylight hours (B) are shown. Data were obtained from the daily weather report of the Gunsan Meteorological Station (36°00' 19.1" N, 126°45' 40.9" E) (KMA, 2020).

Table 1. Phenotypic characteristics of sunflowers at different growth stages

Parameters	<i>p</i> -value	Jun 09 (R3)	Jul 01 (R5.8)	Jul 16 (R7)	Aug 04 (R9)
Plant height (cm)	< 0.001	86.7 ± 4.1 ^c	128.0 ± 5.8 ^a	120.3 ± 8.8 ^a	105.7 ± 7.2 ^b
Stem diameter (cm)	0.698	2.4 ± 0.4 ^a	2.8 ± 0.5 ^a	2.8 ± 0.6 ^a	2.8 ± 0.5 ^a
No. of leaves / plant	0.134	28.7 ± 3.9 ^a	39.0 ± 8.2 ^a	45.7 ± 12.2 ^a	-
No. of flowers / plant	0.614	-	5.0 ± 3.3 ^a	7.4 ± 3.4 ^a	4.9 ± 3.2 ^a
Flower size (cm)	0.434	-	25.7 ± 1.9 ^a	26.1 ± 4.4 ^a	22.6 ± 3.4 ^a

Data are presented as means ± standard deviations (*n* = 3). *P*-values are based on one-way ANOVA. Values within rows followed by the same letters are not significantly different at *p* < 0.05 per Duncan's test.

Changes in distribution of arthropod species according to growth stage of sunflowers

A total of 2,350 arthropod individuals including insects and spiders were collected from sunflower-cultivated fields. They belonged to 10 orders, 71 families, and 134 species (Table 2). Numbers of individuals collected during R3, R5.8, R7, and R9 phases were 638, 468, 638, and 606, respectively. During the R3 phase, individuals from four orders (Araneae, Hemiptera, Coleoptera, and Diptera) of arthropods accounted for 78.0% of total families and 82.0% of total species. Among these arthropods, 247 Diptera (38.7%), 188 Coleoptera (29.5%), and 160 Hemiptera (25.1%) individuals made these overwhelmingly dominant orders, accounting for over 93.3% of total arthropods captured. Among total samples collected at the R5.8 phase,

Hemiptera, Coleoptera, and Araneae accounted for the largest proportion of arthropods, representing 37.3%, 18.6%, and 15.3% of total species, respectively. The most prevalent order, Hemiptera, had 255 individuals, accounting for more than half of total arthropods collected. The abundance of Hemiptera, Araneae, and Coleoptera, which constituted about 37.3%, 18.6%, and 16.9% of the total species, respectively, was higher during phase R7. A total of 520 Hemiptera individuals (81.5% of total individuals) belonged to 11 (31.4%) families and 22 (37.3%) species. The R9 phase showed a similar pattern to the R7 phase. During the R9 phase, numbers of species and individuals in the order of Hemiptera were 21 (51.2% of total species) and 531 (87.6% of total individuals), respectively.

Table 2. Numbers of species and individuals of each arthropod order surveyed at each sampling date

Sampling date (Growth stage)	Order	No. of Families	No. of species	% of species	No. of individuals	% of individuals
Jun 11 (R3)	Araneae	6	8	13.1	9	1.4
	Lepidoptera	3	4	6.6	7	1.1
	Hemiptera	9	17	27.9	160	25.1
	Coleoptera	10	18	29.5	188	29.5
	Orthoptera	1	1	1.6	1	0.2
	Hymenoptera	4	5	8.2	25	3.9
	Odonata	1	1	1.6	1	0.2
	Diptera	7	7	11.5	247	38.7
Subtotal no.		41	61	100.0	638	100.0
Jul 01 (R5.8)	Araneae	5	9	15.3	21	4.5
	Lepidoptera	4	5	8.5	20	4.3
	Hemiptera	13	22	37.3	255	54.5
	Coleoptera	7	11	18.6	71	15.2
	Hymenoptera	3	4	6.8	64	13.7
	Odonata	2	2	3.4	2	0.4
	Dermaptera	1	1	1.7	1	0.2
	Diptera	4	4	6.8	33	7.1
	Chrysopidae	1	1	1.7	1	0.2
Subtotal no.		40	59	100.0	468	100.0
Jul 16 (R7)	Araneae	6	11	18.6	43	6.7
	Lepidoptera	2	2	3.4	11	1.7
	Hemiptera	11	22	37.3	520	81.5
	Coleoptera	5	10	16.9	39	6.1
	Orthoptera	3	4	6.8	4	0.6
	Hymenoptera	2	3	5.1	13	2.0
	Odonata	2	2	3.4	3	0.5
	Diptera	4	5	8.5	5	0.8
Subtotal no.		35	59	100.0	638	100.0
Aug 04 (R9)	Araneae	5	11	26.8	34	5.6
	Lepidoptera	1	1	2.4	2	0.3
	Hemiptera	11	21	51.2	531	87.6
	Coleoptera	2	5	12.2	29	4.8
	Orthoptera	1	1	2.4	2	0.3
	Odonata	1	1	2.4	1	0.2
	Diptera	1	1	2.4	7	1.2
Subtotal no.		22	41	100.0	606	100.0
Total no.		71	134		2350	

The abundance of ten most predominantly observed arthropod species in each growth stage of sunflowers is displayed in Table 3. Among arthropod species, Chironomidae sp. (Diptera; Chironomidae—decomposer insect guild) showed the highest occurrence (37.3%), followed by *Harmonia axyridis* (Coleoptera; Coccinellidae) of a predatory insect guild and *Nysius plebejus* (Hemiptera; Lygaeidae) of a herbivorous insect guild (accounting for 14.4% and 14.3%, respectively). In the R5.8 phase, *Apis mellifera* (Hymenoptera; Apidae) of a pollinator guild showed the highest density at 11.8%. Subsequently, herbivorous insects *Dolycoris baccarum* (Hemiptera; Pentatomi-

dae), *Adelphocoris suturalis* (Hemiptera; Miridae), *N. plebejus*, and *Cletus punctiger* (Hemiptera; Coreidae) accounted for 11.3%, 11.3%, 7.9%, and 7.9%, respectively. *Pochazia shantungensis* (Hemiptera; Ricaniidae) and *D. baccarum* were dominant species during the R7 phase, accounting for 27.1% and 14.9%, respectively. In addition, *Corythucha marmorata* (Hemiptera; Tingidae), *Nysius hidakai Nakatani* (Hemiptera; Lygaeidae), and Cicadellidae sp. (Hemiptera; Cicadellidae) accounted for 6.9%, 5.5%, and 5.0%, respectively. During phase R9, *P. shantungensis* of the herbivorous group was the most abundant one, accounting for 71.9%.

Table 3. Abundance of ten most-observed arthropod species on each sampling date

	Jun 11 (R3)	Jul 01 (R5.8)	Jul 16 (R7)	Aug 04 (R9)
1	Chironomidae sp. 37.3%	<i>Apis mellifera</i> 11.8%	<i>Pochazia shantungensis</i> 27.1%	<i>Pochazia shantungensis</i> 71.9%
2	<i>Harmonia axyridis</i> 14.4%	<i>Dolycoris baccarum</i> 11.3%	<i>Dolycoris baccarum</i> 14.9%	<i>Nysius plebejus</i> 2.5%
3	<i>Nysius plebejus</i> 14.3%	<i>Adelphocoris suturalis</i> 11.3%	<i>Corythucha marmorata</i> 6.9%	<i>Nysius hidakai Nakatani</i> 2.3%
4	<i>Nonarthra cyanea</i> 3.9%	<i>Nysius plebejus</i> 7.9%	<i>Nysius hidakai Nakatani</i> 5.5%	<i>Plagiodera versicolora</i> 2.3%
5	<i>Coccinella septempunctata</i> 3.4%	<i>Cletus punctiger</i> 7.9%	Cicadellidae sp. 5.0%	<i>Dolycoris baccarum</i> 2.0%
6	<i>Propylea japonica</i> 2.5%	<i>Apolygus lucorum</i> 6.4%	<i>Adelphocoris suturalis</i> 4.5%	<i>Propylea japonica</i> 1.7%
7	<i>Athalia rosae ruficornis</i> 2.4%	Chironomidae sp. 6.2%	<i>Apolygus lucorum</i> 3.3%	<i>Chlorita flavescens</i> 1.7%
8	<i>Malachius prolongatus</i> 2.2%	<i>Harmonia axyridis</i> 4.5%	<i>Yemma exilis</i> 3.0%	<i>Psammotettix striata</i> 1.5%
9	<i>Apolygus lucorum</i> 2.2%	<i>Propylea japonica</i> 3.2%	<i>Chlorita flavescens</i> 3.0%	Tipulidae sp. 1.2%
10	<i>Adelphocoris suturalis</i> 1.9%	<i>Coccinella septempunctata</i> 2.8%	<i>Nysius plebejus</i> 2.4%	<i>Eurydema dominulus</i> 1.2%

Analysis of biodiversity and arthropod community composition

In this study, the McNaughton’s dominance index (McNaughton, 1967), Margalef species richness index (Margalef, 1958), Shannon-Weaver diversity index, and Pielou’s species evenness index (Pielou, 1975) were compared to determine differences in biodiversity of arthropod

communities according to sunflower growth stage (Table 4). The dominance index of the arthropod community was significantly higher at phase R9 than that at other phases. By contrast, indices for richness and diversity were significantly greater at phases R3, R5.8, and R7 phases than at phase R9. The evenness index showed no significant differences ($p = 0.075$) according to sunflower growth stage.

Table 4. Analyses of arthropod communities surveyed on each sampling date

Indices	p-value	Jun 11 (R3)	Jul 01 (R5.8)	Jul 16 (R7)	Aug 04 (R9)
Dominance index	0.007	0.26 ± 0.27 ^b	0.03 ± 0.01 ^b	0.12 ± 0.08 ^b	0.66 ± 0.17 ^a
Richness index	0.049	7.70 ± 0.59 ^a	7.27 ± 0.44 ^a	7.24 ± 0.51 ^a	5.37 ± 1.54 ^b
Diversity index	0.041	3.22 ± 0.11 ^a	3.26 ± 0.10 ^a	3.23 ± 0.13 ^a	2.64 ± 0.45 ^b
Evenness index	0.075	0.92 ± 0.02 ^a	0.95 ± 0.01 ^a	0.94 ± 0.02 ^a	0.87 ± 0.06 ^a

Data are presented as means ± standard deviations ($n = 3$). P-values are based on one-way ANOVA. Values within rows followed by the same letters are not significantly different at $p < 0.05$ per Duncan’s test.

NMDS analysis showed distinct groups depending on sampling dates and methods (Fig. 3). In addition, PERMANOVA indicated that the structure and composition of arthropod communities significantly varied based on sampling date ($p = 0.001$), sampling method ($p = 0.001$), and interactions between sampling date and sampling method ($p = 0.001$) (Table 5).

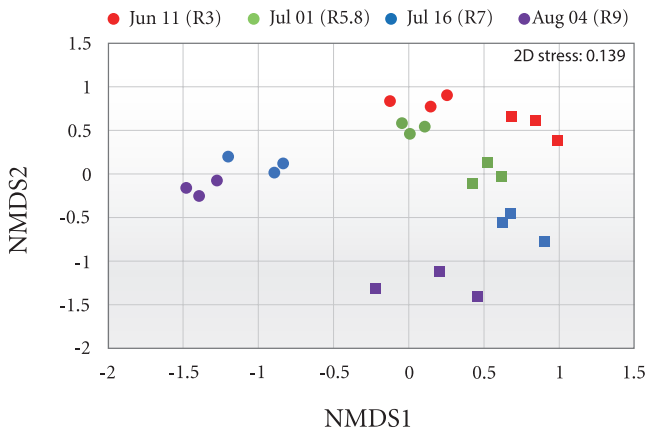


Fig. 3. Non-metric multidimensional scaling (NMDS) ordination plot of Bray-Curtis community dissimilarities based on arthropod species surveyed at each sampling date with different sampling methods (stress value = 0.139). Square, direct observation using an insect net and suction trap; Circle, manned observation using a sticky trap

Discussion

The diversity and composition of arthropods in the field are primary components of ecological risk assessments for transgenic plants because they reflect relationships between organisms and the environment (Wang & Guan, 2020). Many studies have reported no significant differences in arthropod communities between transgenic and non-transgenic plant fields, suggesting that transgenic plants present a low risk of negative consequences

(Chen *et al.*, 2006; Oh *et al.*, 2018; Zuo *et al.*, 2018). However, in one study, although no cumulative effect was observed after two consecutive years of investigation in the field, transgenic maize expressing phytase showed a significant decrease in the number of species belonging to the herbivorous group compared to the number in non-transgenic maize fields (Wang & Guan, 2020). Here, we monitored the abundance and composition of arthropod communities in a field cultivated with sunflowers that were candidates for use in environmental remediation. Results showed that the number of families and species of arthropods was reduced in phase R9, whereas the number of individuals declined in the R5.8 phase. Furthermore, the dominance index of arthropod communities was significantly higher in phase R9 than in other phases, whereas richness and diversity indices were considerably lower in phase R9.

Numerous studies have reported a significant correlation between seasonal variation and arthropod abundance (Campuzano *et al.*, 2020; Shakir & Ahmed, 2015). Climatic factors are directly related to seasonal changes. They can influence the population and community dynamics of insects including their survival, fecundity, and development (Khaliq *et al.*, 2014). The average precipitation during our experiment was elevated by 61.8% compared to the 30-year mean, especially in the period from plant flowering to physiological maturity of seeds. Shakir and Ahmed (2015) have found that high soil temperatures and low relative humidity can elevate the abundance of soil arthropods, while total rainfall is not correlated with their abundance. Zhu *et al.* (2014) have indicated that increased or decreased precipitation can significantly alter the biomass of the whole plant community and lead to changes in insect diversity and trophic structure, especially in herbivores. Combined with these results, our research suggests the importance of long-term field trials over several years under different seasons and weather conditions to draw valid conclusions about the impact of these factors on insect ecosystems.

Table 5. Permutational multivariate analysis of variance (PERMANOVA) results for arthropod species surveyed at each sampling date with different sampling methods

Source	DF ¹	SS ²	MS ³	F ⁴	P ⁵
Sampling date	3	21288	7096.1	5.872	0.001
Sampling method	1	14072	14072.0	11.644	0.001
Date × Method	3	13289	4429.6	3.666	0.001
Res	16	19335	1208.4	-	-
Total	23	67984	-	-	-

DF¹: degrees of freedom; SS²: sum of squares; MS³: mean square; F⁴: F-statistic; P⁵: P-value.

A major ecological concern about the environmental release of transgenic plants for environmental remediation is bioaccumulation of pollutants into higher trophic levels of the food chain from transgenic plants (Peralta-Videa *et al.*, 2009). Butt *et al.* (2018) have reported that the accumulation of Zn, Cd, and Pb from host plants is much higher in grasshopper *Ailopus thalassinus* than in aphid *S. avenae* owing to different feeding behaviors such as sucking vs. chewing of these two insects. Zhang *et al.* (2009) have demonstrated that considerable amounts of Hg, Cd, and Pb are accumulated in carnivorous insects in the *Locusta migratoria manilensis*–spider and –mantis food chain, but not in the *Acrida Chinensis*–spider and –mantis food chain. These studies suggest that the bioaccumulation of heavy metal in insects can vary by feeding style, species, and type of pollutants. In the present study, dominant insects differed greatly according to the growth stage of sunflowers. Therefore, bioaccumulation of environmental pollutants should be considered at various plant growth stages to effectively assess the risk posed by transgenic plants used for environmental remediation.

The abundance of arthropods collected from fields is also influenced by collection methods, including sweeping, beating, hand collecting, vacuum sampling, branch clipping, chemical knockdown, and sticky trapping (Moir *et al.*, 2005). Different collection methods can result in differences in the effort and efficacy of arthropod biodiversity investigations, such as differences in species and individual numbers, the time spent to collect and sort samples, and damage to plants (Swart *et al.*, 2017). Our NMDS and PERMANOVA results showed that the composition of arthropod communities was significantly affected by whether direct or indirect sampling methods were employed and by the growth stage of sunflowers. Therefore, it is important to select an optimal method for arthropod collection depending on the objective of each study (Yi *et al.*, 2012).

Previous studies have revealed that stress conditions can alter the accumulation of key metabolites in host plants and subsequently influence metabolite profiles, growth, and reproduction of insects at higher trophic levels (Nam *et al.*, 2017). Accordingly, in addition to accumulation of environmental pollutants, abiotic stress that can affect host plants and associated insects should be considered in risk assessments of transgenic plants used for environmental remediation. In the present study, we identified the biodiversity and composition of arthropod communities in sunflower fields without treating plots with any inorganic or organic contaminants. Further studies under contaminated environmental conditions are needed to provide valuable insights to support the development of guidelines for assessing potential risks posed by transgenic plants to insect ecosystems.

Conflicts of Interest

The authors declare that they have no competing interests.

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