Environ. Biosafety Res. 9 (2010) 101–112
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DOI: 10.1051/ebr/2010010

Available online at:
www.ebr-journal.org

Assessing the effects of cultivating genetically modified glyphosate-tolerant varieties of soybeans (*Glycine max* (L.) Merr.) on populations of field arthropods

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We assessed the effects of cultivating two genetically modified (GM) glyphosate-tolerant soybean varieties (*Glycine max* (L.) Merr.) derived from Event 40-3-2 and a Japanese conventional variety on arthropods under field conditions, with weed control using glyphosate and conventional weed control for two years. Plant height and dry weight of the conventional variety were significantly larger than those of the GM varieties, but the GM varieties bore more pods than the conventional variety. We found arthropods of nine taxonomic orders (Araneae, Acari, Thysanoptera, Homoptera, Heteroptera, Coleoptera, Diptera, Lepidoptera, and Hymenoptera) on the plants. The arthropod incidence (number per plant unit weight pooled for each taxonomic order) on the soybean stems and leaves generally did not differ significantly between the GM and conventional varieties. However, the incidence of Thysanoptera and total incidence (all orders combined) were greater on the GM variety in the second year. The weed control regimes had no significant influence on the arthropod incidence on the soybean stems and leaves. The number of flower-inhabiting Thysanoptera (the dominant arthropod in the flowers) was not significantly different between the GM and conventional varieties. *Asphondylia yushimai* (Diptera, Cecidomyiidae) was more numerous on the pods of the GM variety in both years. Neither the soybean variety nor the weed control regime significantly affected the density of soil macro-organisms. However, the glyphosate weed control affected arthropods between the rows of plants by decreasing the abundances of Homoptera, Heteroptera, Coleoptera and Lepidoptera, and diversity of arthropods.

Keywords: biodiversity / arthropods / environmental impact / genetically modified crops / glyphosate / herbicide tolerant crops / soil macro-organism / weed control

INTRODUCTION

Commercial cultivation of genetically modified (GM) crops is increasing, reaching 134 million ha in 25 countries in 2009 (James, 2009), equivalent to *ca.* 8.8% of the world's cropland. Among those crops, GM soybeans had the largest cultivated area (69.2 million ha), corresponding to 52 and 72% of the total area of GM crops and of the total of world soybean cultivation, respectively. Although the area cultivated using insect-resistant crops with a single resistance trait, such as *Bacillus thuringiensis* delta endotoxins (*Bt*), has begun to stabilize, the cultivation of crops with a single trait for herbicide tolerance (HT) and of those with multiple traits (*i.e.*, HT and insect-resistance) is still increasing, reaching 83.6 million ha and 28.7 million ha, respectively; thus, 83% of GM crops have HT traits (James, 2009). To protect human health and the environment from the possible adverse effects of the products of modern biotechnology, the Cartagena Protocol on Biosafety was adopted in 2000 under the Convention on Biological Diversity (Convention on Biological Diversity, 2000). An assessment of risks to biodiversity is therefore essential whenever we introduce GM crops (Andow and Zwahlen, 2006). Japan ratified the Cartagena Protocol and enacted the domestic Cartagena Law in 2003 (Ministry of Environment, 2003). According to this law, environmental risk assessments are required for GM crops prior to commercial cultivation or importation.

Many studies have assessed risks of GM crops to biodiversity (see reviews by Ammann, 2005; Andow and Hilbeck, 2004; Andow and Zwahlen, 2006; Conner et al., 2003; Dale, 2002; Dale et al., 2002; Devos et al.,

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2008; Lövei and Arpaia, 2005; Marvier et al., 2007; Saito and Miyata, 2005; Shirai, 2007), although some of these studies chose an overly broad definition of biodiversity that included the diversification by GM crops with novel genes (Ammann, 2005) rather than the conservation of endemic biodiversity. However, most of these studies have focused on insect-resistant GM crops, including those bearing the *Bt* trait (*e.g.*, Conner et al., 2003; Lövei and Arpaia, 2005; Marvier et al., 2007; Saito and Miyata, 2005; Shirai, 2007), and studies of the impact of GM herbicide-tolerant (GMHT) crops on arthropods have been relatively scarce (Bitzer et al., 2002; Buckelew et al., 2000; Carpenter et al., 2002; Dale et al., 2002). Recently, the latter studies have become more numerous (Albajes et al., 2009; Bourassa et al., 2010; Brondani et al., 2008; Cárcamo and Blackshaw, 2007).

Large-scale field evaluations of the effects of GMHT beets, maize, and oilseed rape on farmland biodiversity ("farm-scale evaluations") were carried out in the UK (Brooks et al., 2003; Champion et al., 2003; Firbank, 2003; Firbank et al., 2003; Hawes et al., 2003; Heard et al., 2003a, 2003b, 2006; Perry et al., 2003; Roy et al., 2003; Squire et al., 2003). These studies surveyed 14 taxonomic groups (higher plants, gastropods, mites, spiders, springtails, and nine insect groups) as biodiversity indicators in fields cultivated with GMHT and conventional varieties. They concluded that: (1) Weed diversity was little affected by the GMHT or conventional cropping systems, but that the GMHT treatments generally decreased the weed seed bank. The exception was GMHT corn, for which weed density was higher than in the conventional treatment, leading to large long-term effects on weed populations (Heard et al., 2003a, 2003b). (2) GMHT crop management affected the counts of many surface-active invertebrates, with either increasing or decreasing captures depending on the crops and on the phenology and ecology of the species (Brooks et al., 2003). (3) GMHT management had no strong effects on the majority of the higher taxa of aerial and epigeal arthropods, but clearly affected the pollinators (bees and butterflies) and the detritivores (Collembola), with either increasing or decreasing captures depending on the crop and on the phenology and ecology of the species (Haughton et al., 2003). The cover, flowering, and seed production of plants were lower in the GMHT field margins than in the margins of the conventionally managed fields, although the reverse was true in corn, and butterfly populations were adversely affected in field margins of GMHT spring oilseed rape (Roy et al., 2003). (4) The changes in weed communities that resulted from the introduction of new herbicide regimes affected herbivores, detritivores, pollinators, predators, and parasites through trophic relations (Hawes et al., 2003). The mechanistic causes of the observed results in the "farm-scale evaluations", however, remained obscure due to the imperfect experimental design (Andow, 2003).

Researchers also noted that their findings could not be extrapolated to other crop traits and socio-environmental systems (Crawley et al., 1993; Firbank et al., 2003). Andow (2003) pointed out that the "farm-scale evaluations" of GMHT crops in the UK were a first step toward understanding the effects of these crops on the environment, but the prevailing scientific consensus is that the environmental risks of GM organisms must be assessed on a case-by-case basis (Andow and Hilbeck, 2004; Convention on Biological Diversity, 2000; Craig et al., 2008). Furthermore, the direct impacts of growing GMHT crops and the associated indirect effects of the new weed control regime used for cultivation of GMHT crops were not separately assessed in those studies. The distinction between these impacts is crucial for an adequate assessment of the environmental risk posed by GMHT crops.

Soybeans (*Glycine max* (L.) Merr.) are one of the most important foods and food materials in Japan, where 4 Mt of soybeans are consumed annually. The soybean crop that originated in East Asia has more diverse communities of associated arthropods in this region (including Japan) than in other areas where this crop was introduced (Kogan, 1981). In addition, a wild species (*Glycine soja* Sieb. et Zucc.) that can cross with soybeans grows in East Asia, resulting in a potential introgression of transgenes. Thus, biodiversity assessment to determine the impact of GM soybean is important in East Asia, including Japan. The guidelines for field assessments under the domestic Cartagena Law (Ministry of Finance et al., 2003), which includes guidelines for Type-1 Use of GM organisms (GMOs) and measures to prevent their spread beyond the facilities that are investigating them, require an assessment of the following properties of GM plants: (1) competitiveness, (2) production of harmful substances, (3) crossability with other species, and (4) other properties, such as ones that affect wildlife by changing the ecosystem structure or functions. These guidelines have adopted the concept of "substantial equivalence" (Kuiper et al., 2001; OECD, 1993, 1998): if the crop species to which the recipient belongs has long been cultivated in Japan, as is the case for soybean, the assessment may be based on a comparison between the recipient variety and conventional varieties. In terms of crossability, Yoshimura et al. (2006) confirmed that a 10-m isolation distance was sufficient to prevent pollen flow from GM soybeans to conventional soybeans based on field surveys of the out-crossing rate and of pollinators.

The present study focused on assessing the production of harmful substances and on the impacts on arthropods caused by the changing ecosystem that results from cultivation of GM soybean (*i.e.*, properties (2) and (4) in the Japanese guidelines). These assessments must identify whether there is any harmful effect observed in the farm environment and if so whether it is caused by the production of one or more harmful substances by the GM crop or by the weed management system introduced to sustain cultivation of the GM crop. Thus, we built on previous research by assessing the effects of cultivating glyphosate-tolerant GM soybeans on the arthropods that are present on the plants, between the rows of plants, and in the soil. To do so, we established a factorial experiment that used both GM and conventional varieties under two weed control regimes (glyphosate or conventional weed control) for two years. Since GMHT crops have been developed to facilitate weed control, the influences of their cultivation on arthropods, if any, are considered to be non-target effects.

RESULTS

Soybean varieties

The plant height and dry weight of the conventional variety (Tachinagaha) were significantly larger than those of either GM variety (plant height: $F_{1,\,56\,(2004)} = 46.223$, $F_{1,\,56\,(2005)} = 35.562$, $p < 0.01$; dry weight: $F_{1,\,56\,(2004)} = 30.666$, $F_{1,\,56\,(2005)} = 11.448$, $p < 0.01$). The soybean growth was better in 2004 than 2005. The number of flowers per soybean plant differed significantly between the varieties in 2004 ($F_{1,\,12} = 66.438$, $p < 0.01$; conventional > GM) but not in 2005 ($F_{1,\,12} = 1.394$, $p > 0.05$). The number of pods per plant was significantly larger in the GM variety in both years ($F_{1,12\,(2004)} = 31.203$, $F_{1,\,12(2005)} = 12.613$, $p < 0.01$). However, the dry weight of pods per plant did not differ significantly between the GM and conventional varieties ($F_{1,12\,(2004)} = 0.697$, $F_{1,\,12\,(2005)} = 0.585$, $p > 0.05$). Glyphosate application significantly increased the number of pods in 2004 ($F_{1,\,12} = 9.672$, $p < 0.01$), resulting in a significant difference in pod dry weight ($F_{1,\,12} = 8.129$, $p < 0.05$). There were no significant interactions.

Arthropods on the soybean stems and leaves

Because the plant size varied during the growing period and between the varieties, we standardized the arthropod abundance on the soybean stems and leaves as the number per dry weight of stem and leaves (*i.e.*, the incidence). The incidences of the arthropod orders changed during the growing period, but differences were only significant for Thysanoptera in 2005, Homoptera in 2004, and the total incidence (all orders combined) in 2005 ($p < 0.01$; Tab. 1, Appendix 1 available online at www.ebr-journal.org). There was no significant difference in the incidence of arthropods between the GM and conventional varieties, except for Thysanoptera and the total incidence in 2005; both incidences were significantly larger on the GM variety ($p < 0.01$). The weed control regimes had no significant effect on the arthropod incidences. There were no significant interactions.

Arthropods in flowers

Arthropods from the Acari, Thysanoptera, Homoptera, Heteroptera, Lepidoptera, and Hymenoptera were found on or in the flowers. However, except for the Thysanoptera, we found few of these taxa. Thus, we only analyzed the number of Thysanoptera. The number of Thysanoptera per flower did not differ significantly between the GM and conventional varieties or between weed control regimes in either year, and there were no significant interactions (Tab. 2, Appendix 2 available online).

Arthropods in pods

Arthropods from the Araneae, Acari, Thysanoptera, Homoptera, Heteroptera, Coleoptera, Diptera, Lepidoptera, and Hymenoptera were found on or in the pods. Although the soybean pod gall midge (*Asphondylia yushimai* Yukawa et Uechi, Diptera, Cecidomyiidae), a dipterous gall former, was dominant, the numbers of individuals from other taxa were low. Thus, we analyzed the incidence of *A. yushimai* per unit pod dry weight and the total incidence pooled over all the taxa. The incidence of *A. yushimai* was significantly larger in the GM pods in both years ($p < 0.01$; Tab. 2, Fig. 1, and Appendix 2 available online). When the total incidences were compared, a similar tendency was observed, but the difference was not significant (Fig. 1). Glyphosate application also significantly increased the incidence of *A. yushimai*, particularly in pods of the GM variety in 2004, but not in 2005 (Tab. 2).

Soil macro-organisms

Although the density of the soil organisms fluctuated significantly during the growing season ($p < 0.05$ or $p < 0.01$; Tab. 3, Appendix 3 available online), there were no significant differences between the soybean varieties or between the weed control regimes in either year, and no significant interactions.

Arthropods between the rows

Table 4 shows that two arthropod orders surveyed between the rows in 2005 were significantly influenced by

Table 1. Analysis of variance results for the number of arthropods per unit dry weight of soybean stems and leaves ("incidence"), enumerated at the order level, in the 2004 and 2005 experiments.

Arthropod order	Year	Factor (56)[1]			
		Month (2)[2]	Variety (V, 1)[3]	Weed control regime (W, 1)	$V \times W$ (1)
Araneae	2004	0.013[4]	0.001	0.003	0.002
	2005	1.886	0.072	1.254	0.004
Acari	2004	2.057	0.170	0.048	0.003
	2005	3.069	3.708	2.013	0.613
Thysanoptera	2004	1.568	1.544	0.031	0.014
	2005	127.173**	15.763**	0.228	0.011
Homoptera	2004	9.455**	0.687	0.716	0.602
	2005	0.929	2.648	0.325	0.070
Heteroptera	2004	0.343	0.053	0.001	0.145
	2005	2.897	2.093	0.071	0.983
Coleoptera	2004	0.527	1.111	1.077	0.756
	2005	0.259	0.017	0.209	0.044
Diptera	2004	0.411	0.264	0.135	0.205
	2005	0.448	0.019	1.458	0.140
Lepidoptera	2004	0.018	0.299	0.012	0.086
	2005	2.464	1.383	0.193	0.180
Hymenoptera	2004	0.437	0.200	0.077	0.939
	2005	0.232	1.437	0.543	0.086
Total incidence	2004	2.618	0.732	0.389	0.257
	2005	75.797**	18.703**	0.007	0.003

[1]Degrees of freedom for the errors pooled over 2004 and 2005.
[2] Numbers in parentheses indicate degrees of freedom (d.f.).
[3] The GM varieties in 2004 and 2005 were AG3701RR and AG3802RR, respectively.
[4] Numbers are F-values.
**, $p < 0.01$.

Table 2. Analysis of variance results for the number of arthropods per flower or per unit of pod dry weight in the 2004 and 2005 experiments.

Arthropod	Year	Factor (12)[1]		
		Variety (V,1)[2,3]	Weed control regime (W, 1)	$V \times W$ (1)
Flowers				
Thysanoptera	2004	4.264[4]	0.003	0.001
	2005	0.351	0.569	0.654
Pods				
Asphondylia yushimai	2004	12.928**	6.716*	6.834*
	2005	30.408**	0.128	0.252
Total incidence	2004	4.353	1.515	1.073
	2005	3.661	0.710	0.184

[1]Degrees of freedom for the errors pooled over 2004 and 2005.
[2] The GM varieties in 2004 and 2005 were AG3701RR and AG3802RR, respectively.
[3] Numbers in parentheses indicate the degrees of freedom (d.f.).
[4] Numbers are F-values.
**, $p < 0.01$; *, $p < 0.05$.

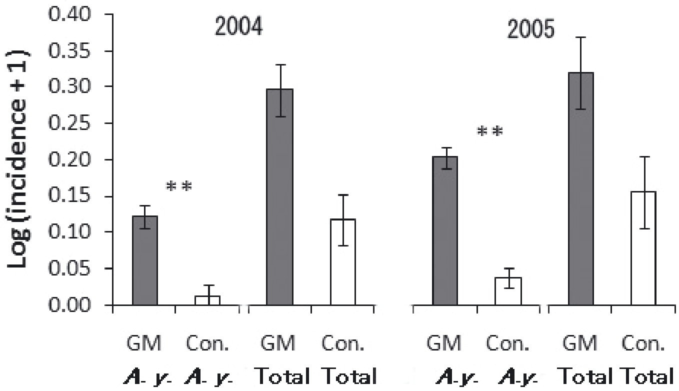

Figure 1. *Asphondylia yushimai* (*A.y.*) and total arthropod (Total) incidences (the number of individuals per unit dry pod weight) in the pods of the GM soybean varieties and of the conventional soybean variety (Con.) in 2004 and in 2005. Values represent the least-squares-means estimated by fitting a linear model for ANOVA; range bars represent the standard errors. **, significantly different at $p < 0.01$.

Table 3. Analysis of variance results for the number of soil macro-organisms, enumerated at the order level, in the 2004 and 2005 experiments.

		Factor (56)[1]			
Organism order	Year	Month (2)[2]	Variety (V, 1)[3]	Weed control regime (W, 1)	$V \times W$ (1)
Tubificida[4]	2004	1.862[5]	0.273	0.085	2.226
	2005	0.520	0.070	0.020	0.031
Acari	2004	7.033**	0.379	0.958	0.140
	2005	0.146	1.071	0.068	0.114
Collembola	2004	4.750*	0.457	0.012	1.326
	2005	3.335*	2.465	2.662	0.445
Psocoptera	2004	9.335**	0.098	0.007	1.705
	2005	6.267**	0.101	0.001	0.209
Coleoptera	2004	1.819	0.685	1.181	0.815
	2005	3.106	1.037	0.046	0.181
Lepidoptera	2004	0.891	0.001	0.472	0.129
	2005	11.239**	0.006	0.881	0.032
Total number	2004	6.561**	0.382	0.711	1.320
of individuals	2005	1.985	1.587	1.129	0.196

[1] Degrees of freedom for the error pooled over 2004 and 2005.
[2] Numbers in parentheses represent the degrees of freedom (d.f.).
[3] The GM varieties in 2004 and 2005 were AG3701RR and AG3802RR, respectively.
[4] Phylum Annelida.
[5] Numbers are F-values.
**, $p < 0.01$; *, $p < 0.05$.

the soybean variety (Collembola and Homoptera; $p < 0.05$) and that the total number of orders and four specific orders were significantly influenced by the weed control regime (Homoptera, Heteroptera, Coleoptera, and Lepidoptera; $p < 0.01$ or $p < 0.05$). Collembola were more abundant between the rows of the conventional variety ($p < 0.05$), and glyphosate application significantly decreased Collembola between the rows of the GM variety but increased them between the rows of the conventional variety ($V \times W$ interaction; $p < 0.01$). In contrast, Homoptera were significantly more abundant between the rows of the GM variety ($p < 0.05$). Glyphosate application significantly decreased Homoptera abundance between the rows, particularly in the GM variety ($p < 0.01$;

Table 4. Analysis of variance results for arthropods collected between the rows in the 2005 experiment.

Arthropod	Factor (6)[1]		
	Variety (V, 1)[2]	Weed control regime (W, 1)	$V \times W$ (1)
Araneae	0.294[3]	0.486	8.214*
Collembola	10.907*	3.047	16.082**
Orthoptera	1.745	1.168	0.519
Homoptera	6.604*	27.823**	15.384**
Heteroptera	2.359	31.718**	4.922
Coleoptera	0.002	12.436*	0.975
Diptera	0.149	0.665	1.550
Lepidoptera	1.067	13.067*	1.067
Hymenoptera	2.915	1.640	15.872**
Number of orders	0.610	15.244**	7.049*

[1] Degrees of freedom (d.f.) of the error.
[2] Numbers in parentheses represent the degrees of freedom (d.f.). The GM varieties in 2004 and 2005 were AG3701RR and AG3802RR, respectively.
[3] Numbers are F-values.
**, $p < 0.01$; *, $p < 0.05$.

Fig. 2). Similarly, glyphosate application significantly decreased the abundances of Heteroptera, Coleoptera, and Lepidoptera between the rows ($p < 0.01$ or $p < 0.05$; Fig. 2). Hymenoptera seemed to respond positively to weed abundance (data not shown) between the rows. Glyphosate application also significantly decreased the total number of arthropod orders found between the rows ($p < 0.01$; Fig. 2), particularly in the GM variety.

DISCUSSION

Because the two GM soybean varieties (AG3701RR and AG3802RR) were derived from the same source (Event 40-3-2), they were quite phenotypically similar and were significantly smaller than the conventional variety (Tachinagaha) as measured by plant height and dry biomass. The better growth of the soybeans in 2004 increased the number of flowers more in Tachinagaha, resulting in a significant difference between the two varieties. The GM varieties bore significantly more pods than Tachinagaha. However, the pods on the GM varieties were significantly smaller than those of Tachinagaha, resulting in comparable pod production (dry weight). Glyphosate application significantly increased the number of pods and pod dry weight in 2004 because the glyphosate application decreased weeds more than the conventional weed control. A similar tendency was observed in 2005. The observed varietal differences can be ascribed mainly to differences between the Japanese and American varieties.

Plant architecture, including the size and growth form, is known to influence arthropod abundance and diversity on plants (*e.g.*, Andow and Prokrym, 1990; Lawton, 1983; Price et al., 1980; Rudgers and Whitney, 2006), and Price et al. (1995) suggested correlations between plant size and insect abundance. Buckelew et al. (2000) reported that soybean plant height and leaf area were positively correlated with the abundance of some canopy insects and negatively correlated with that of others in GMHT and conventional plants. Thus, the incidence parameter (*i.e.*, the number of arthropods per unit plant weight) used in this study appears to be an appropriate measure for evaluating arthropod abundance on crop varieties with different dimensions or architectures.

In field evaluations of the impacts of GMHT beets, maize, and oilseed rape on farmland biodiversity (Firbank, 2003), the weed management regime used for the GMHT crops had no strong effects on the majority of the higher taxa of aerial and epigeal arthropods other than the pollinators and the Collembola detritivores (Haughton et al., 2003). Rosca (2004) also reported that the cultivation of Roundup Ready maize had no influence on the main species of natural enemies of arthropods living on maize plants. Buckelew et al. (2000) mentioned that GMHT varieties of soybean did not seem to strongly affect insect populations in field trials, but that the associated weed management system did. Jasinski et al. (2003) also concluded that few negative impacts on non-target arthropods could be directly associated with the production of GM soybeans. However, these studies did not separately evaluate the effects of weed control regimes and cultivars (*i.e.*, GM *vs.* conventional) on the arthropods on the soybean plant or in the fields as a whole.

In the present study, there were significant seasonal changes in the incidence of some arthropod groups on the soybean stems and leaves, but the weed control regimes did not affect the incidence significantly in either year. The varietal difference also did not significantly affect arthropod incidence, except for significantly larger incidences of Thysanoptera and total arthropods on the GM variety in 2005. *Orius sauteri* (Poppius) (Heteroptera, Anthocoridae), which was frequently seen on soybean plants in this study, is an effective predator of thysanopterous insects (Ohno and Takemoto, 1997; Yasunaga et al., 2001). However, no significant difference in the incidence of *Orius* spp., including *O. sauteri*, was detected between the GM and conventional varieties in either year. Thus, bottom-up effects such as morphological and chemical differences among the soybean varieties could be responsible for the difference (Hara and Ohba, 1981; Hart et al., 1983; Hulburt et al., 2004; Khan et al., 1986; Kobayashi, 1972; Liu et al., 1992; Smith,

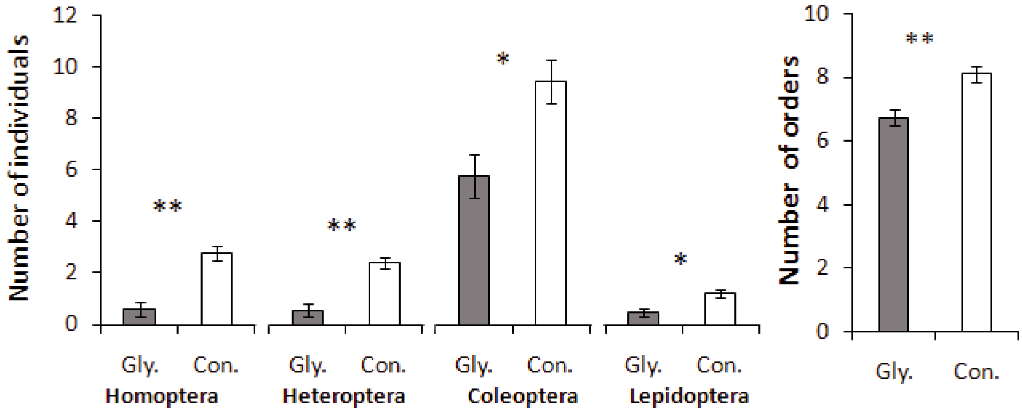

Figure 2. Significantly different arthropod abundances observed for the Homoptera, Heteroptera, Coleoptera and Lepidoptera (left) and for the total number of arthropod orders (right) between the rows in the Glyphosate (Gly.) and conventional (Con.) weed control plots. Values represent the least-squares-means estimated by fitting a linear model for ANOVA; range bars represent the standard errors. ** and *, significantly different at $p < 0.01$ and $p < 0.05$, respectively.

1985; Tester, 1977; Turnipseed, 1977). However, we did not attempt to identify the factor or factors responsible for the differences in the incidence of Thysanoptera or in the total arthropod incidence, both of which were significantly larger on the GM varieties. It is likely that morphological and chemical differences among conventional varieties that are capable of affecting arthropod incidences are ubiquitous, independent of genetic modification (Hara and Ohba, 1981; Hulburt et al., 2004; Khan et al., 1986; Liu et al., 1992; Smith, 1985; Tester, 1977; Turnipseed, 1977).

Thysanoptera were the only dominant arthropod group in the flowers. Yoshimura et al. (2006) also reported that Thysanoptera are dominant flower visitors in fields of GM and conventional soybeans in Tsukuba, Japan. Neither the varieties nor the weed control regimes significantly affected their incidence in the present study.

Asphondylia yushimai is one of the most serious pod pests in Japan (Kuwayama, 1953). The incidence of *A. yushimai* was significantly larger on the GM variety in both years in this study. Tamura (1941) noted that the pubescence characteristics of the pods – such as whether pubescence was present and, if so, the density, orientation, and length of the hairs – were related to the infestation rate by *A. yushimai*, but aspects of plant architecture such as height and canopy size did not affect its abundance. In the present study, both varieties had pubescent pods, but we did not examine details of their characteristics. Thus, whether or not pubescence was related to this difference was not clear in our study. Glyphosate application also significantly increased the incidence of *A. yushimai*, particularly in the GM variety in 2004. A similar tendency was observed for the total incidence (all arthropods combined), but the difference was not significant. In 2004, the weed density was markedly higher in the conventional weed control plots than in the glyphosate plots from August to September, particularly in the GM plots. Dense weeds during the pod growth and maturation periods in the conventional weed control plots possibly decreased the incidence of *A. yushimai.* Altieri et al. (1981) reported that the pod pest *Nezara viridula* was less abundant in weedy than weed-free plots in a conventional soybean variety field.

The release of GM crops may potentially affect soil meso- and macrofauna by introducing gene products and root exudates into the soil (Angle, 1994). Jasinski et al. (2003) found more soil-inhabiting mites in one non-HT soybean field in a comparison of six paired non-HT and HT soybean fields. In the present study, however, the soybean varieties and the weed control regimes did not significantly affect densities of soil macro-organisms. Similarly, Powell et al. (2009) observed no persistent negative effects of GMHT maize and soybean crops and their management on soil biota. Although Saxena et al. (1999) demonstrated the possibility that *Bt* corn would exert a toxic effect on non-target organisms through exudates from its roots, such an effect is unlikely in GMHT soybeans.

Although we used different GM varieties in 2004 and 2005, the effects on the arthropods on the soybean plants and on the macro-organisms in the soil did not appear to differ between the years. Thus, we could not detect cumulative effects of growing GM soybeans on the arthropod community found on the plants or on the soil macro-organisms over two years. A similar conclusion was reported in a 2-year experiment with GMHT maize in the UK (Heard et al., 2006).

The soybean varieties and the weed control regimes influenced the arthropods between the rows in 2005. Detritivorous Collembola were significantly more abundant between the rows of conventional plants, probably because the glyphosate application in the conventional plots supplied more decaying weeds and the closed canopy of the larger conventional variety kept the decaying weeds fresh (significant $V \times W$ interaction in Tab. 4). Bitzer et al. (2002) also suggested that the differences in the abundance of epigeal Collembola in GMHT soybean fields with different forms of weed management were caused by the beneficial accumulation of fresh organic matter from killed weeds rather than directly from herbicide toxicity. Homoptera were significantly more abundant between the rows in the GM plots, particularly with conventional weed control in the present study (significant $V \times W$ interaction in Tab. 4). Because the conventional weed control regime allowed weeds to grow more abundantly in plots containing shorter and smaller GM variety, the abundant weeds present between the rows obviously increased the density of the phytophagous Homoptera. For the same reason, glyphosate application significantly decreased the abundance of Heteroptera, Coleoptera, and Lepidoptera between the rows because there were fewer weeds in the glyphosate plots. The glyphosate application also significantly decreased the overall diversity of arthropods (number of arthropod orders) between the rows, particularly in the GM plots with glyphosate, where the fewest weeds were present. Buckelew et al. (2000) concluded that the differences in insect populations in GMHT soybean fields resulted more from the effects of weed control than from the herbicides used in those fields. In the "farm-scale evaluations", Hawes et al. (2003) also stated that changes in the weed communities as a result of the introduction of new herbicide regimes affected arthropods, including herbivores and detritivores, through trophic interactions.

In the present study, we separately assessed the impact of growing GMHT soybean and that of the weed control regime used for cultivation of the GMHT soybean. As our study showed, the cultivation of GM glyphosate-tolerant soybean varieties will not have direct adverse short-term effects on the biodiversity of epigeal arthropods and soil macro-organisms in fields. Carpenter et al. (2002) also concluded that biodiversity is maintained in GMHT soybean fields based on a summary of relevant studies. However, the present study demonstrated that the weed management regime used for the cultivation of GM glyphosate-tolerant soybean potentially decreases the arthropod biodiversity between the rows by reducing weed populations. Similarly, Watkinson et al. (2000) predicted possible adverse effects of field usage of GMHT crops on wild birds such as skylarks that depend on weed seeds for their diet, although contrary evidence has also been reported (Butler et al., 2007; Carpenter et al., 2002; Firbank and Forcella, 2000; Freckleton et al., 2004). We observed no adverse effect caused by the GM soybean variety on the arthropod biodiversity between the rows in the present study.

Because small-scale field experiments such as the present study (with a limited number of sites, a short term of only two years, and a relatively small field) are not sufficiently sensitive to detect anything but obvious local ecological effects, more large-scale and long-term assessments are needed (Andow and Zwahlen, 2006). As Firbank et al. (2003) suggested, soil organisms may take a long time to respond to a new GMHT cropping regime. The accumulation of more such studies to confirm the results of the present study may contribute to a broader public acceptance of GMHT crops, as Batie and Ervin (2001) suggested.

MATERIALS AND METHODS

General field management and experimental procedures followed the guidelines for Type-1 Use of GMOs (Ministry of Finance et al., 2003).

Experimental field

We established an experimental field (90 m × 30 m) at the Nasu Research Station of the National Institute of Livestock and Grassland Science, Nasushiobara, Tochigi, Japan. We separated the field into 12 square 200-m^2 plots (six plots in two rows), each separated by *ca.* 2 m, and divided the plots into three blocks, each composed of four plots. We applied cattle manure (60 $t.ha^{-2}$) and compound fertilizer (20 $kg.ha^{-2}$ N, 80 $kg.ha^{-2}$ P, and 80 $kg.ha^{-2}$ K) as basal fertilizers. We randomly assigned the following four treatment plots to each block, each replicated three times (*i.e.*, a randomized complete block design with three replications): (1) a GM glyphosate-tolerant soybean (*Glycine max* (L.) Merr.) variety (see below) sprayed with glyphosate for weed control; (2) the same GM soybean variety with a conventional weed control regime (see below); (3) a conventional soybean variety sprayed with glyphosate for weed control; and (4) the same conventional soybean variety with a conventional weed control regime. No insecticides were used in the fields. We carried out the experiment in two successive years (2004 and 2005) and used the same plot arrangement and procedures in both years, except for the GM varieties and arthropod sampling between the rows.

Soybean varieties

The conventional soybean was Tachinagaha, which is planted in more than 90% of the soybean growing area in Tochigi Prefecture. The GM glyphosate-tolerant soybean varieties were AG3701RR in 2004 and AG3802RR in 2005, both derived from the same Event (40-3-2) and developed by the Monsanto Company (St. Louis, Missouri, USA); both varieties incorporate the 5-enolpyruvyl-shikimate-3-phosphate synthase gene from *Agrobacterium* spp. strain CP4, and are therefore tolerant to glyphosate.

We hand-sowed the seeds in 18 rows at 75-cm spacing between rows on 10 June in each plot. After thinning, the number of seedlings was about 12 plants.m^{-2}.

Weed control

In the conventional weed control plots, Linuron (3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea) was sprayed as a pre-emergence application at the rate of 2 kg (AI).ha^{-2}, and tillage between the rows was performed using a cultivator two months after seeding. Thereafter, weeds taller than the soybean plants were removed manually three times during the growing period. The weeds removed by hand were removed from the fields. In the glyphosate plots, Roundup (N-phosphonomethyl glycine; ammonium salt; Monsanto) was sprayed one month after seeding at a rate of 1.025 kg (AI).ha^{-2}; in the plots where the conventional variety was growing, Roundup was carefully sprayed between the rows so as to avoid direct contact with the soybean plants. Weeds were more abundant in the plots with glyphosate application than in the conventional weed control plots during the early growing season, but after the Roundup spray, weeds were effectively suppressed and were less abundant in the sprayed plots, particularly in the GM plots.

Field sampling and survey

We sampled the soybean plants on 13 and 14 July, 3 and 4 August, and 14 and 15 September in 2004 and 2005, respectively. These dates corresponded to the early growing, flowering, and pod-growing stages, respectively. To obtain our samples, we quickly covered the plant canopy with a polyethylene bag, closed the bag above the root and cut the stem. On each day, we systematically sampled four plants within a central 5 × 5 m area in the plot to avoid edge effects (Olson and Andow, 2008). Soon after the sampling, we stored the samples at –30 °C in a freezer until they could be examined.

We also collected a soil sample from the surface to a depth of 5 cm around the roots of the first three sampled plants in the plot using a 100-mL core sampler (51 mm deep, 50 mm in diam.; Daiki Rika Kogyo, Kounosu, Japan).

On 13 September 2005, we surveyed arthropods on the plants and the ground surface between the rows using the covering method (Southwood and Henderson, 2000). We put an open-topped transparent acrylic resin box (50 × 50 cm, 70 cm deep) with a gauze sleeve at the top between the rows and we sucked the arthropods contained in the box using a modified electric vacuum cleaner for 5 min (Imura and Morimoto, 2004). We took three samples between the rows in the central area of the plot to avoid edge effects. The collected arthropods were kept in the freezer until they could be examined.

Laboratory examination

We examined the arthropods obtained from the sampled soybean plants and from between the rows under a binocular microscope. We also separately examined the arthropods found in the flowers and pods by dissecting these plant parts under the microscope. Some arthropods on the flowers and pods might have moved to other plant parts after the sampling. However, their number was considered to be small because most of these arthropods live inside the flowers and pods. We also counted the numbers of flowers and pods. The stems, leaves, and pods were oven-dried at 105 °C for 72 h and weighed to determine the biomass and pod yield.

We extracted soil macro-organisms from the soil samples using a Tullgren funnel (New, 1998) for 72 h. The extracted organisms were preserved in glass vials containing 70% ethanol.

We counted all collected arthropods, including their immature stages, at the order level (Homoptera and Heteroptera were counted separately) and used these counts in our subsequent analyses.

Statistical analyses

Because the dry weights of the soybean plants (stems and leaves) and pods varied significantly across months and varieties, we used a standardized incidence parameter for arthropod abundance of the plants and pods: the number per unit dry weight of the plant or pods. We averaged the measurements within a plot and performed ANOVA using the standard least-squares method provided by the JMP 6 software (SAS Institute, Cary, NC, USA). The variables measured in both years were analyzed by pooling the errors over the two years. The dry weight of soybean, arthropod abundance on soybean plants, and number of soil macro-organisms underwent log (n+1) transformation to ensure homoscedasticity prior to the ANOVA. To test for independence of the data between months, we examined correlations between the data. Although we found 9% (8 in 90) and 7% (3 in 42) significant correlations ($p < 0.05$) between months for data from arthropods on the plants and those in the soil, respectively, none of the arthropod orders had more than one significant correlation.

ACKNOWLEDGEMENTS

We thank S. Kaneda and T. Murakami, Research Branch of the National Agriculture Research Center for Tohoku Region at Fukushima, for their help extracting the soil organisms with the Tullgren funnel, and M. Araki, M. Matsui, T. Matsumura, and M. Miyazaki, National Institute of Agro-Environmental Science (NIAES), for identifying the arthropods and soil organisms. M. Siyomi, Ibaraki University, provided us with statistical advice. K. Yasuda of NIAES provided constructive criticism of an earlier version of the manuscript. We also thank M. Iwakoshi and N. Takano for their field and laboratory assistance. The study was funded by Japan's Ministry of Agriculture, Forestry, and Fisheries ("Assurance of safe use of genetically modified organisms").

Received September 1, 2010; accepted December 12, 2010.

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