Project Number 289706

COLLABORATIVE PROJECT

Assessing and Monitoring the Impacts of Genetically modified plants on Agro-ecosystems

Deliverable 5.1 Establishment of relative abundance of NTOs in maize and potato

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PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
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Summary

The most common natural enemies in all regions studied belong to the orders Coleoptera, and the Araneae (spiders). The most numerous predators, again in all regions, are members of the family of ground beetles (Carabidae), rove beetles (Staphylinidae), and spiders belonging to the families of Lycosidae and Lyniphiidae. The project did not have sufficient resources to reach a higher taxonomic resolution. Analysis of the most abundant NTO-taxa in the four biogeographical regions revealed differences between the regions. For brevity, a few examples are reported. Among herbivores *Acheta* sp. were the most abundant herbivores in maize fields in Spain whereas in Denmark and Sweden aphids (Homoptera) were found to be the most abundant in pitfall trap catches. Homopterans were the second most frequently caught herbivores in Spain. Among predators Aranea (spiders), in particular of the families Lycosidae and Linyphiidae, and Carabidae were found most frequently in pitfall traps. In Spain earwigs (Dermaptera) of the genus *Labidura* were found whereas these were absent in Denmark and Sweden. In Spain very low numbers of parasitoids were found, whereas in Denmark and Sweden parasitic Hymenopterans were trapped, in Denmark in ca. 30-fold higher numbers. Detritivores and fungivores were abundant in all regions, Collembola (springtails) and Mesostigmata (Acarina) being the most abundant taxa. Differences were relatively high numbers of orbatid mites in Spain, whereas these were rare in Denmark and Sweden. In Denmark three other taxa of detritivores have been found, Diplopoda, Protura and Cryptophagidae, which are mainly fungivores, that were all three virtually absent in Spain. A main conclusion is that Spain (mediterranean) on the one hand and Denmark (atlantic) differ clearly in arthropod fauna dwelling in maize fields. This leads to the recommendation that region-specific NTO-indicator species are included in ERA-protocols. The second arthropod survey method applied was direct visual observation which provided qualitatively different data on both herbivorous and predaceous arthropods than pitfall trapping. Thus, visual observations provide valuable information that is additional rather than redundant compared to the pitfall trap data, in particular for plant-dwelling taxa such as aphids, leafhoppers and predatory chrysopid larvae. Overall, relative abundance of arthropods in maize and potato as observed in AMIGA field surveys corresponds with that of the EFSA European Arthropod Database and provides significant additions for underrepresented countries, biogeographical zones and functional groups.

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1. General introduction

Natural control of crop pests by parasitic and predatory arthropods is a crucial feature of the trophic structure of agroecosystems and one of their key ecosystem services. Parasitic, predatory, non-target herbivorous and detritivorous arthropods constitute the main groups of non-target organisms (NTOs). Representatives of other NTO-functional groups are earthworms and nematodes in the soil and pollinators that are covered in reports relative to AMIGA work packages 4 and 6 respectively.

AMIGA NTO-field trials have resulted in a wealth of standardised data over three years and five biogeographic zones in insect resistant GM-maize (Mon810) and late blight (*Phytophthora infestans)*–resistant (DuRPh) GM-potatoes that have been planted next to isogenic non-GM lines. In **WP 5 - Trophic structures in agro-ecosystems**, six partners contributed: INIA, AU, LU, SAU and INCDSB and WU. Data have been aggregated to four functional groups (herbivores, parasitoids, predators and detritivores) and to order and as much as possible family level. For some of the most abundant taxa, e.g. predatory beetles of the family Carabidae, higher taxonomic resolution (genus or species level) has been achieved.

Data on relative abundance of arthropods collected in the AMIGA project in 2013 are accessible through the GEOSYS-database. Data collected in 2012, 2014 and 2015 are available as MS Excel-files upon request from the authors of this report.

2. Potato

2.1. Pitfall trapping in field trials with conventional, cis- and transgenic potato at Carlow, Ireland and Valthermond, The Netherlands

To increase the rigour of potato NTO-field trials within AMIGA's framework, we conducted field experiments both in The Netherlands at the WUR-experimental farm Valthermond and in Ireland at Teagasc (Irish Agriculture & Food Development Authority, Carlow Oak Park Crops Research Centre, hosted by Dr. Ewen Mullins), according to the EFSA ERA Guidance Document (2010).

Here we report the composition of the NTO-arthropod community based on pitfall trap data for Ireland and Valthermond, The Netherlands in 2013 and 2014. The experimental design was the same at both sites (Appendix 1). Pitfall traps were deployed for one week in the months July, August and September at both sites.

In the interest of a comprehensive overview of the data, the presentation is limited to the most abundant taxa within each of four functional groups. In 2013, aphids (Aphidoidea) were the most abundant herbivorous arthropods. Carabidae were the most abundant predators, followed by predatory Staphylinids. Among detritivorous taxa, Poduromorpha (Collembola) were dominant. The most abundant arthropods in the 2014 pitfall traps at Carlow were the minute clear or spotted detritivorous Mesostigmata mites, followed by the Collembola, suborder Entomobryomorpha. The third most abundant organisms in 2014 in Ireland belonged to the Carabidae, followed by members of the parasitoid family Scelionidae. Aphids (Aphidoidea) were the most abundant herbivores, however, they were the 12th most abundant taxon found in the 2014 Irish plots. Subsequently, each taxon identified has been assigned to a functional group, resulting in the following tables for the 2013 and 2014 field seasons at Teagasc, Carlow.

Table 1 - Pitfall trap catches (Carlow, Ireland, 2013). Summary totals for arthropod functional categories. Total is the numbers of specimens caught in 108 plots (1 double pitfall trap/plot), deployed over a total area of 486 m^2.

	July	**Aug**	**Sept**	**Total**
Predators	2034	555	1968	4557
Detritivores	1146	221	810	2177
Parasitoids	63	43	501	607
Herbivores	1992	575	81	2648
Other	186	183	441	810
Total	5421	1577	3801	**10799**

Table 2 - Pitfall trap catches (Carlow, Ireland, 2014). Summary totals for arthropod functional categories. See Table 1.

	July	**Aug**	**Sept**	**Total**
Predators	2612	2753	2114	7479
Detritivores	5547	3640	3009	12196
Parasitoids	985	1704	528	3217
Herbivores	530	370	121	1021
Other	307	215	216	738
Total	9981	8682	5988	**24651**

Table 3 – Three most abundant arthropod taxa per functional group found in pitfall traps at Carlow, Ireland in 2013 and 2014. Totals: the summed number of specimens caught in 108 plots (1 double pitfall trap/plot; total area of 486 m^2), deployed during one week in July, August and September.

Taxon	2013 **Totals**	**Taxon**	2014 **Totals**
Predators		***Predators***	
Carabidae	1941	Carabidae	2740
Staphylinidae	1300	Araneae	1842
Araneae	854	Staphylinidae	1671
Detritivores		***Detritivores***	
Poduromorpha	1083	Mesostigmata	5006
Mesostigmata	319	Entomobryomorpha	3329
Megadrila[1]	246	Symphypleona	993
Herbivores		***Herbivores***	
Aphidoidea	1632	Aphidoidea	365
Thysanoptera	291	Thysanoptera	73
Mollusca	26	Mollusca	59
Parasitoids		***Parasitoids***	
Scelionidae	465	Scelionidae	2726
Proctotrupidae	37	Ceraphronidae	233
Braconidae	49	Proctotrupidae	36

1 – Annelida

An apparent difference between the two years is that more than twice as many specimens have been collected in 2014 than in 2013. Regarding the functional groups, in 2014 a higher

abundance of detritivores and herbivores and lower abundance of herbivores contributed most to this difference.
For the field experiments in Valthermond, NL in 2013 and 2014, the following data have been collected.

Table 4 - Pitfall trap catches (Valthermond, 2013). Summary totals for arthropod functional categories.

	July	**Aug**	**Sept**	**Total**
Predators	1832	1504	458	3794
Detritivores	1509	12839	3862	18210
Parasitoids	187	673	513	1373
Herbivores	362	109	158	629
Other	163	615	429	1207
Total	4053	15740	5420	**25213**

Table 5 - Pitfall trap catches (Valthermond, 2014): Summary totals for arthropod functional categories.

	July	**Aug**	**Sept**	**Total**
Predators	4009	1796	692	6497
Detritivores	6117	2612	1788	10517
Parasitoids	526	758	305	1589
Herbivores	1094	80	65	1239
Other	308	346	328	982
Total	12054	5592	3178	**20824**

Table 6. Three most abundant arthropod taxa per functional group found in pitfall traps at Valthermond, NL, 2013 and 2014. Totals: the summed numbers of specimens caught in 108 plots (1 double pitfall trap/plot; total area of 486 m^2), deployed during one week in July, August and September.

Taxon	2013 **Totals**	**Taxon**	2014 **Totals**
Predators		***Predators***	
Staphylinidae	1309	Staphylinidae	2776
Carabidae	1123	Carabidae	1425
Araneae	675	Araneae	1700
Detritivores		***Detritivores***	
Mesostigmata	16516	Mesostigmata	2385
Entomobryomorpha	345	Entomobryomorpha	4468
Poduromorpha	264	Latridiidae	1613
Herbivores		***Herbivores***	
Aphidoidea	337	Aphidoidea	1065
Diptera larvae	215	Diptera larvae	149
Cicadellidae	77	Thysanoptera	47
Parasitoids		***Parasitoids***	
Scelionidae	880	Scelionidae	1207
Diapriidae	142	Diapriidae	43
Braconidae	93	Eucoilidae	148

Both the Carlow and Valthermond sites are located in the atlantic biogeographic zone, nevertheless results differed between them. Whereas at Carlow carabidae were the most abundant predators, at Valthermond, Staphylinidae were more abundant. At both sites, aphids were the most abundant herbivores trapped. Detritivorous communities were similar for the two most abundant taxa at both sites but their communities differed among the two years at both sites. Taken together these and other data indicate differences between years and sites.

2.2 Direct visual observations - Valthermond 2013 and 2014

In 2013, the total number of insects observed was 2237, by sampling two plants per plot mid-season. The most abundant insects were aphids, mostly *Myzus persicae* Sulzer (Hemiptera: Aphidoidea); second were Hymenoptera, which cannot be identified by plain eyesight, most abundant were aphid parasitoids. Next most abundant were thrips (Thysanoptera), dipteran eggs, lacewing (*Chrysopa* (Neuroptera*:* Chrysopidae)) eggs and Coleoptera. We also counted CPB larvae and adults in the plots at each collection point. The visual observations showed a few minor differences among the three potato genotypes and germplasms and the management regimes (Fig. 1).

In 2014, the total number of insects observed was 2063, by visually sampling two plants per plot three times in the middle of the growing season. At the early and mid-season collection dates, the most abundant insects were aphids, mostly *Myzus persicae;* and lacewing (*Chrysopa*) eggs and larvae. Mid-season we also observed more dipteran eggs and thrips. Late season, hemipteran nymphs (mainly Pentatomidae), leafhoppers and Colorado potato beetles were the most abundant.

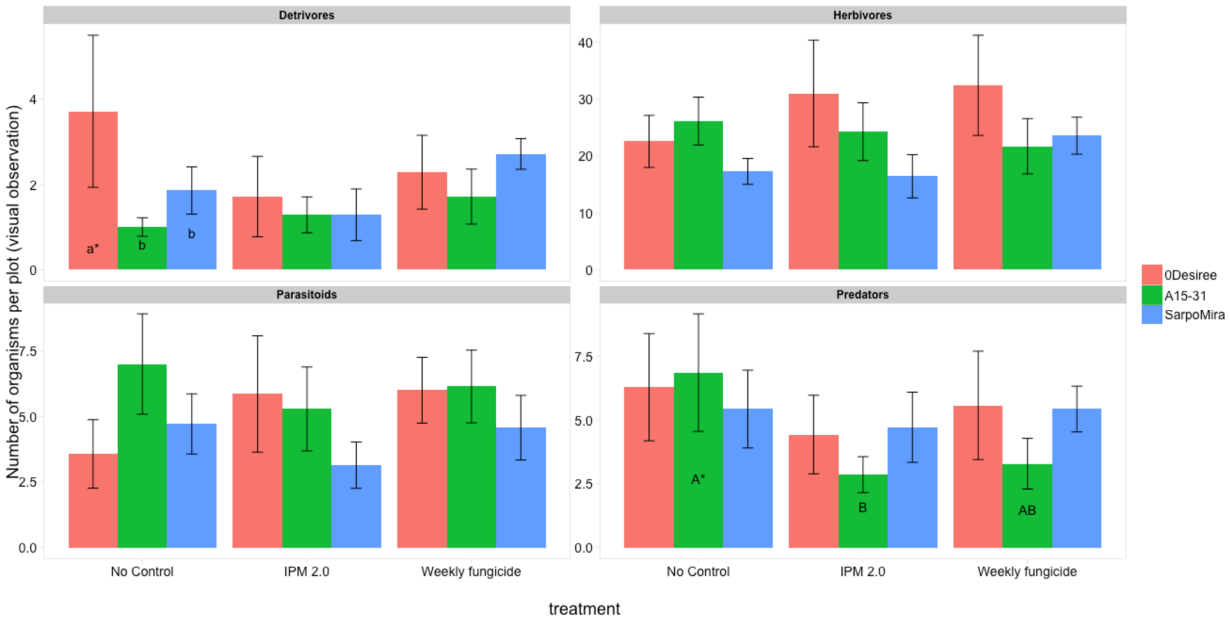


Figure 1 - Direct visual observations of number of organisms per plot in each of four functional catgories, Valthermond 2013. Small letters indicate differences between plant genotype within a treatment, and capital letters indicate differences in plot treatment within a genotype. *indicates a strong trend ($p < 0.1$). Total number of organisms observed in all 6 replicates per treatment was 2237.

2.3 Potato genotypes and crop management regimes

At both field sites, three crop management regimes and three potato genotypes were investigated, resulting in 9 management regime x genotype combinations (Appendix 1). Means and variation for each of these combinations haven been plotted in Fig. 2 for Ireland and The Netherlands. It can be readily seen that overall differences between treatments and potato genotypes were small or non-existent for each arthropod functional category.

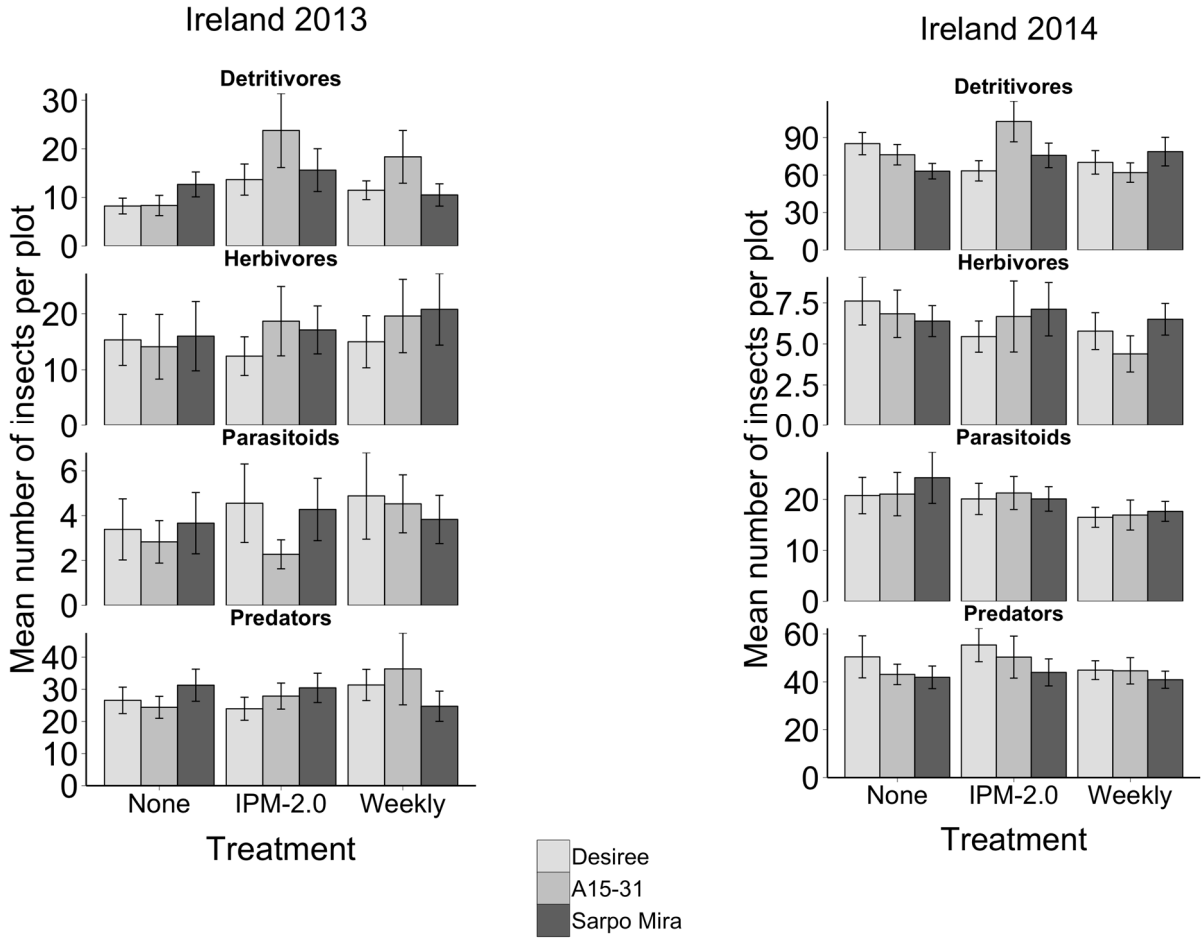


Figure 2A. Mean number (± SEM) of insects per plot caught in pitfall traps in Carlow, Ireland, depicted for three crop management regimes (treatments: none – no management, IPM-2.0 and weekly fungicide spray) and three potato genotypes (Désirée, *P. infestans*-resistant cis-genic line A15-31 and the conventional *P. infestans*-resistant cultivar Sarpo Mira).

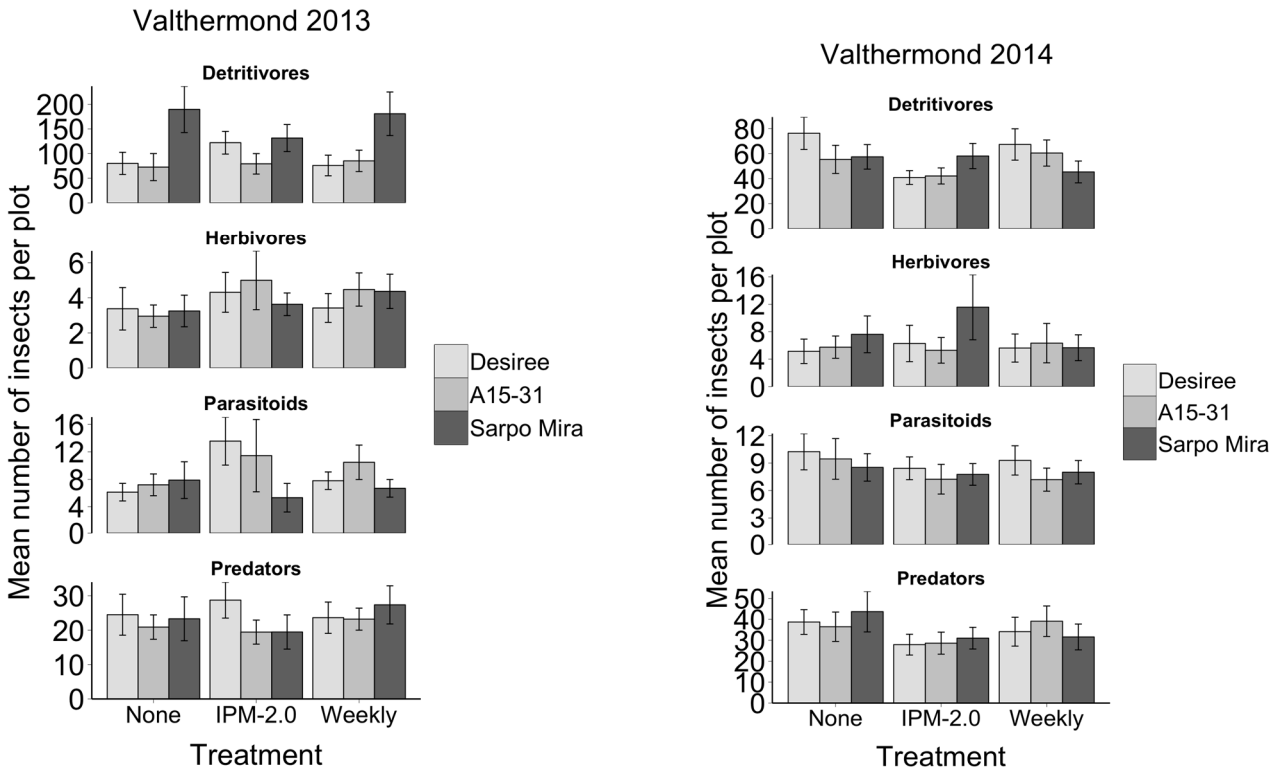


Figure 2B. Mean number (± SEM) of insects per plot caught in pitfall traps in Valthermond, Netherland, depicted for three crop management regimes (treatments: none – no management, IPM-2.0 and weekly fungicide spray) and three potato genotypes (Désirée, *P. infestans*-resistant cis-genic line A15-31 and the conventional *P. infestans*-resistant cultivar Sarpo Mira).

3. Maize

3.1 NTO field trials in insect-resistant Bt-maize in four biogeographiczones

NTO-trials on maize plots have been carried out in five biogeographic zones in three years in the period 2012-2015. Five partners contributed: INIA (mediterranean), AU (atlantic), LU (boreal), SAU (continental) and INCDSB (balkan). Details of the experimental sites are presented in Appendix 1**.**
Sampling of arthropods in *Bt* and isogenic maize plots were accomplished by pitfall trapping (ground-dwelling arthropods) and visual surveys (plant-dwelling arthropods). At least three mid-season 1-week pitfall trappings were carried out (see report on D5.2), upto a maximum of nine weekly trappings (Spain).

3.2 Detailed analyses: Spain as a case study

Pitfall trap data for soil-dwelling arthropod faunal composition in the field trials in Spain (Seseña) have been summarised in Tables 7 and 8 for three mid-season sample times in 2013 and 2014. For the sake of a concise overview, in summarising these data, a threshold has been applied (min. 2 specimens per taxon per trap per week). Consistent data were obtained between the two years for the most abundant taxa in three of the four arthropod functional categories. Main differences between the years were a shift from Delphacidae to Cicadellidae as the second most abundant herbivores in maize; higher abundance of parasitic Mymaridae and predatory Formicidae in 2014.

Table 7. Pitfall trap catches of soil-dwelling arthropods (# specimens/trap/week) in NTO-field trials in Seseña, Spain; July – September 2013.

	19 July				14 August				10 September			
	ISO		***Bt***		**ISO**		***Bt***		**ISO**		***Bt***	
Herbivores	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Acheta sp.	14.1	2.89	16.1	8.46	8.3	3.77	5.5	2.83	1.4	0.84	1.4	1.02
Cicadellidae	-		-		-		-		-		-	
Delphacidae	0.8	0.82	1.0	0.69	3.8	2.29	4.4	1.94	3.1	1.71	5.2	2.75
Predators												
Lycosidae	7.8	2.96	8.5	3.59	2.4	1.81	3.2	1.76	1.5	1.11	0.9	0.57
Lycosidae[&]	6.1	12.02	13.4	20.29	2.45	4.09	3.9	5.70	10.1	10.62	2.8	8.34
Phytoseidae	1.5	1.41	2.1	2.11	1.8	1.78	1.4	0.70	1.1	0.46	1.8	1.69
Labidura sp.	6.6	1.97	6.4	2.50	3.8	1.49	2.3	2.56	-		-	
*P. rufipes**	2.0	0.93	3.0	2.24	17.4	9.43	26.5	12.22	12.9	4.61	11.6	6.6
Formicidae	-		-		-		-		-		-	
Parasitoids												
Mymaridae	-		-		-		-		-		-	
Detritivores												
Oribatida	51.5	42.3	51.9	21.3	14.7	10.4	17.3	7.02	2.2	0.95	2.2	1.75
Collembola	72.8	59.94	96.5	81.44	71.5	44.10	130.0	115.59	35.9	22.2	29.4	12.1
Phoridae	3.5	2.19	6.0	7.47	0.7	0.54	1.3	1.40	-		-	

ISO: non-GM isoline of Mon810; Bt: Mon810; - : below threshold (see text) or not found.).
[&] Lycosidae immature stages; **Pseudophonus rufipes* (Carabidae).

Table 8. Pitfall trap catches (# specimens/trap/week) of soil-dwelling arthropods in NTO-field trials in Seseña, Spain; July – September 2014.

	18 July				13 August				10 September			
	ISO		***Bt***		**ISO**		***Bt***		**ISO**		***Bt***	
Herbivores	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Acheta sp.	21.2	7.00	20.10	6.20	6.85	4.63	6.55	4.23	3.35	2.07	3.45	2.33
Cicadellidae	7.20	6.10	4.75	3.20	2.30	1.77	2.90	1.15	1.80	1.21	1.60	0.99
Delphacidae	-		-		-		-		2.5	2.39	1.9	0.81
Predators												
Lycosidae	5.80	2.50	4.80	2.00	5.25	2.61	6.40	3.92	-			
Lycosidae im[&]	4.10	6.50	0.90	2.30	-				-			
Phytoseidae	4.10	2.30	2.25	2.35					1.45	1.04	1.6	1.1
Labidura sp.	4.80	3.40	3.35	2.45					1.6	1.07	1.3	0.82
*P. rufipes**	2.30	1.30	4.30	2.26	1.85	1.29	2.80	2.21	4.00	2.98	3.15	3.01
Formicidae	3.70	3.84	5.05	5.06	3.90	3.77	9.80	8.87	3.30	4.44	4.25	4.60
Parasitoids												
Mymaridae	1.40	0.77	1.35	0.85	3.25	1.34	3.30	1.49	-			
Detritivores												
Oribatida	79.30	39.12	84.5	37.9	1.75	1.30	1.00	0.75	14.65	9.49	19.25	18.62
Collembola	75.60	43.77	95.7	125.0	13.70	6.72	17.40	8.44	180.9	110.9	216.5	61.43
Phoridae	5.55	2.14	8.9	6.08	-		-		-		-	

ISO: non-GM isoline of Mon810; Bt: Mon810; - : below threshold (see text) or not found.
**Pseudophonus rufipes* (Carabidae).[&] Lycosidae immature stages

3.3 Comparisons between isoline and GM-line

In both years the abundance of arthropods belonging to the four functional categories was essentially similar in fields planted with the conventional isoline and those with GM *Bt*-maize Mon810. The degree of variation among replicate plots (n = 10) is overall high, showing coefficients of variation between 50 – 100%, in some cases up to 200%. This indicates a limited sensitivity to detect statistically significant differences in arthropod abundance among treatments for these field trials. Statistical comparisons are the subject of **D9.4/D9.5** and are not presented in this report. For this reason, the numbers trapped in the isoline- and GM-plots have been summed in subsequent tables.

3.4 Temporal patterns in pitfall trap catches in Spain

In Sesena nine 1-week pitfall trappings have been carried out in 2012, 2013 and 2014. The time pattern in abundance of different functional groups (Fig. 3) was consistent over the three years (only 2012-data are shown). A total of 155.000 specimens were captured in 2012. The most abundant predators were spiders and carabids. Staphylinids (rove beetles) maintain low populations throughout the sampling period (about 1 per trap and sampling week in 2012). Over 90% of captured carabids (ground beetles) belong to the species *Pseudophonus (Harpalus) rufipes* and *Pseudophonus griseus*. The spiders families Lycosidae, Gnaphosidae and Linyphiidae accounted for over 90% of spiders. Collembola are the most abundant taxon in the category detritivores (134.000 individuals in 2012). Captures of the four trophic groups as a whole (herbivores + predators + parasitoids + "other arthropods") showed similar patterns of abundance in *Bt* and conventional maize plots.

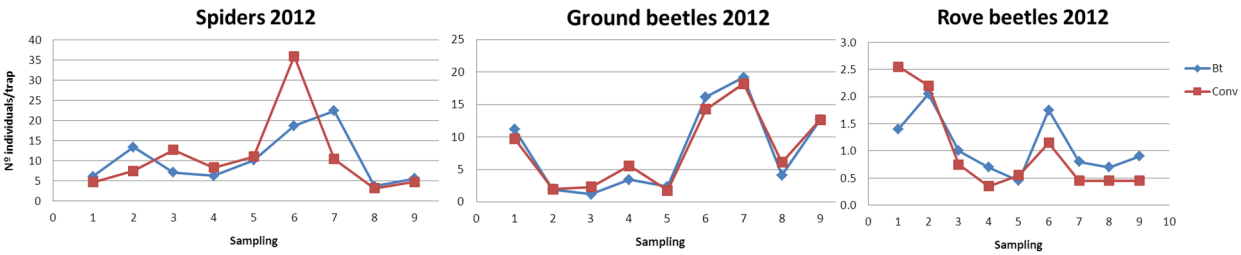


Figure 3. Pitfall trap catches of the most important groups of predatory arthropods in *Bt* and conventional maize plots in 2012. Sampling times after traps had been in the field for 7 days: 1 – 20 June; 2 – 4 July; 3 – 20 July; 4 1 August; 5 – 16 August; 6 – 29 August; 7 – 12 Sept.; 8 – 26 Sept.; 9 – 10 Oct. Based on 155.000 specimens caught; note the differences in scales of the Y-axes in the three panels.

Similar temporal patterns have been observed in other biogeographic regions. An example is given for Carabidae and Arachnida in Romania in 2014 and 2015 (end of June – mid October) (Fig. 4). In both years, spiders in the family Lycosidae were most abundant early in the season and then declined, whereas Opiliones were below threshold (1 specimen/trap per week) or low in June but populations were higher in Sept-Oct. The most abundant predators were Carabidae, that had peak populations in July. Two species of Carabidae (Pseudophonus rufipes and Anchomenus dorsalis, out of 6 -29 species found)) represented over 80% of total numbers all Carabidae species. These phenological patterns indicate that the probability of catching certain taxa varies over the season.

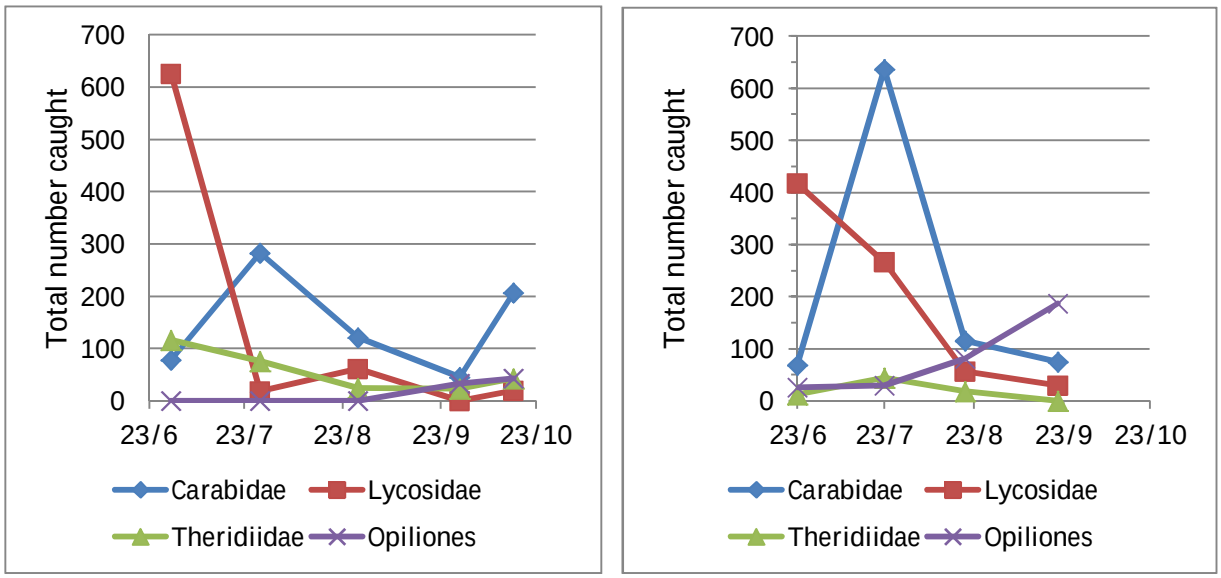


Fig. 4. Pitfall trap catches of the most important groups of predatory arthropods in *Bt* and isoline maize plots in 2014 and 2015. Numbers of specimens trapped were added for the 10 replicate *Bt*- and 10 repliacte isoline plots (see 3.3).

3.5 Direct visual observations

From direct visual observations, 26% of captured specimens were herbivores, 29% predators and 45% were classified in the group "other arthropods" (Fig. 5). Most of the recorded herbivorous specimens belong to the family Cicadellidae (82%) followed by Thysanoptera (8%) and Aphididae (6%). The most abundant predators were Chrysopidae (34%), Coccinellidae (18%) and Cecidomyiidae (15%). Numbers of herbivores, predators, parasitoids

and "other arthropods" recorded in *Bt* and conventional maize plots seem not to be different. It is important to note that the visual observations provide valuable information that is additional rather than redundant compared to the pitfall trap data, in particular for plant-dwelling taxa such as aphids, leaf hopers and predatory chrysopid larvae.

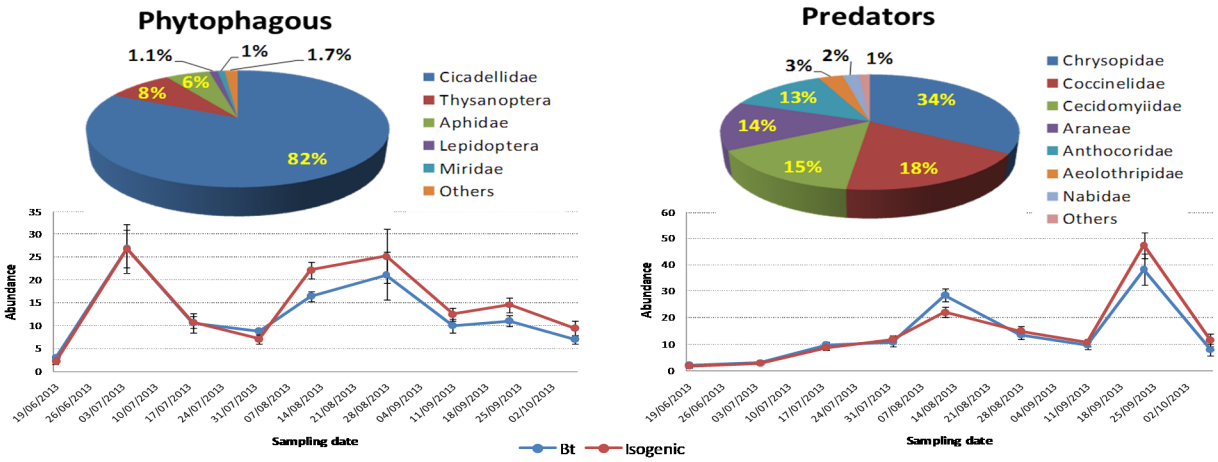


Fig. 5. Composition and abundance of herbivorous and predatory arthropods found in visual surveys in *Bt* and conventional maize plots in Sesena, Spain in 2013.

3.6 Comparison among five biogeographical regions for maize NTO field sites

A selective analysis was performed of the most abundant NTO-taxa in five biogeographical regions (**Table 9**). The comparison shows that:

1. **Herbivores***: Acheta* sp. were the most abundant herbivores in maize fields in Spain (mediterranean) whereas in Denmark (continental) and Sweden (boreal) Homoptera were found to be the most abundant in trap catches. Homoptera were the second most frequently caught order of herbivores in Spain.
2. **Predators**: Araneae, in particular of the families Lycosidae and Linyphiidae, and Carabidae were found most frequently in pitfall traps. In Spain earwigs of the genus *Labidura* were found whereas these were absent in Denmark and Sweden. Arachnid species belonging to the family Theridiidae have only been consistently found in Romania.
3. **Parasitoids**: In Spain very low numbers of parasitoids were found, whereas in Denmark and Sweden parasitic Hymenopterans were much more abundant, in Denmark in ca. 30-fold higher numbers.
4. **Detritivores/fungivores**: This functional group was most abundantly occurring in trap catches in Spain and Denmark. Collembola being most abundant. Differences were relatively high numbers of orbatid mites in Spain, whereas these were rarely found in Denmark and Sweden. In Denmark three other taxa of detritivores have been found, Diplopoda, Protura and Cryptophagidae, which are mainly fungivores, that were all three virtually absent in Spain and only the last taxon was found in Sweden, albeit at 9-fold lower numbers. The main conclusion of this comparison is that Spain on the one hand and Denmark and Sweden on the other hand differ clearly in arthropod fauna dwelling in maize fields. This provides an argument to include region-specific NTO-indicator species in ERA-protocols.

Table 9: Pitfall trap catches summed for 20 traps per site (# specimens caught/week/2000 m^2) of soil-dwelling arthropods in NTO-maize field trials in five sites in five biogeographic European regions. Most abundant taxa per functional group found in pitfall traps in mid July.
From the pitfall trap catches in Slovakia and Romania only Arachnida and Carabidae have been identified.

	Spain	**Sweden**	**Denmark**	**Romania**	**Slovakia**
Herbivores					
Acheta sp.	413	-	-		
Cicadellidae	120	139	9520[&]		
Chrysomelidae	-	-	287		
Thysanoptera	-	-	449		
Predators					
Arachnida	157	-	-		
Lycosidae	156	-	2100[#]	-	73
Linyphiidae	-	1560	-	-	-
Theridiidae	-	-	-	76	-
Phytoseidae	64	-	-		
Labidura sp.	82	-	-		
Carabidae	66	612	2350	283	902
Staphylinidae	-	125	1798		
Formicidae	88	-	91		
Parasitoids	-	64	1230		
Detritivores					
Oribatida	1638	-	-		
Collembola	1713	583	30730		
Phoridae	145	-	-		
Cryptophagidae	-	97	880		
Protura	-	-	216		
Diplopoda	-	-	899		

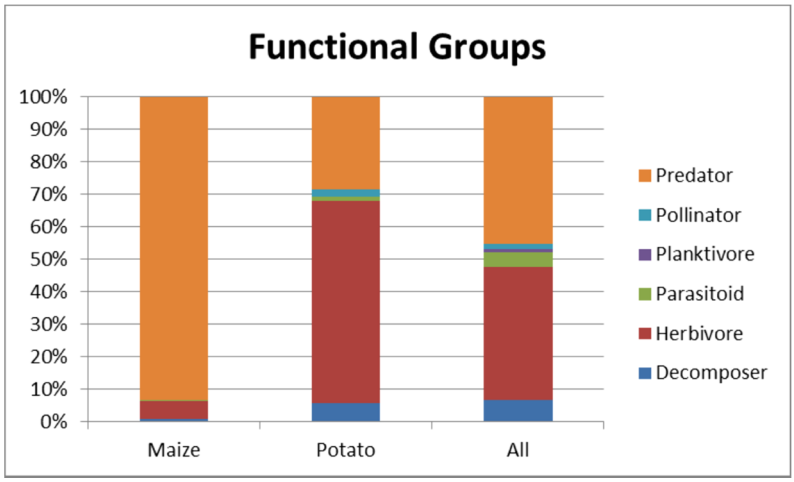
- : not found
& The numbers for Cicadellidae given for Denmark refer to all Homoptera pooled.
# The numbers for Lycosidae for Denmark refer to all Araneae pooled.

4. Comparison between AMIGA NTO-field data and the European Arthropod Database

Overall, relative abundance of arthropods in maize and potato as observed in AMIGA field surveys corresponds with that of the EFSA European Arthropod Database (Meissle et al., 2012). The latter database contains 3030 arthropod species documented in 1067 references. Some of these references include data for more than one crop and around half of them contain records of only one or two species per crop. In total 14.762 records are available in the databse. The database contains 5499 records of 1679 species from maize and 2637 records of 793 species from potato. The records come from 31 countries, with the highest number of records from Germany (3095). Fifteen methods have been used to collect these data, with pitfall traps being by far the most frequently used method (4470 records), followed by visual observation (1943 records), the two methods were also used by AMIGA WP5. EU countries underrepresented in the database relative to the surface areas of maize and/or potato cultivation are Slovakia, Denmark, Sweden and Ireland, thereby the AMIGA data sets contributing in an important way to filling these biogeographical gaps. Also when taking the perspective of biogeographical zoning, AMIGA NTO abundance data supplement the EFSA database to a significant extent for the boreal, mediterranean and balkan zones.

From the perspective of functional groups (Fig. 6), AMIGA has generated data on abundance of detritivores (decomposers) that are virtually absent in the EFSA database for maize and underrepresented for potato.

Figure 6. proportions of records on functional groups in maize, potato and all crops available in the European Arthropod Database (Meissle et al. 2012).



Finally, the arthropod abundance data sets provided by the AMIGA project have the advantage of allowing quantitative comparisons among biogeographic regions since survey methods were strictly standardised and plot sizes were identical at all NTO-field sites.

5. References

Meissle, M., Álvarez-Alfageme, F., Malone, L.,A. & Romeis J. (2012) Establishing a database of bio-ecological information on non-target arthropod species to support the environmental risk assessment of genetically modified crops in the EU. Available at: http://www.efsa.europa.eu/en/search/doc/334e.pdf; 28/04/2013.

Appendix 1. Design and crop management of NTO field trials

Pitfall trapping in NTO-field trials with conventional, cis- and transgenic potato at Carlow, Ireland and Valthermond, The Netherlands

Field design

Factor	Levels	
Potato genotype	3	Desirée, A15-031 (*cis*-genic *Phytophthora infestans (Pi)*-resistant line) and conventional *Pi*-resistant cultivar, Sarpo Mira
PLB control strategy	3	No intervention, Standard practice, IPM2.0 (see WP8)
Replicates	6	Total of 3 * 3 * 6 = 54 plots
Plot size	3 x 3 m	
Inter plot distances	6 m on all sides	Interplot crop was grass mowed regularly
Rotation	1:4	Plots shift 6 m each year due to max. space regulation (1 ha)

Maize field trials

INIA (Spain) WP5 AMIGA field assay:

20 Plots: AMIGA 2012 field assay Spain 01 Plot 41..…60;

10 x 10 m, distance between plots = 5 m, 14 maize rows per plot,

0.75 m inter-row distance

Corn maturity class (FAO Units): 700

10 GM Bt Corn: Variety DKC6451YG

10 Conventional Corn: Variety DKC6450

AMIGA_TUE_Soil:	24/01/2012	Disk harrow
	27/01/2012	Disk harrow

31/01/2012 Subsoiler

09/02/2012 Chisel plough with roller

14/02/2012 Rotovator

AMIGA_TUE_Sowing: 11/05/2012 Density: 80,000 seeds/ha

AMIGA_TUE_Fertilization: 13/02/2012 Basal dressing N:P:K (8-15-15) 300 kg/ha

08/06/2012 Top dressing Urea-46 300 kg/ha

AMIGA_TUE_Irrigation: From 09/06/2012 to physiological maturity, by flooding (Each 10 days approx.)

AMIGA_TUE_Protection: 07/06/2012 1 Herbicide treatment:

Primextra 3l/ha (S-metoalachlor 31.25% + terbutilazine 18.75%) + Elite Plus 0.75 l/ha (nicosulfuron 6%) + Callisto 0.5 l/ha (mesotrione 10%)

Flowering date: 24/07/2012

Plant height (m): 2.6

AMIGA_TUE_Harvest: 05/12/2012

AMIGA_Target: Weeds: weed species and weed density.

4 sampling times

2 samples/plot

Pitfall traps: Arthropods: classified to morphospecies.

9 sampling times

AMIGA 2013 field assay INIA, Spain

20 Plots: AMIGA field assay Spain 01 Plot 41.…60;

10 x 10 m, distance between plots = 5 m, 14 maize rows per plot,

0.75 m inter-row distance

Corn maturity class (FAO Units): 700

10 GM Bt Corn: Variety DKC6451YG

10 Conventional Corn: Variety DKC6450

AMIGA_TUE_Soil: 20/03/2013 Disk harrow

09/04/2013 Subsoiler

16/04/2013 Rotovator

AMIGA_TUE_Sowing: 09/05/2013 Density: 80,000 seeds/ha

AMIGA_TUE_Fertilization: 15/04/2013 Basal dressing N:P:K (8-15-15) 300 kg/ha

15/06/2013 Top dressing

CAN 27% (Calcium Ammonium Nitrate) 500 kg/ha

AMIGA_TUE_Irrigation: From 02/06/2013 to physiological maturity, by flooding (Each ten days approx..)

AMIGA_TUE_Protection: 11/06/2013 1 Herbicide treatment:

Primextra 3l/ha (S-metoalachlor 31.25% + terbutilazine 18.75%) + Elite Plus 0.75 l/ha (nicosulfuron 6%) + Callisto 0.5 l/ha (mesotrione 10%)

Flowering date: 03/08/2013

Plant height (m): 2.7

AMIGA_TUE_Harvest: 09/01/2013

AMIGA_Target: Weeds: weed species and weed density.

5 sampling times

2 samples/plot

Pitfall traps: arthropods: classified to morphospecies.

9 sampling times

Visual surveys: arthropods registered.

9 sampling times

1 whole plant/plot

AMIGA 2014 field assay Spain 01

20 Plots: AMIGA field assay Spain 01 Plot 41.…60;

10 x 10 m, distance between plots = 5 m, 14 maize rows per plot, 0.75 m distance inter-row

Corn maturity class (FAO Units): 700

10 GM Bt Corn: Variety DKC6451YG

10 Conventional Corn: Variety DKC6450

AMIGA_TUE_Soil: 06/03/2014 Disk harrow

10/03/2014 Disk harrow

14/03/2014 Subsoiler

29/03/2014 Rotovator

AMIGA_TUE_Sowing: 19/05/2014 Density: 80,000 seeds/ha

AMIGA_TUE_Fertilization: 27/03/2014 Basal dressing N:P:K (8-15-15) 300 kg/ha

01/07/2014 Top dressing Urea-46 600 kg/ha

AMIGA_TUE_Irrigation: From 17/06/2014 to physiological maturity, by flooding (Each ten days approx..)

AMIGA_TUE_Protection: 30/06/2014 1 Herbicide treatment:
Primextra 3l/ha (S-metoalachlor 31.25% + terbutilazine 18.75%) + Elite Plus 0.75 l/ha (nicosulfuron 6%) + Callisto 0.5 l/ha (mesotrione 10%)
Flowering date: 07/08/2014
Plant height (m): 2.75
AMIGA_TUE_Harvest: 30/12/2014
AMIGA_Target: Weeds: weed species and weed density.
5 sampling times
2 samples/plot
Pitfall traps: arthropods: classified to morphospecies. Ongoing 9 sampling times
Visual surveys: arthropods registered. Done
9 sampling times
1 whole plant/plot

Romania NTO- Maize experimental field location (Secuieni, 2014 & 2015)

Maize hybrids used:
1. Conventional (near-isogenic line) – hybrid DKC 3871;
2. Transgenic MON810 (YieldGard) – hybrid DKC 3872 YG.

Location: Secuieni, Neamt county, Romania
GPS coordinates: 46°85'99"N / 26°86'32.9"E

A.2. NTO-oriented experimental field plan (Secuieni, Romania) (2014, 2015)
Each hybrid was sown in 10 repetitions.
Each plot had a size of 10 x 10 m.
Each plot was isolated from the other by a strip of 5 m of soil with grass (i.e. baley) (kept short).
Mandatory isolation distance: 200 m linear (on all sides).
Number of plants/ plot: approx. 750 (13 rows with 58 plants/ row).

NTO-oriented experimental field plan:

NTO experiment		**2014**		**2015**	
		INPUTS			
Seeds	Seeding rate (seeds/ha)	65,000 seeds/ha		65,000 seeds/ha	
Fertilizer	Amount of fertilizer / ha	NPK 20:20:20 / 250 kg/ha			
Plant Protection Products	Herbicides (product used +active substances)	➔ Buctril Universal (bromoxinil 280 g/l + acid 2.4-D (ester) 280 g/l) ➔ Equip (foramsulfuron 22.5 g/l + isoxadifen etil (safener) 22.5 g/l)			
	Number of applications	2			
	Rate of applications	➔ Buctril Universal: 0.8 l/ha ➔ Equip: 1.75 l/ha			
		OUTPUTS (kg/ ha; reported at 14% humidity)			
		2014		**2015**	
Hybrid		DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)	DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)
Mean yield (kg/ ha)		9,059	8,261	5,477	5,286