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COLLABORATIVE PROJECT

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

D8.2 Development and assessment of IPM Strategies for European GM maize

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Key concepts for development of a sustainable GM maize IPM strategy for Europe

Introduction: The evolution of IPM in the EU (based on the 8 key Principles of IPM)

IPM was originally defined as "applied pest control which combines and integrates biological and chemical control" (Stern et al. 1959). The concept was initially developed by entomologists faced with indiscriminate broad-spectrum insecticide use and insect outbreaks caused by the elimination of natural enemies and the emergence of pesticide resistance. IPM now applies to all aspects of plant protection and is the object of renewed attention as European farming policy, research and extension efforts across the European Union. The EU Framework Directive 2009/128/EC on the sustainable use of pesticides addresses this challenge, providing an updated definition of IPM largely inspired from the definition given by the Food and Agriculture Organization of the United Nations.

The European Commission (EC) has defined IPM as follows: "IPM means careful consideration of all available plant protection methods and subsequent integration of appropriate measures that discourage the development of populations of harmful organisms and keep the use of plant protection products and other forms of intervention to levels that are economically and ecologically justified and reduce or minimise risks to human health and the environment. IPM emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms".

The new EU IPM definition substitutes the concept of "pest control techniques" with "plant protection methods" and adding the concept of "ecological justification" to that of economic justification. These recent modifications reflect an increasing interest in understanding and working with ecological processes to make farming methods more sustainable. The EU Framework Directive requires that all EU Member States develop a National Action Plan which ensures that a set of eight general principles of IPM (Barzman et al., 2015) are implemented by all professional pesticide users starting January 1, 2014 (European Union 2009a). The new set of legislation - the so-called "EU pesticides package" includes two Directives and two Regulations and aims at risk reduction during the use phase of pesticides and demands that all pesticide users adopt IPM. IPM, is a multi-facetted approach drawing on many disciplines and involving several economic sectors and addressing the diverse needs of European agriculture across multiple climatic zones, farm sizes, variable pest pressures and changing farming practices, (Birch Begg & Squire 2011; Barzman et al., 2015).

IPM has come a long way since the introduction of "integrated control" defined as "applied pest control which combines and integrates biological and chemical control" (Stern et al. 1959). The concept was initially developed by entomologists faced with indiscriminate broad-spectrum insecticide use and insect outbreaks caused by the elimination of natural enemies and the emergence of pesticide resistance. IPM now applies to all aspects of plant protection. It is the object of renewed attention as European policy, research and extension efforts strive to mainstream it across the European Union. The EU Framework Directive 2009/128/EC on the sustainable use of pesticides takes on this challenge. It provides a definition of IPM largely inspired from the definition given by the Food and Agriculture Organization of the United Nations, substituting the concept of "pest control techniques" with "plant protection methods" and adding the notion of "ecological justification" to that of economic justification (Fig. 1). These recent modifications reflect an increasing interest in understanding and working with ecological processes. The EU Framework Directive requires that all EU Member States develop a National Action Plan which ensures that a set of eight general principles of IPM (Table 1) are implemented by all professional pesticide users starting January 1, 2014 (European Union 2009a). In addition to the Directive, Regulation 1107/2009/EC on the placing of plant protection products on the market requires that pesticides be "*used properly*", where proper use *"shall also comply with general principles of integrated pest management"* (European Union 2009b). The new set of legislation; the so-called "EU pesticides package includes two Directives and two Regulations, aims at risk reduction during the use phase of pesticides and demands that all pesticide users adopt IPM. This, however is a multi-facetted approach drawing on many disciplines and involving several economic sectors, so is a long term strategy that evolves to address changing pest threats, pesticide use restrictions, food chain demands, climatic changes and variable economic conditions.

Implementation of IPM principles: A dynamic, long term agroecosystems systems-based approach

IPM is a holistic, systems-based approach that creates synergies by integrating complementary preventive methods drawn from a diverse array of approaches. It builds on agronomic, mechanical, physical and biological principles, resorting to selective pesticide use when pest problems cannot be successfully managed with other systems. Reliance on a wide diversity of evolving solutions is needed to ensure the long-term sustainability of pest control measures. Over-reliance on a single pest control method (eg a single resistance gene or pesticide mode of action) can also cause a shift in the composition of a pest community towards species less susceptible to that method. The higher the selection pressure exerted by the control method, the more rapid the process. Pest complexes change over time (within and between growing seasons), so the IPM toolbox also needs to be dynamically adaptive. The application of IPM principles at farm or regional level requires a broad perspective that considers farming strategies operating within cropping systems over an extended spatial and temporal scale, rather than tactical control applied to individual crops. Many of the factors that are key to achieving robust agro-ecosystems are to be found at the cropping system level and at larger scales, as in area-wide IPM and landscape ecology. Approaching the problem from an agroecosystems angle makes it possible to design less pest-vulnerable cropping systems, move away from prophylactic control towards longer term prevention and suppression of key pests (including multi-pest complexes) in the system over multiple fields, farms and seasons (Barzman *et al*., 2015).

IPM is shaped according to site-specific factors such as regional cropping pattern, field size, type and availability of semi-natural habitats, the broader landscape, cultivation practices, pest pressure, R&D efforts, availability of training, farmer attitude, and economics. Benbrook et al. (1996) introduced the idea that farmers can evolve along an IPM continuum ranging from "no IPM" to "high or bio-intensive IPM". The continuum includes the integration of optimized pesticide use combined with non-chemical strategies in current crop production systems as well as more radical redesigns of production systems involving pest-resistant plant varieties (eg *Bt* expressing), crop rotations to avoid regional pest build up or to break the development of resistance breaking populations), landscape features and new IPM-compatible technologies. The "ultimate IPM", ideal is perhaps an unattainable situation, where the cropping system has been so well designed and implemented so that no crop protection intervention is needed once it is in place (Ratnadass and Barzman 2014). In reality, individual farmers practice IPM via a process of continuous stepwise improvements and integration of innovative solutions over several years. Gradual adaptation enables them to meet changing pest threats, agricultural policies, market pressures and financial incentives. Researchers and farm advisers can develop lasting and stable strategies by extending crop protection over larger spatial and temporal scales via stepwise improvements with farmers.

Adopting the 8 (P)rinciples of IPM in Europe: (Barman et al., 2015)

The driving principles of IPM in Europe are defined as:

- **P1 Prevention and suppression** (eg use of crop rotations, choice of pest-resistant crop varieties, pest suppressive landscape features)
- **P2 Monitoring** (eg use of traps to detect ‘hotspots’ of key pests or to trigger action thresholds for essential pesticide applications)
- **P3 Decision making (DSS)** (e.g. use of monitoring data and thresholds to guide farmers towards optimal IPM tools)
- **P4 Non chemical methods** (e.g. use of trap crops, insect pheromones, bio-fumigation, pest suppressive soil enhancement, conservation and augmented biocontrol)
- **P5 Pesticide selection** (eg advise on most selective product that is least harmful to humans, birds, other wildlife and pollinators and natural enemies providing key ecosystem services to local farming communities)
- **P6 Reducing pesticide use and reliance as the first tool of choice** (eg good advice and training on other IPM options)
- **P7 Pesticide / genetic resistance management (including *Bt* and HT traits and associated pesticide inputs)** eg use of high dose/refugia for Bt crops coupled with annual monitoring of pest populations, especially in ‘hotspot’ areas where resistance is most likely to be selected; stacking of pest resistance traits with differing modes of action.

- **P8 Evaluation** (e.g. annual and multi-year review of on-farm pesticide reduction success, yield losses, cost:benefit assessment of current IPM plans, new IPM products via advisors, new IPM training courses, inter-farmer knowledge exchange and cooperation, network building, area-wide IPM strategies via coordinated pest suppression at regional scale eg *Bt* maize growers suppressing ECB in the region for non Bt-maize growers, regional environmental and health benefits, trade-offs with other components affecting regional economy, environment and social/health criteria.

Specific aims of EU IPM systems: Translation from principles to implementation on farms

- **Reduce reliance on pesticides** (insecticides, herbicides, fungicides, others) to control key agricultural pests, including herbicide-resistant weeds.
- **Provide regionally and seasonally adaptive toolboxes of complementary IPM tools (used in optimal combinations)** that are readily available. There is no 'one size fits all' IPM solution, so the strategy must by dynamic and adaptive to changing circumstances.
- **Support prevention as primary goal** (eg rotations for WCR to reduce selection pressure, IRM, herbicide MOA switching, long term pest suppressive measures at farm and landscape scales**). Coordinate regional measures including rotations to create 'pest suppressive landscapes':**. Rotating maize to a diversity of non-maize crop species helps farmers avoid the development of variants of the Western corn rootworm (WCR) that are referred to as "rotation resistant" because of their propensity to oviposit in non-maize crops. The maize-soybean rotation in the USA corn belt routinely applied over large areas for many years has selected for a WCR strain that has lost its preference for laying eggs in maize, resulting in damage in maize following soybean crops (Levine and Oloumi-Sadeghi, 1996; Gray et al., 1998; Levine et al., 2002).
- **Suppress primary (target) *and* secondary pests**, which can be sporadic but can be problematic and add complexity to IPM programmes.
- **Promote biodiversity, especially NTOs** that contribute to Ecosystem Services (eg biocontrol of key pests and pollination of regional plants). Beyond pesticide reduction, promoting floral resources and NTO habitats on-farm (e.g. flowering or grassy margins, flowering strips, beetle banks, hedges, woods, etc).
- **Optimise crop agronomy to promote plant vigour, biotic and abiotic stress tolerance and reduce attractiveness for pests:** Many crop management practices apparently unrelated to pest management actually have a significant impact on the vulnerability of cropping systems to pests. Fertilization is known to affect sap-sucking insects and mites (Altieri and Nicholls 2003), plant pathogenic fungi (Snoeijer et al. 2000) and bacteria (Lamichhane et al 2013). Mechanical weeding can damage crop tissue and favor diseases (Hatcher and Melander 2003). Crop residue management can affect the overwintering capacity of pests (Sojka et al. 1991). Tillage systems often

determine abundance and composition of weed communities and soil-borne diseases (Norris 2005).

- **Reduce impacts of crop protection strategies on key pollinators** (future IPPM development, protecting key pollinators in agroecosystems), which safeguards key pollinators of crops and wild plants, especially in conservation areas/nature reserves.
- **Have no significant yield penalty** compared with currently approved good practice for crop protection in each production region (but dependent on pest pressure, IPM uptake and regional economics).
- **Provide regionally adaptive maize toolboxes** (taking into account: regional crop management, climate, readily available IPM tools, variable pest complexes).
- **Design of area-wide IPM programmes via farmer cooperation, using spatial and temporal deployment of selected IPM tools (eg growing pest-resistant varieties, coordinated spraying of pesticides), aided by Decision Support Systems /predictive pest epidemiology models.**
- **Promote training of independent, regional IPM advisors**; IPM needs to be 'hands on' (using on farm demonstrations) and not an academic subject.
- **Economically viable and practical for farmers** (based on regional cost:benefit analysis provided by independent IPM advisors).
- **Relate to pest pressure via regular monitoring of key pests**. Use forecasting tools and DSS will aid selection of best IPM options (dynamic over time and regional adjustments).
- **Maximise resilience and durability of crop protection systems**, particularly use of regionally adapted pest-resistant varieties which are managed for counter-adaptation in pests due to high selection pressure (eg pest adaptations to insecticidal *Bt* genes, insecticides and herbicides; countering via stacking resistance genes and reducing selection pressure via using a range of IPM tools, rather than just one).
- **Promotes mitigation measures**, including monitoring of pest 'hotspots' in areas of intensive cultivation, use of well managed refugia (Bt crops in cultivated Europe and elsewhere) and other landscape management measures that support biocontrol and pollination (eg floral resources and overwintering habitats on-farm).
- **Special attention for integrated weed management and sustainable use of herbicides,** reducing threats to biodiversity that underpins ES (eg pollinators, predators, parasitoids) and possible health risks (eg glyphosate over-use).

The potential role of GM maize in EU IPM: Specific issues to be addressed (*Bt* and HT traits).

- **Key 'target' pests of current *Bt* maize Mon 810 in EU = 'target pests' for approved Bt maize Mon 810** are European corn borer = ECB (*Ostrinia nubilis*; widespread; target lepidopteran pest for *Bt* Mon 810 expressing cry1Ab), Mediterranean corn borer = MCB (*Sesamia nonagroides*; Mediterranean region; Lepidopteran target pest for *Bt* Mon 810), Western corn rootworm (WCR) (*Diabrotica virgifera virgifera*; introduced pest, coleopteran pest of maize, spreading rapidly; potential target pest for cry34Ab1, cry35Ab1, cry1F expressing maize events still under evaluation for EU cultivation by EFSA and EC). However, WCR has already developed resistance to *Bt* maize expressing cry3Bb1 in USA maize growing regions faster than predicted by models. This is probably due to low dose expression of Bt protein in roots, presence of resistance alleles in higher frequency than expected, non-random mating in irrigated crops (humidity affects behaviour) and lack of fitness costs associated with resistance (Devos *et al*., 2013). IPM-compatible strategies to combat resistant WCR include the use of gene pyramiding and using seed mixtures; however recent research (Carriere *et al*., 2015) indicate that these strategies may be less successful since many WCR populations have lower susceptibility to currently available Bt toxins and cross resistance to pyramided toxins is common. Hence field resistance to *Bt* maize events evolved in 4-6 years in the USA, indicating an urgent need for additional IPM tools, including rotation.

- **Other key pests not targeted by currently available *Bt* maize crops = 'non target or secondary pests for current *Bt* crops'** (often these are regionally sporadic, they are not controlled by *Bt* maize and therefore require additional control measures using IPM). These include noctuid lepidopteran pests (eg *Sesamia* spp), coleopteran pests (eg wireworms), cereal leaf beetles, sap feeding beetles, corn weevil and white grubs, flies and midges, spider mites, aphids (with their associated viruses), leafhoppers and thrips. In Spain, more aphids and leafhoppers were found on Bt maize plots than on the isogenic variety (farm scale study; Pons et al., 2005). For some of these pests, good IPM tools (e.g. biocontrol agents like parasitoid wasps, pheromones, conventionally bred varieties with generic resistance to individual/groups of pests) are available and widely used. For other pests, good IPM tools are not yet developed or widely used, so public and private sector research effort is ongoing (e.g. development of biopesticides that are compatible with other IPM tools in the regional toolbox). In addition, the enhancement of endemic predators and parasitoids in *Bt* maize crops, achieved via reduced insecticide applications, must be an IPM priority for integrated maize production in Europe (Meissle et al, 2011).

- **Choice of control measure(s) driven by local economics, availability of IPM tools, changing pest pressures and availability of an independent advisory service.** For example, if pest pressure in a particular season is low but farmers are risk averse, they may invest in early season crop protection measures (eg GM seeds, seed coatings) that are not required, so would incur a cost penalty that is not offset by an increased yield. On the other hand, effective, long term use of *Bt* maize (or a conventionally bred resistant variety) will probably lower pest pressure over multiple seasons (thus reducing cost/benefit of this tool), raising the suitable mix of GM and non-GM (temporarily and/or spatially) would be optimal (modelling tools can assist with such predictions, because experimental evidence is too costly and time consuming to be a realistic option). In Spain (2002-2004) the economic benefit to farmers growing *Bt* maize varied significantly between regions and seasons (Gomez-Barbero *et al*., 2008), in relation to changing pest pressure and *Bt* maize seed cost premiums (which can be regionally adjusted to promote uptake of the technology). However, in farmer surveys farmers indicated that risk reduction and better yields (less mycotoxin contamination) were key drivers for paying the *Bt* maize seed premium in areas of highest ECB abundance (eg Zaragova province) where 11.8% yield gains were observed. The largest gross margin increase recorded was 122 euros/ha where pest pressure was highest, but this was lower in other regions with low pest pressure and reduced pesticide costs. Areawide pest suppression (AW-IPM) in areas of high Bt maize uptake can reduce costs for neighbouring non-*Bt* maize growers (i.e. shared economic benefit, but not shared costs). These landscape scale suppressive effects also add incentives to plant non-*Bt* maize refugia, since the **Bt** maize apparently offers a halo of protection at farm scale. Cumulative benefits over 14 years in 3 US states were estimated to range between $2.4-3.2 billion, with >50% benefit going to non-*Bt* maize growers (Hutchinson et al., 2010). Thus the benefits can be shared between adopters and non-adopters at regional scale.

- **Choice of regionally adapted maize varieties with appropriate genetic backgrounds and grown with agronomic practices to ensure high dose expression of *Bt* toxins across the whole season, as part of IRM** (high dose expression of *Bt* in relevant plant tissues is important to delay resistance evolution in pest populations; see lessons learnt with WCR resistance in USA; Carriere et al 2015).

- **Integration of IPM to include weeds that are difficult to control with currently available herbicide regimes:** HT crops including HT maize offer new tools for post-emergence weed control and where certain weeds are difficult to control due to existing herbicide resistance issues due to over-use and selection pressure (Powles 2008; Cerdeira et al., 2007; Heap 2016). An additional risk of HT maize is over-use of herbicides near conservation areas containing non-crop host plants for protected insect species. Studies on milkweed in USA show that monarch butterfly decline is linked increased use of glyphosate on plantings of HT soybean and HT maize (Pleasants *et. al*., 2013). If managed well and herbicide usage is closely regulated, biodiversity on farms can increase. Conversely, HT crops have also led reduction in on farm biodiversity, negatively affecting non-target organisms including pollinators and biocontrol agents (e.g.GM HT beet and GM HT oilseed rape; Firbank et al 2003). In these UK Farm Scale Evaluation (FSE) trials, testing HT maize (genetically engineered for resistance to glufosinate-ammonium) against conventional maize (Firbank et al., 2003), both the density and biomass of broad leaved weeds were three times higher in the GM than in conventional maize, producing twice as many seeds for on-farm wildlife. Other potential benefits of HT maize include reduction in fuel costs and in soil erosion if conservation tillage is practiced within an Integrated Weed Management (IWM) system. In IWM, selection pressure for herbicide resistance in weed populations could be avoided by using a range of complementary weed management tools in multiple strategies (Lamichhane et al 2016), including rotation, cultivation, mechanical or robotic weeding, use of new bio-herbicides and growing maize varieties that compete more effectively with weeds for resources. The long term aim is to reduce herbicide use, but IWM like IPM is case by case, management dependent and will need to be regionally adaptive to cope with changing weed types and competitive pressures. This requires a better knowledge of the biology and ecology of weeds and socio-economic stabilisation of IPM and IWM systems for maize in each production area in Europe using knowledge transfer from researchers to advisors and farmers and economic incentivisation programmes.

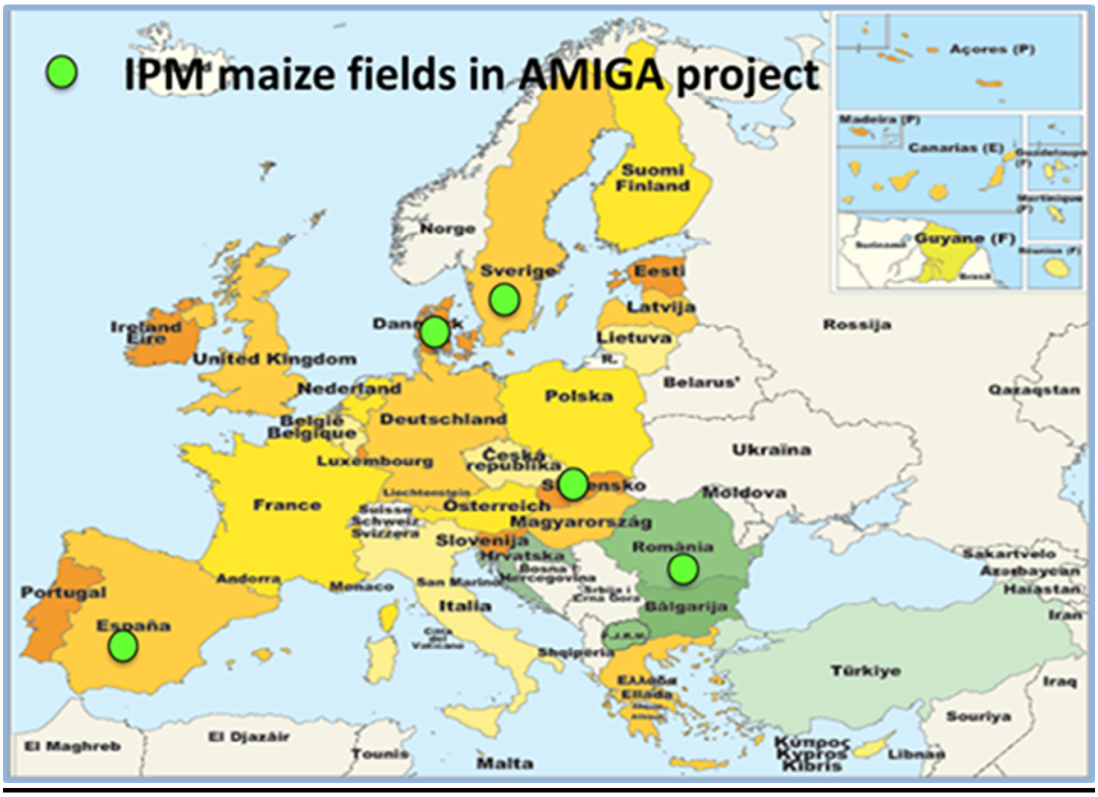
- **Compliance with licence requirements:** GM crops in general have more administrative burden and legal restrictions on use than conventionally bred varieties. For example, farmers growing *Bt* maize need to follow strict regulations on planting refugia (non-*Bt* maize that reduces selecting pressure on regional populations of the target pest) and monitoring the crop for any pest, disease or abnormal phenotypic issues. They also need to follow obligatory isolation distances between GM and non GM maize fields to comply with coexistence regulations that minimise gene flow by Bt maize pollen spread (which varies by member state, from 25m in the Netherlands to several hundred meters in Luxemburg; Meissle et al., 2011). *Bt* maize pollen deposited on non-crop plants which are hosts to protected species also poses a risk to *Bt*-sensitive non-target lepidopterans in protected habitats close to *Bt*-maize fields (Lang 2015; Hoffmann 2016), so a 20m isolation distance between the edge of *Bt* maize fields and such habitats is currently recommended (EFSA 2011, 2012).

Summary

- *Bt* maize has the potential to be a valuable IPM tool to suppress both ECB (eg Mon 810) and WCR (if approved for cultivation), if it it is managed sustainably, with particular attention to insect resistance management, rotations, monitoring of primary and secondary pests and additional IPOM tools to suppress other pest species in the regional pest complex (including weeds).
- HT maize could (if approved for cultivation) be a valuable new toll in EU for pest-emergence weed control and could benefit on-farm biodiversity if managed sustainably and weeds are monitored for herbicide resistance. IWM must take into account a better knowledge of biology and ecology of weeds, a more efficient use of herbicides with mixtures and rotations of different mode of action, crop rotation if possible in the area, the use of various tillage systems and mechanical weeding. Without forgetting that any IPM system must be done case by case. The agricultural, environmental and socio-economic situation are the basis on which any IPM system must be established

- Non target pests (not controlled by the GM traits under consideration; *Bt* and HT) need to be monitored regionally and IPM tools need to be included in the regional maize IPM toolboxes to suppress them.
- On-farm biodiversity that delivers biocontrol and pollination could be enhanced through careful design and implementation of IPPM strategies to reduce pesticide inputs. However, on-farm and semi-natural habitats that provide essential resources for beneficial organisms need to carefully managed, especially if sited close to fields of *Bt* of HT maize. Adequate isolation distances need to be ensued to prevent either *Bt* maize pollen or herbicide sprays adversely affecting habitats for NTOs, especially protected species and those under threat (e.g. bees and other pollinators).
- Successful uptake of GM crops within IPM frameworks will probably require economic incentives (e.g. regional seed price adjustments), better training of advisors and farmers and greater acceptance by consumers and environmentalists (a long term and ongoing societal debate). It will also be dependent on variable pest pressures over space and time (affecting economics and farmers' risk management behaviour), changing pesticide use legislation at EU and MS levels, impacts of climate change on regional cropping systems and pest impacts and on global markets for food, feed and derived products produced from GM crops; so a highly dynamic and flexible approach will be required.

AMIGA WP8 case studies: Testing maize IPM options in several geographic regions



In this reports data relative to maize experimental fields are only partially described, since the wealth of ecological data collected will require additional time due to the long lasting taxonomic classification necessary before running the complete statistical analyses.

Case study 1: Integrated weed management for Bt-HT maize in Spain (INIA)

The AMIGA project in its WP8 aims to: (a) identify IPM components which could be affected by adoption of GM crops; (b) assess the environmental impact of management options for in our case Bt and HT maize and (c) propose measures by combining IPM tools that reduce selection pressure on weeds and reduce pesticide use in terms of active ingredients.

The INIA team in AMIGA WP8 evaluated and compared the effect of conventional and innovative weed management practices, including *Bt* and HT maize, on weeds and arthropods

Weed competition has been considered to be one of the main biotic constrains limiting crop production (Oerke 2006). In most arable crops, the dependence on herbicides prevails on intensive farming systems. In all major crops, as maize, herbicides have been the main tool for weed control. Recent studies indicate that 90% of the total maize in 11 European regions has been treated with herbicides at least once in a season (Meissle et al 2010). The over-use of one or few herbicides leads to the development of herbicide resistant weed populations and/or to shifts in the weed species communities.

The introduction of GMHT technology, involving the usage of an approved broad-spectrum herbicide such as glyphosate or glufosinate-ammonium (Owen 2000), will provide new alternatives for in crop weed control. Both herbicides are widely used and non-selective, especially glyphosate; used for weed control in no or reduced tillage systems, in perennial crops and in no cropping areas. Glyphosate is one of the most effective herbicides available but from this high effectivity, many concerns can arise if it is not properly used. The HT crops, their associated herbicides and the strategies used to integrate them in weed control systems could be in the origin of hazards and benefits reviewed for maize by Dewar (2009).

HT maize could be used a new tool for weed control with good efficacy that can be used in post-emergence, when a glyphosate tolerant crop is available. This could help in the control of HR weeds to other herbicides and weeds difficult to control in a crop. Also HT crops and the associated herbicide regime can benefit biodiversity of plants, arthropods and the food web if properly managed in an IWM system. Besides this, agronomic practices such as conservation tillage were favoured by HT maize with reduction of soil erosion, fuel use, carbon mobilisation and contamination of water.

The main risk of HT maize is the development of herbicide resistance in weeds because of the over-frequent use of a single mode of action in large areas (Powles 2008). At present, glyphosate resistance was mentioned for around 260 populations of 35 weed species (Heap 2016). Some of them appeared in areas were glyphosate tolerant crops dominate (Cerdeira et al 2007). Weed shifts produced by glyphosate management in HT crops allow less sensitive weed species to proliferate and modify weed communities (Westra 2005, Loureiro et al 2011) creating another potential risk. Besides, this glyphosate tolerant volunteers remaining in the field from a previous HT crop will be a major problem in a scenario of one or various glyphosate tolerant crops in rotation. The risk of HR trait transfer by gene flow, could be associated with the above mentioned risks, HR genes can move from crop to crop or weed to weed (Loureiro et al 2012; Loureiro et al 2016). The direct and indirect risks identified, as any one-weed control agricultural practice can affect biodiversity of plants, arthropods and the food web.

Spain has about half a million hectares for maize production. Maize is grown in specific areas very diverse in their characteristics, which means that maize from 200 to 800 maturity classes are sown. Pest incidence is very different in the different areas. Currently, GM maize (Bt maize) is cultivated only in some regions (Aragon, Cataluña and Extremadura) due to the high pressure of corn borer pests, not efficiently controlled by conventional insecticides. In those areas, Bt maize increases yields and reduces insecticide use. While weeds represent a major threat in all maize producing areas, in these areas, there is a very high share of maize in crop rotations. In these circumstances, it is difficult to include crop rotations on IPM strategies without significantly reducing the benefits to the farmer. Taking into account the maize scenario in Spain, our IPM proposal was an IWM including conventional; Bt and BT-HT glyphosate tolerant maize varieties.

Field trials were located near Madrid (Fig. 1). The first one (red mark) was in a 5 ha Bt maize field in Seseña , province of Toledo in the Jarama river plain, a maize cropping area. The second one (blue mark) was in a 2 ha HT-Bt maize field in Alcalá de Henares, province of Madrid in the Henares river plain, a non-typical maize cropping farm belonging to INIA.

Figure 1. Location of the two field trials

LA CANALEJA HT-Bt-MIR MAIZE FIELD

This trial, for economy and safety reasons,_was conducted in the INIA farm La Canaleja in 2013 and 2014 with the non-yet authorized stacked event: Bt11 x MIR604 x GA21 and its isogenic line provided by Syngenta. In 2014, this field was attacked by birds on July 2014 and destroyed by us according to the Spanish legislation on 24th September of 2014. Data were collected only for one year and will not be presented.

SESEÑA Bt MAIZE FIELD

A three-year study was conducted in a farmer's field located in Seseña, during the maize growing seasons in 2012, 2013 and 2014. This is one of the traditional maize producing areas within central Spain, and therefore the field had already been cultivated with maize for years before the beginning of the experiments. The regional climate is continental Mediterranean, with mean annual rainfall of 425 mm, mostly distributed from October to May and almost absent during the summer cropping season. For this reason irrigation is necessary, so the maize field was flooded every 8-10 days. Other standard farming practices for maize production, including fertilization, were used as required.

The experimental field was divided into 30 x 20 m plots, separated by 3m wide lanes of bare soil. The experimental set up was a completely randomized design involving eight managements combining Bt (MON810) and the conventional variety with five different herbicide treatments, each of them with five replicates. The location of each plot was identical for the three consecutive years of the study.

The maize sown were the conventional (**C**) and Bt (**Bt**) maize varieties DKC6450 and DKC6451YG (http://www.dekalb.es/dekalb/index.jsp?siteCode=SPAIN). Maturity class (FAO units): 700

The five-herbicide treatments, one conventional and four potential Integrated Weed Management (IWM) approaches were:

C The Conventional herbicide treatment (pre + post application) is the standard treatment conducted by farmers according to the recommended regional practices for maize production

HR The Herbicide Reduced reduces both the herbicide application to only one and the doses of the herbicides employed in the conventional treatment.

Pr+G The Conventional pre-emergence + glyphosate includes a pre-emergence application at the lowest registered rate of a conventional herbicide, followed up by a post-emergence application of glyphosate

G+G Two glyphosate post-emergence applications

HR+G Includes two treatments: the above mentioned HR treatment and a second treatment with glyphosate, both post-emergence applications

The combination of the treatments including glyphosate and conventional maize would mimic the use of one herbicide tolerant maize variety, while combined with a Bt maize variety, they would mimic the use of an stacked (Bt + HT) tolerant variety.

The herbicides and doses applied in each treatment are summarized in Figure 2.

amiga **Bt Maize WP8** INIA

	Herbicide treatments	Dose (L ha^{-1})
CONVENTIONAL-PRE	S-Metholachlor 31.25% + Terbutilazine 18.75%	4.0
CONVENTIONAL-POST	Nicosulfuron 6%	0.65
	Mesotrione 10% w/v	1.00
HERBICIDE REDUCED		
	S-Metholachlor 31.25% + Terbutilazine 18.75%	3.0
	Nicosulfuron 6%	0.75
	Mesotrione 10% w/v	0.50
GLYPHOSATE		
	Glyphosate 36% (2 treatments)	3.0
CONVENTIONAL-PRE + GLYPHOSATE		
	Conventional-pre + Glyphosate 36% (1 treatment)	
HERBICIDE REDUCED + GLYPHOSATE		
	Herbicide reduced + Glyphosate 36% (1 treatment)	

Figure 2. Herbicides and the doses of herbicide applied in each treatment

Treatments **C**; **HR** and **Pr+G** were applied to **Bt** and **C** conventional maize, treatments **G+G** and **HR+G** were applied only to **Bt** maize.

The effect of the combined treatments of seed variety and herbicide treatment was studied on target pests, weed control and non-target arthropods.

Target pests:

In the area of the trial, corn borers attack is not a common problem (low pest pressure in most seasons), however the presence of corn borers (*Sesamia nonagrioides* and *Ostrinia nubilalis*) was evaluated. From each plot, the cob of 10 selected plants per row was classified according to the percentage of surface damaged by corn borers, using a scale from 1 to 7. In addition, the number of larvae of corn borers per plant and the number of tunnels per plant were counted.

As expected, the incidence of corn borers was very low in our trial in the two years (Fig 3)

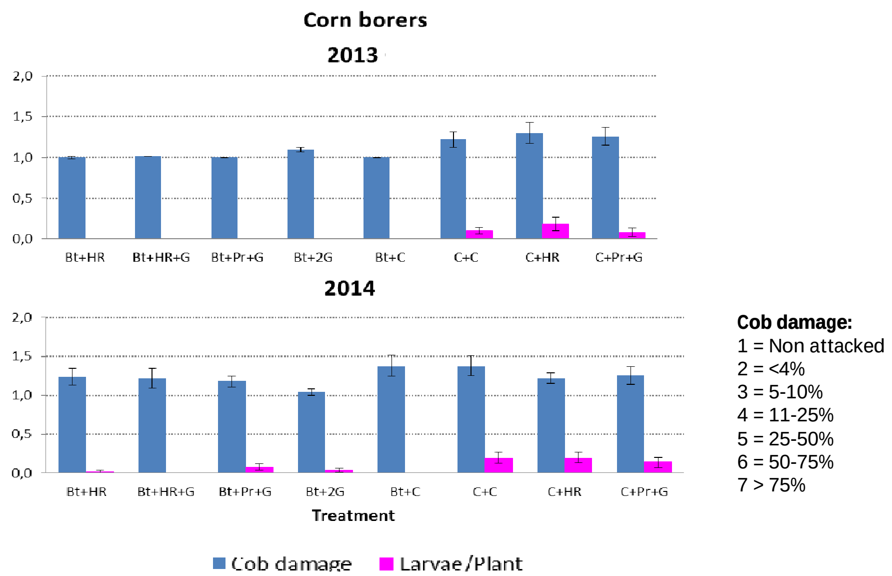


Figure 3. Incidence of corn borers in 2013 and 2014 field trials

Secondary pests

The incidence of **spider mites and aphids** was evaluated (figure 4). From each plot, 5 plants of 2 maize central rows were scouted at the end of the cultivation, but when the plants were still green. Plants were classified as: 0 = free of aphids and mites; 1= 1-2 small colonies; 2=several and/or large colonies.

In 2013, a high incidence of spider mites and low population levels of aphids were recorded. On the contrary, in 2014, mite populations were negligible and incidence of aphids was much more important. This could be explained by the different climatic conditions in the two years: summer of 2013 was much drier and warmer than in 2014.

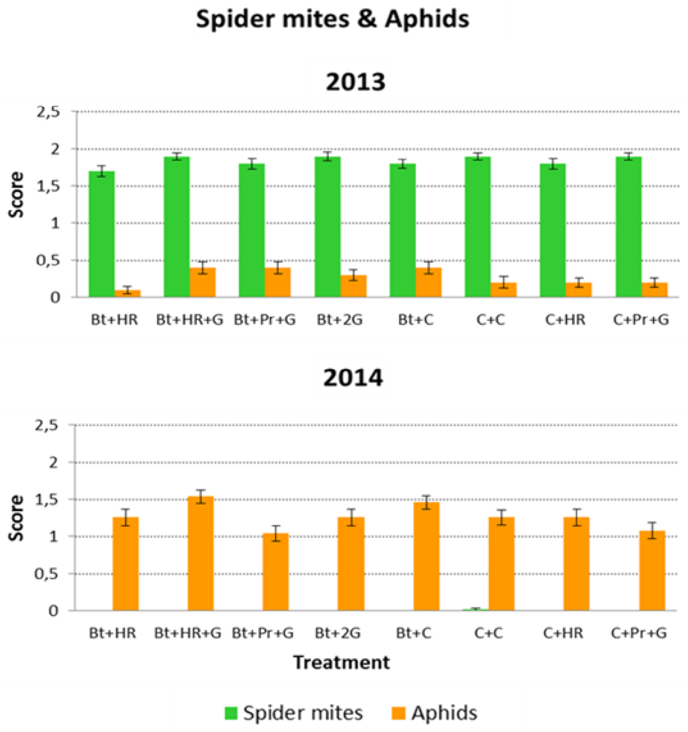


Figure 4. Incidence of spider mites and aphids in 2013 and 2014 field trials

Soil insects: wireworms and cutworms. From each plot, 2 maize central rows were chosen and all plants were scouted at the 4th leaves stage (V4), registering the number of damaged plants. Incidence of wireworms and cutworms was very low in the two years (lower than one damaged plant per row).

Estimated Production (plot yields)

The field trial was conducted in a farmer's own field. For this reason, it was not possible to assess individually the production of each plot. The production in grain dry weight was assessed indirectly through the weight of 10 cobs collected at random in each plot and the estimated number of adult plants per plot in 2013 and 2014. This was the only way available with our facilities. There were not significant differences among the five herbicide managements for the estimated production.

Weed abundance and diversity

In each plot, 12 0.25 m^2 quadrats were used for the weed assessments, which means an area of 3 m^2 per plot. The parameters measured were weed species, weed density/number per species. Weed seedlings were counted but not removed to check post-emergence weed control effect and final weed density. Weeds were sampled before the first herbicide treatment, 15 days after each herbicide treatment and at physiological maturity of the crop.

The weed species present in the field along the three years were the typical weeds of maize crop in the area, of them 19 species were dicotyledoneous and 5 monocotyledoneous.

The abundance of weeds was evaluated over the whole-cropping period. Figure 5 shows the mean abundance of weeds for each treatment for the last sampling, done at physiological maturity of the crop.

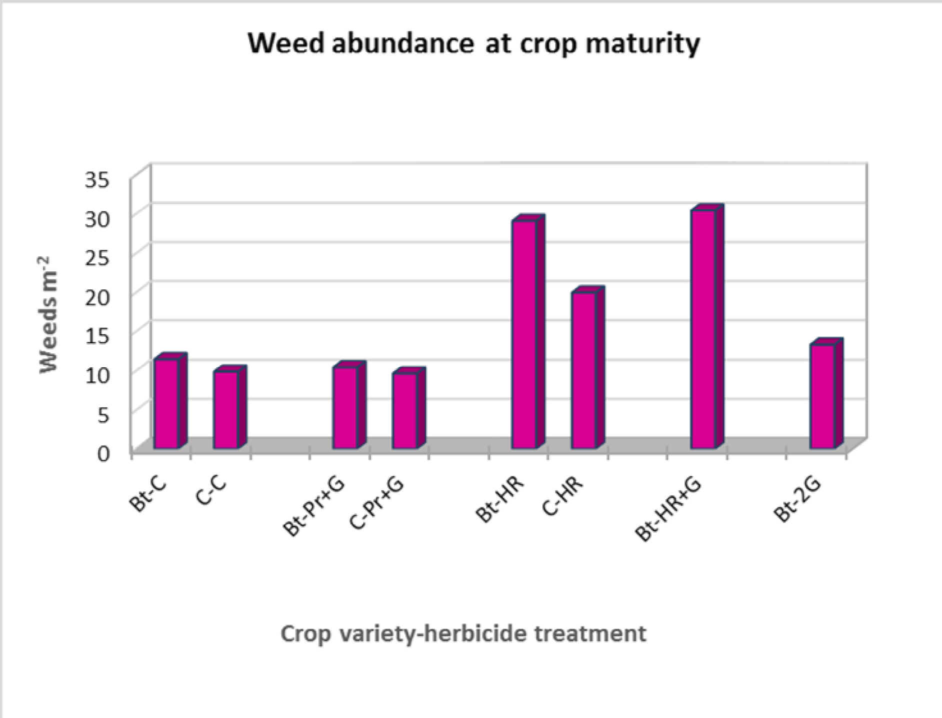


Figure 5. Weed abundance at crop maturity by treatment (mean of 3 years)

In order to test differences in the response of weed populations and the estimated yield to the different herbicide applications, an ANOVA (Analysis of the Variance) was performed. The analysis was conducted on data from 3 surveys. Survey 0 (S0): before the first herbicide treatment; S3: 15 days after the last herbicide treatment and S4: at physiological maturity of the crop. The eight treatments formed combining seed variety and herbicide management show that all treatments that include a pre-emergence herbicide followed by a post-emergence application show a better control of weeds irrespective of the post emergence herbicide applied. The treatments done only in post emergence HR, glyphosate and the combination of both allow a higher abundance of weeds.

The data show that although the number of grass species is low, their abundance is higher than that of the broadleaf species at the end of the cropping period.

Seedbank study

For the 3 years of the study, soil for seed bank analysis was sampled in the field. The analysis was completed for 2012 and 2013, 2014 is under study. Data were not analysed yet.

The protocol used was “A Handbook of Field Sampling Protocols for Biodiversity Indicator Monitoring” (James Hutton Institute, 2011) with some modifications. A sample of 2 litre of soil was collected in the middle of each field plot; seedbank diversity was evaluated using the germination method for three years after soil collection. The number of individuals of every weed species that germinate is recorded, until no further weeds germinate.

Non-target arthropods

The presence of non-target (NT) arthropods was monitored in 2012, 2013 and 2014 with three different survey technics: visual samplings, pitfall traps and yellow sticky traps. The data presented are only from 2013.

Visual samplings. Inspection was made of one entire maize plant per plot, selected randomly in situ, at intervals of 4 weeks starting from early June until the middle of October, with a total of 5 sampling dates per year. Individuals were classified in three different functional groups: phytophagous, predators and others.
The functional group “Other arthropods” (with different feeding habits to phytophagy, predation or parasitism) was the most frequently found in visual inspections of plants (Figure 6), includingspringtails (Collembola) the most abundant group by far. Phytophagous insects found were mostly Cicadellidae and Thysanoptera. The most abundant groups of predators were Cecidomyiidae, Chrysopidae and Araneae.

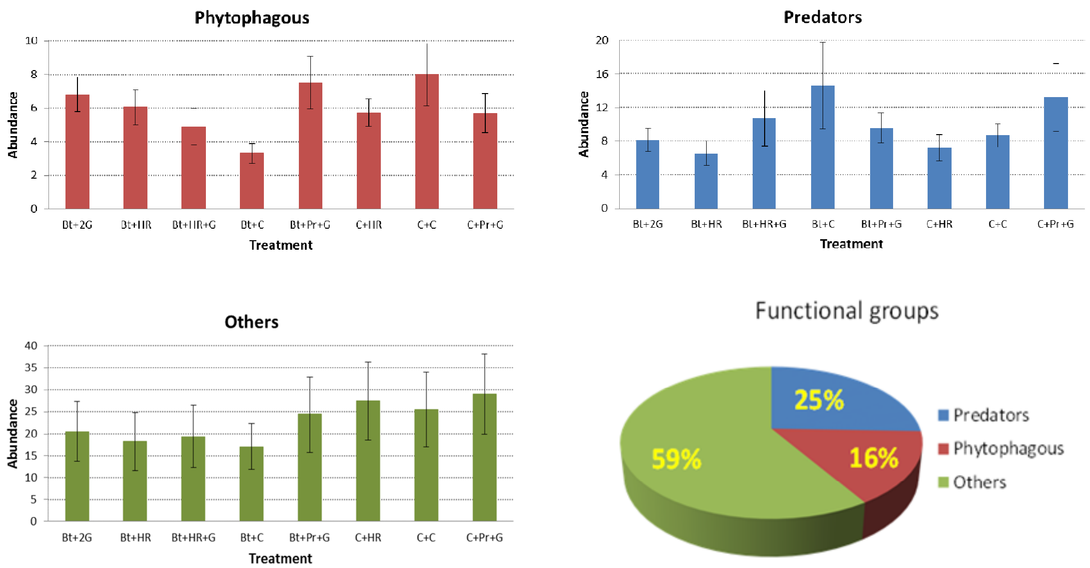


Figure 6. Abundance of arthropods of the different functional groups, found in the different treatments by visual inspection

Pitfall traps. Above ground arthropods were monitored by pitfall traps. Two traps per plot were arranged diagonally in the middle of each plot. Traps were operative for 2 days every two weeks. A total of nine sampling dates per year were performed from the end of May to mid-October. All the individuals collected in the pitfall traps were taxonomically identified to the genus/species level in the predominant groups and to at least the family level in the others. All individuals were classified into four categories namely as phytophagous, predators, parasitoids and others.

Predators were the group most frequently captured in pitfall traps (Figure 7), being Araneae and Carabidae the most abundant groups. Almost all phytophagous arthropods captured were omnivorous Gryllidae. Within the functional group "Other arthropods", Collembola was also the most abundant taxon found with this sampling method. Some parasitoids were also captured in pitfall traps, all of them *Baeus* spp. (Hymenoptera: Scelionidae), parasitoids of spider egg sacs.

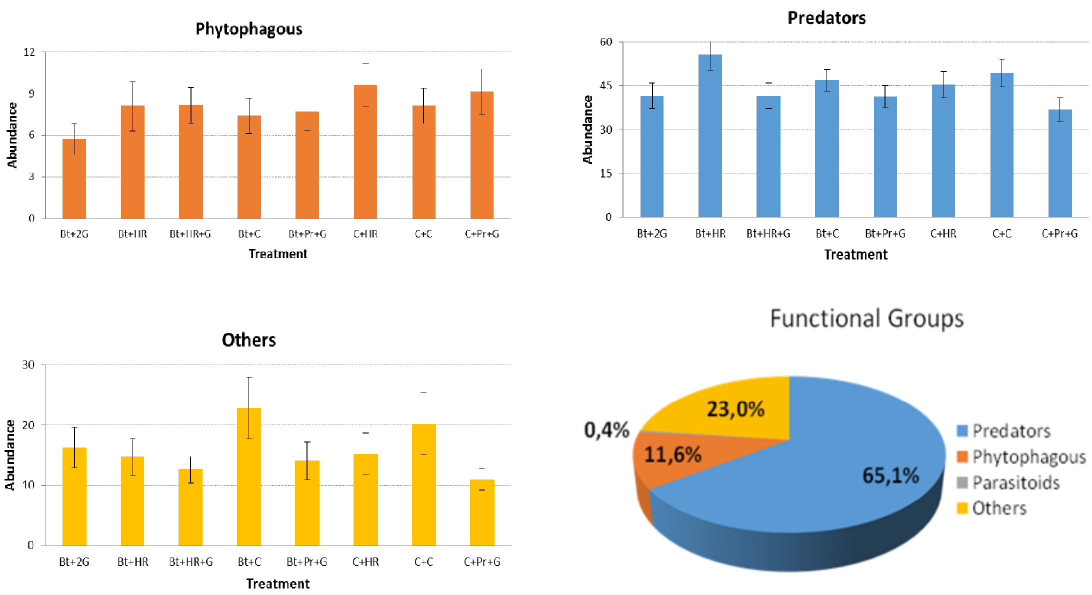


Figure 7. Abundance of above ground arthropods of the different functional groups, found in the different treatments by pitfall traps caching

Yellow sticky traps. Yellow sticky traps were used to monitor flying arthropods. Two cards (20 cm x 24.5 cm) per plot were fastened to woody stakes, arranged diagonally in the centre of each plot, and adjusted periodically to the height of the canopy. Traps were operative for 3 days every 4 weeks and then returned to the laboratory for processing. A total of five sampling dates per year were performed from the end of May to mid-October. A 10 cm x 10 cm square portion was cut out from the whole card, always the same part, and all the individuals in that portion were taxonomically identified to at least the family level. Individuals were also classified in four different functional groups: phytophagous, predators, parasitoids and others.

The arthropods most frequently found in yellow sticky traps were phytophagous (mainly Cicadellidae) (Figure 9). Parasitoids were also frequently captured, being the most abundant by far, those belonging to the family Mymaridae (parasitoids of Cicadellidae). The most abundant groups of predators in these traps were Aeolotripidae, Coccinellidae and Anthocoridae. In this case, Chloropidae was the family most frequently captured belonging to the functional group "Other arthropods".

The abundance of arthropods of the different functional groups, obtained by yellow sticky traps, in the different treatments is shown in **Figure 8.**

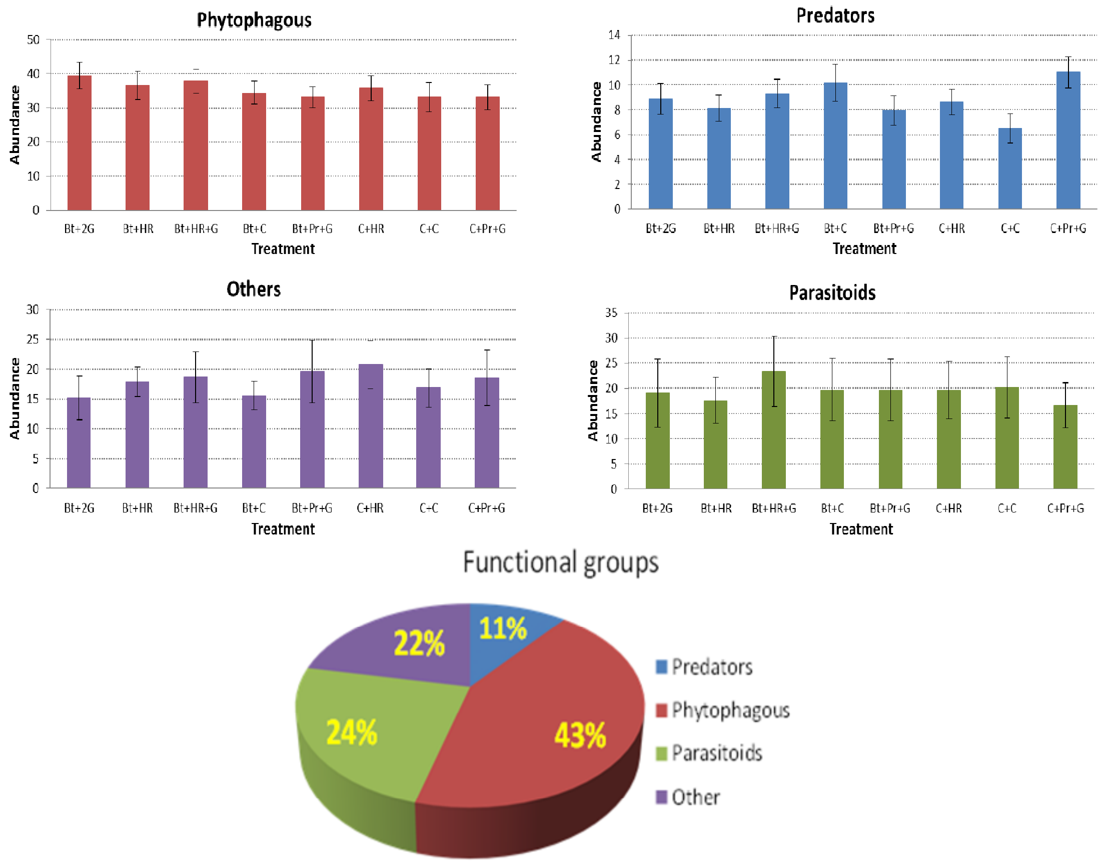


Figure 8. Abundance of above ground arthropods of the different functional groups, found in the different treatments by sticky traps caching

The INIA group has so far only partially analysed the large number of data obtained from field studies. At this point, we cannot make definitive conclusions, we can only point out trends that will have to be confirmed or rejected once the analysis of all data is completed.

Related to weed control, main findings so far are:

- There were no significant differences among the five herbicide managements for the estimated production.

- In all weed management systems tested, at the end of the cropping period grass weeds were more abundant than broadleaf weeds.
- Treatments that include a pre-emergence herbicide followed by a post-emergence herbicide showed a better control of weeds, irrespective of the post-emergence herbicide applied.
- The treatments done only in post emergence as HR, glyphosate and the combination of both allow a higher abundance of weeds in the field at least at the end of the cropping period.

Related to pest and non-target arthropods, main findings so far are:

- Very low incidence of corn borers and soil insect pest (wireworms and cutworms) in the area of study, regardless of weed management methods and maize seed (Bt or isogenic).
- Highly variable incidence of spider mites and aphids, depending on the climatic conditions of the year.
- Extremely high abundance and diversity of non-target organisms in our area, by all sampling methods.
- High population levels and diversity of ground-dwelling predators (especially spiders and carabid beetles).
- Extremely high abundance of Collembola in ground and plants.
- High population levels of the secondary pest leafhoppers (Cycadellidae) and their parasitoids (mainly Mymaridae)
- .

The data presented are only a small part of data collected. Sampling of weeds and arthropods is completed, so only the analysis of seed bank is incomplete and needs one more year.

Analysis of the whole data and relatioships amongst the different parameters evaluated is ongoing.

PRELIMINARY CONCLUSIONS (Spanish maize IPM experiments)

Most of the data analysis are in progress. The preliminary conclusions presented are partial and therefore only indicative. The relationships between maize variety, pest control, herbicide treatment, weed control and their effects on non-target arthropods needs a deep analysis over the whole cropping period. In our case, insecticide treatments were not necessary because the low incidence of corn borers (and other pests as wireworms and cutworms). This is most likely a reason for the high abundance and diversity of arthropods, including major groups of natural enemies (ground beetles, spiders, hymenopteran parasitoids).

The use of Bt-HT maize could allow a more diverse weed management in maize. Since no differences in production (plot yields) were detected we can consider that all treatments are acceptable *a priori*. The cropping circumstances would determine the most appropriate weed control system in every case. The reduced herbicide system, has both economic and environmental advantages. The programs that include glyphosate could control weed biotypes resistant to other herbicides and in rotation could avoid or at least delay herbicide resistance development. Treatments applied in post-emergence allow weed control later in the season if weed infestation is low at maize emergence, they also favour more weeds which could be environmentally favourable for beneficial flora and fauna. The abundance and richness of weeds undoubtedly conditions the diversity and abundance of arthropods, so, the conclusion of the study of the relationship between herbicide treatments and arthropods populations can provide important information to select management practices that help to maintain populations of herbivorous controlled by their natural enemies, and to avoid dependence on insecticide treatments. Finally, the conventional treatment could be used to achieve a highly effective control of weeds if needed. Each treatment has advantages and disadvantages. A good weed management would diversify as much as possible weed control methods.

Following Birch et al (2011), the applied research challenge nowadays is to reduce selection pressure on single solution strategies, by creating interactions between IPM components that can increase the durability of individual tools. Diversification in crop systems and weed management tactics reduces the risks of weed control and promotes biodiversity. Therefore, the most effective and sustainable use of HT crops would be as a component of an integrated weed management (IWM) approach. Employing multiple strategies to manage weed populations in a manner that is economically and environmentally sound to suppress weed populations, and to prevent or delay herbicide resistance evolution (Lamichhane et al 2016). These authors review IWM measures, limiting factors and the role of HT crops in the framework of EU directives and their mandatory principles. Based on that, the use of herbicides in intensive production is unavoidable, the efforts on IWM must be focused on herbicide reduction and on lower risks without affecting crop production.

IWM must take into account a better knowledge of the biology and ecology of weeds, a more efficient use of herbicides with mixtures and rotations of different mode of action, crop rotation if possible in the area, the use of various tillage systems and mechanical weeding. It is important to remember that that any IPM system (including IWM) must be done case by case. The agricultural, environmental and socio-economic situation are the basis on which any IPM system must be established.

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Case study 2: Amiga experience from the Swedish maize IPM trial

Choice of control measure(s) driven by local economics – examples from the IPM trial in Sweden

The establishment of the European Corn Borer is ongoing in Sweden, and is continuously monitored by the Swedish Board of Agriculture. A stable establishment of ECB has been seen under the duration of the Amiga project (2012-2015, Figure 1). To prevent establishment of ECB, crop rotation with a 3-4 gap between consecutive maize crops has been suggested (Söderlind et al. 2015). Field observations during the Amiga project however show that it is common to grow maize after maize in the region of Scania. To prevent development of caterpillars in maize stubble, a change in management to 10 cm ploughing after maize harvest, in contrast to the present practice of leaving maize stubble until the next spring, has been suggested (Söderlind et al. 2015). At present, no chemicals for pest control in maize are registered in Sweden. Due to low pest pressure of ECB in Sweden, a simulated best practice was therefore developed with 2 sprayings of Sumi alpha pyritroid to be able to compare the abundance of Non-target Organisms in conventional maize with and without chemical control, and MON 810 maize. The trial showed that similar numbers of arthropods were found in all 3 maize treatments in June, July and August 2014 (Fig. 2).

Birds have been reported to be problematic in organic maize cultivation (Ivarsson, http://www.jordbruksverket.se/download/18.5aec661121e2613852800010896/1370041014943/Jonas%2BIvarson.pdf), and this was also our experience in the Amiga trial. Maize establishment was poor in our trial in 2013, and bird herbivory was observed. Scare crows, dead rooks and netting were unsuccessful in deterring birds in 2013, which led to re-sowing of several plots. In 2014, Mesurol coating was therefore used, which successfully prevented herbivory by rooks and crows. IPM control measures would need to take bird herbivory into account in this region. A large population of wild boar has also made it necessary for farmers in some areas to put electric wire around their maize fields (this was the case for the Dalby field, a conventional field sampled for Amiga). For ECB control, biological control with Bt or *Trichogramma brassicae* has been suggested but at present we are not aware of any experience of biological control in Sweden (Söderlind et al. 2015).

Natural pest control was investigated as the number of aphid mummies. Regular inventories for aphid colonies were performed according to the Amiga protocol. Aphid colonies were not found, but hand picking of leaves discovered aphids and aphid mummies. There was a strong seasonal pattern with more mummies in mid-summer than in early and late samplings, but the number of mummies did not differ between maize cultivars or treatments (Figure 3). There appeared to be a seasonal differenc in the number of aphids on conventional compared to Bt-maize, with more aphids on conventional maize early in the season and more aphids on Bt-maize in the second half of the season. I future, this needs to be analysed in relation to the developmental stage of the maize varieties.

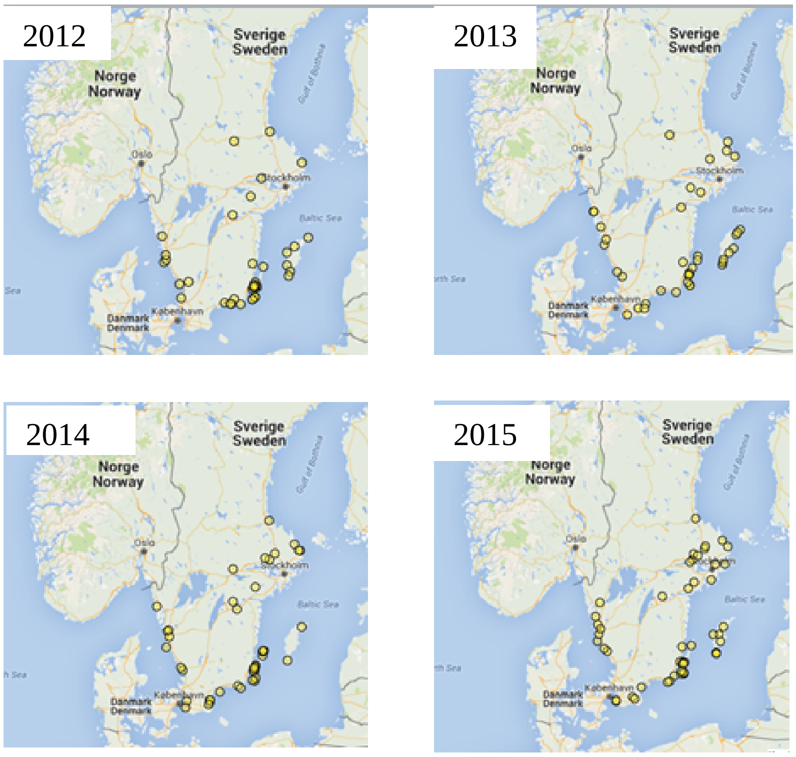

Figure 1. Number of observations of the European Corn Borer reported in Sweden during the period of the Amiga project (2012-2015) according to Artportalen (https://www.artportalen.se).

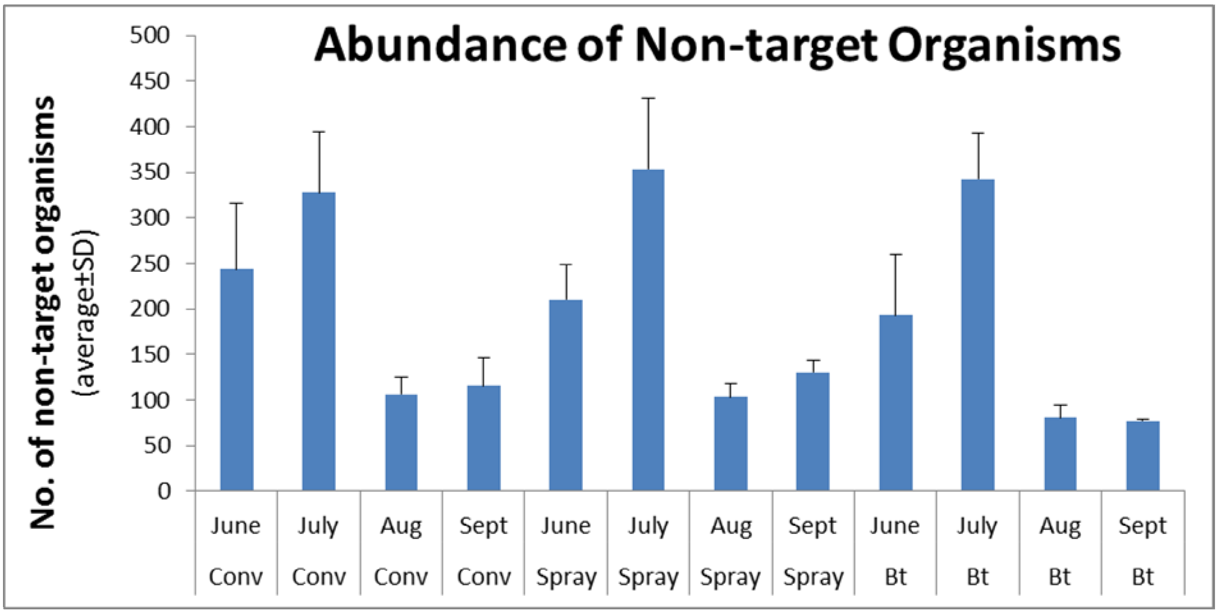

Figure 2. Abundance of Non-target Organisms in conventional maize, conventional maize with pyretroid sprayings and MON 810 maize in the IPM Amiga trial in 2014.

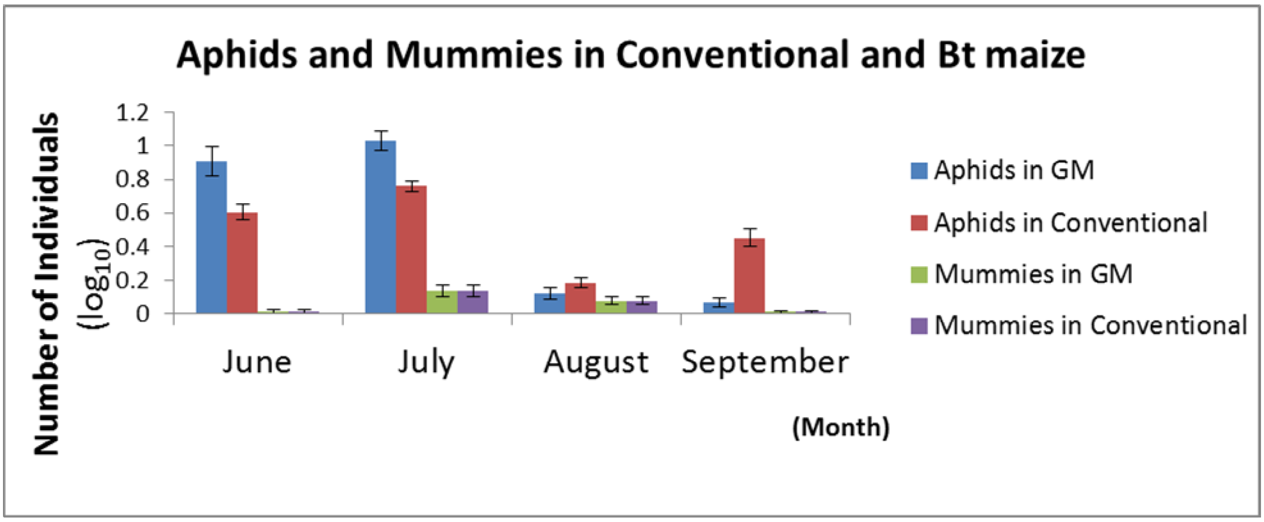

Figure 3. Number of aphids and aphid mummies in conventional and Bt MON 810 maize.

Choice of regionally adapted maize varieties for Sweden

In the Amiga trials, a variety adapted to more southern regions (Slovakia) was used. A number of new silage maize varieties are appearing on the market for cultivation in northern regions with for example early maturation. For northern cultivation of maize, the growing season is a problem. Especially in clay soils, the temperature for sowing may not be warm enough until quite late in the season. At present, maize stubble is left in the ground over winter and provides some cover until the late sowing. However, if stubble will be removed before winter to prevent ECB caterpillar development, late sowing of maize may need to be combined with for example a cover crop to prevent a long period of bare soil which may lead to increased soil erosion.

Integration of IPM to include weeds in Sweden

To encourage establishment of maize, early weed control is needed and MaiSter herbicide is commonly used in Swedish maize cultivation. Two applications of MaiSter were therefore used in the Amiga trials. In combination with increasing maize cultivation and a warmer climate in Sweden, the formerly rare annual weed *Echinochloa crus-galli* has become common in some regions and is expected to increase. This weed was also common in the Amiga trial, together with *Solanum nigrum, Geranium molle, Tripleurospermum perforatum* and *Cirsium arvense*. *Echinochloa crus-galli* is considered a noxious weed globally, and has become problematic in agricultural fields on the island of Öland. Crop rotation with cereals and oilseed crops is suggested as a way to limit the establishment of *Echinochloa crus-galli* (http://www.anpdm.com/newsletter/2645558/44425D447843435A4A71). A suggested IPM strategy for weed control in Sweden would include mechanical row management in comintaion with crop rotation.

PRELIMINARY SUMMARY (Swedish maize IPM case study)

A sustainable IPM strategy would involve crop rotation, mechanical weeding, use of early season varieties for faster maturation and shallow ploughing of stubble before the winter. To avoid a long period of open soil during winter and spring it would be beneficial to include cover crops in the rotation when maize is followed by a spring-sown crop. The use of refuges to encourage populations of natural enemies of for example aphids will be important and could in this region include keeping early-flowering Salix species in the landscape to maintain a high population of gall wasps. Bird herbivory is a problem for maize and deterring birds involves a whole season and very committed approach, including the feeding of birds away from maize fields.

References (Swedish maize IPM)

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Case study 3: Slovakian maize IPM study

Introduction

In general, no comprehensive IPM programmes have been developed to accompany the introduction and use of GM-crops. Field studies, including GM Bt maize and non-GM maize, were conducted in Slovakia to assess alternative IPM-management options for *Bt* maize. Comparison will be against current best non-GM practices for the region(s) of choice.

The Slovak maize IPM trial included three variants with four replications (Table 1). The trial was supported by the results from BT trial (Table 2). Both trials were at the same field, were sown in the same time and managed by the same soil management.
In both trials, pitfall traps were used for the monitoring of insects (mainly Carabidae, Collembola, Spiders).
BT trial in Slovakia (2012-2015). Each hybrid was sown in 10 repetitions. Each plot was 10 m long and 10 m wide. Each plot was isolated from other plot with 5 m wide barley strip (**Table 2**).

Results from Slovakia

Generally the variants of both experiments did not influenced non-target insects.

Bt trial included maize plots in which there were not used nor chemical, nor bioinsecticides. BT maize totally influenced the attack of the European corn borer (ECB) (*Ostrinia nubilalis*). IPM trial showed the same result.

The plots with Bt maize usually produced higher yield compared to their isolines.
Bt maize influenced also the occurrence of the Cotton bollworm (*Helicoverpa armigera*) in 2015, in which there was higher attack of this pest in Slovakia.

Application of chemical insecticide was more effective against the ECB compared to the application of bioinsecticide.

PRELIMINARY CONCLUSIONS FROM SLOVAKIA

- *Bt* maize hybrids principally influence the occurrence of the main pest of maize (ECB) in Slovakia and they have also partial effect to reduce the damage caused by the cotton bollworm.
- *Bt* maize hybrids do not influence the non target insects and they can be recommended as potential options in future systems for IPM of maize in Slovakia.

Table 1. Summary of field experiments with MON810 maize in Slovakia

	2013			2014			2015		
Variant	Conventional	IPM	IPM/BT maize	Conventional	IPM	IPM/BT maize	Conventional	IPM	IPM/BT maize
Hybrid	DKC3871 (near-isogenic line)	DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)	DKC3871 (near-isogenic line)	DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)	DKC3871 (near-isogenic line)	DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)
Insecticides	Karate Zeon 5 CS (lambda-cyhalothrin, 50 g/l)) 0.25 l/ha	Biobit XL (Bacillus thuringiensis ssp. kurstaki), 1.5 l/ha	no	Karate Zeon 5 CS (lambda-cyhalothrin, 50 g/l)) 0.25 l/ha	Biobit XL (Bacillus thuringiensis ssp. kurstaki), 1.5 l/ha	no	Karate Zeon 5 CS (lambda-cyhalothrin, 50 g/l)) 0.25 l/ha	Biobit XL (Bacillus thuringiensis ssp. kurstaki), 1.5 l/ha	no
Elaterid damage	Number of damaged plants were counted during visual surveys. 200 plants per plot were evaluated in June at all plots of IPM12 trial. (percentage of damaged plants)			Number of damaged plants were counted during visual surveys. 200 plants per plot were evaluated in June at all plots of IPM12 trial. (percentage of damaged plants)			Number of damaged plants were counted during visual surveys. 200 plants per plot were evaluated in June at all plots of IPM12 trial. (percentage of damaged plants)		
	0,25a	0,75a	0,75a	0,00a	0,00a	0,00a	0,75a	0,25a	0,75a
Agrotis (Scotia) spp. Damage	Number of damaged plants were counted during visual surveys. 200 plants per plot was evaluated on June 17 in IPM12 trial (percentage of damaged plants)			Number of damaged plants were counted during visual surveys. 200 plants per plot was evaluated on June 17 in IPM12 trial. (percentage of damaged plants)			Number of damaged plants were counted during visual surveys. 200 plants per plot was evaluated on June 17 in IPM12 trial. (percentage of damaged plants)		
	0,00a	0,00a	0,50a	0,00a	0,00a	0,50a	1,25a	1,50a	0,25a
Diabrotica virgifera virgifera	Number of damaged plants and number of beetles per plant were counted during visual surveys. Plants were not damaged. Number of DVV adults.			Number of damaged plants and number of beetles per plant were counted during visual surveys. Plants were not damaged. Number of DVV adults.			Number of damaged plants and number of beetles per plant were counted during visual surveys. Plants were not damaged. Number of DVV adults.		
	0,00a	0,00a	0,00a	0,25a	0,50a	0,25a	1,25a	2,50a	1,75a
Percentage of plants damaged by the European corn borer*1	19,75ab	27,75b	0,00a	8,25ab	11,00b	0,00a	6,00a	12,25b	0,00a
Percentage of plants damaged by Helicoverpa 2	0,00a	0,50a	0,75a	0,25a	0,25a	0,00a	15,5ab	20,00a	7,5b
Harvest	October 24	October 24	October 24	November	November	November	November	November	November
Yield of maize (t/ha)*	6.7775a	6.3325a	5.9650a	11.4825a	11.2450a	12.1475b	5,1425a	5,1175a	5,3625b

Table 2 European corn borer damage and final yield in experimental fields in Slovakia

	2012		2013		2014		2015	
Hybrid	DK440 (near-isogenic line)	DKC4442YG (Bt maize line MON810)	DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)	DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)	DKC3871 (near-isogenic line)	DKC3872YG (Bt maize line MON810)
Percentage of plants damaged by the European corn borer*	60.00b ±19.22	0.00a ±0.00	52.33b ± 19.14	0.00a ± 0.00	35.00b±9.84	0.00a ± 0.00	41.00b±10.15	0.00a ± 0.00
Yield of maize (t/ha)*	11.09a ± 0.928	11.30a ± 0.924	6.08a ± 0.45	6.44a ± 0.44	10.67a±0.45	11.04a±0.58	5.73a±0.16	6.03a±0.22

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Web links for maize IPM: http://www.pure-ipm.eu/node/356