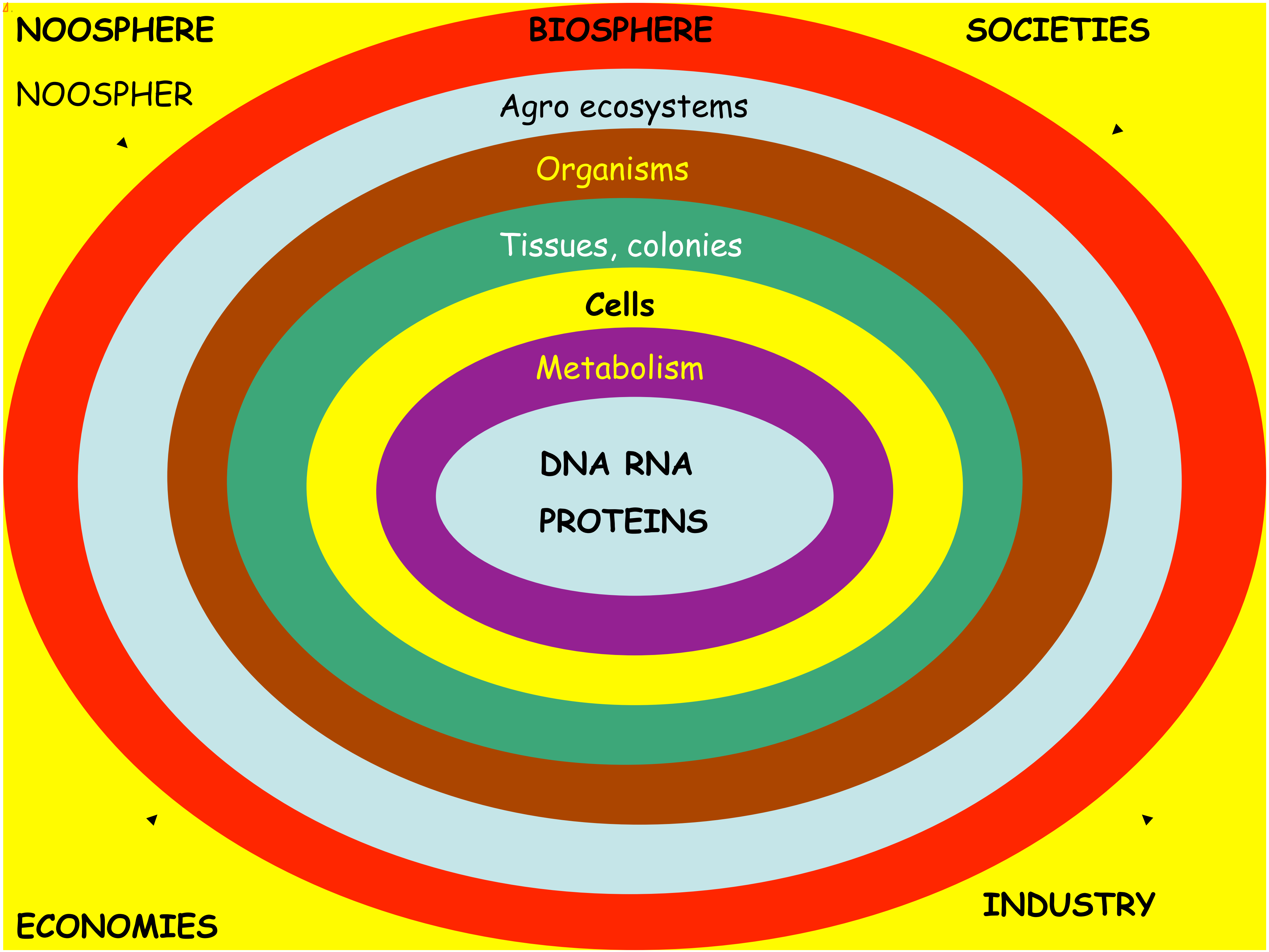
Present genetic engineering assessment practices are not holistic: implications and suggestions for a new integrated approach

The rationale behind the application of "genetic engineering" to domesticated plants and animals has been the same of the "green revolution". Both are based on a mechanistic conception of living beings thought to be "substantially equivalent" to machines liable to be programmed and changed at will without any "unintended effects". During the green revolution breeders were taught to choose the single characters known to be relevant for production, to assemble them designing a theoretically optimal future variety ("Donald's ideotype") and to proceed with selection obtaining the "best possible cultivated varieties". Little or no attention was given to the possible interactions of the change with the plant livng network, of the selected plants with the different environments, agricultures, and in general with human societies. GMOs are the even more rigid molecular version of the same concepts based on the DNA-centric vision of living systems reduced to computers endowed with a single programme whose independent components could be manipulated and substituted one by one with others coming from different species.

The "Biological revolution" of the last decade of the XXth Century and the first of the 3° Millennium has completely changed our vision of life but has not yet entered in the present laws and procedures of risk assessment. A NEW, HOLISTIC APPROACH IS URGENTLY NEEDED.


NOOSPHERE
BIOSPHERE
SOCIETIES
NOOSPHER
Agro ecosystems
Organisms
Tissues, colonies
Cells
Metabolism
DNA RNA
PROTEINS
ECONOMIES
INDUSTRY

-Living systems are organized into a hierarchy of network levels whose components communicate both "horizontally" within each level and "vertically" between levels, every level being "external" as seen from the "internal one" and "internal" to the higher level.

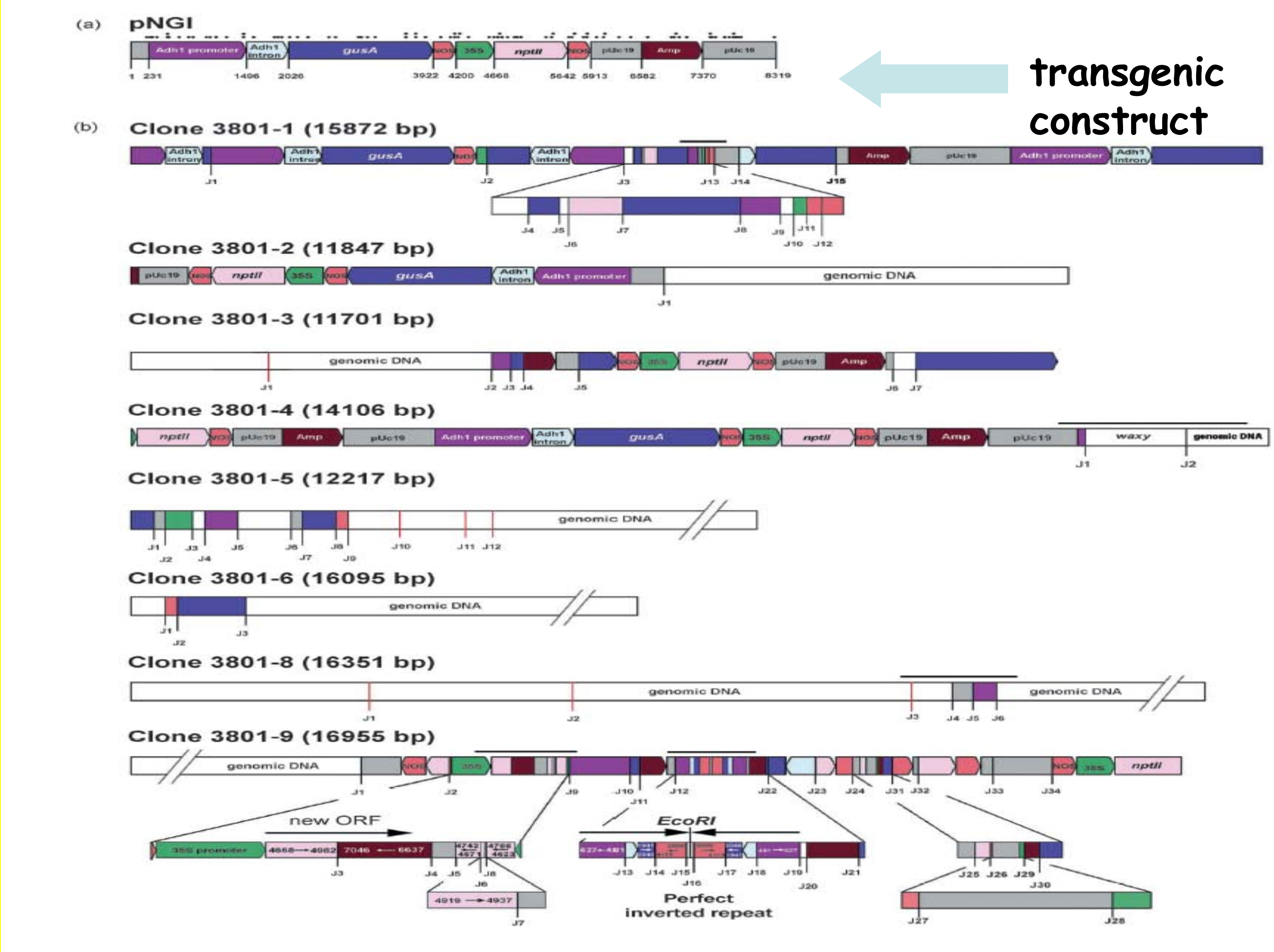
-Variations in all levels of networks will be selected and inherited depending from their level of coherence with the rules of the specific level where they happen and with those of the other levels of the hierarchical organisation of life, all "vertically" connected through a continuous exchange of molecules and energy.

-Therefore, all non-coherent changes will induce highly unpredictable levels of "turbulence" in the systems leading to their reorganization or to death depending from their resilience and robustness levels.

-All this should be taken into account when a gene is introduced into a plant with which it did not co-evolve. A holistic assessment necessarily implies careful studies of the final effects of the change at all levels of the hierarchical organization, from the molecular to the social and economic ones (see article by Hartmut Meyer).

-Thus we propose a completely new approach to risk evaluation suggesting not only the reorganization of EFSA but also the involvement of other agencies as for the 13-July-2010 EU recommendation

LEVEL 1: DNA



transgenic construct

The original construct (a) and nine different re-arranged sequences found in a single transformed oat line

When we insert a construct we cannot predict:

a) how many copies are inserted the analysis being limited to the construct itself and possible extra-copies of it or fragments are never looked for.

b) where are the construct and putative copies or fragments inserted, in a gene or in a regulatory region or elsewhere,

c) whether they are re-arranged and how,

d) which epigenetic changes have happened

f) which changes in expression levels of which genes may have happened

THEREFORE studies are needed among which:

a) Localization of insertion(s) and analysis of the sequences affected including the possible synthesis of “fusion-RNAs”. Transposon methylation levels

b) Transcriptome analysis of expression patterns through micro-arrays

c) Quasi-random whole genome genetic and epigenetic analysis through AFLP and MSAP patterns

LEVEL 2:METABOLISM

A metabonomic study of transgenic maize (*Zea mays*) seeds revealed variations in osmolytes and branched amino acids

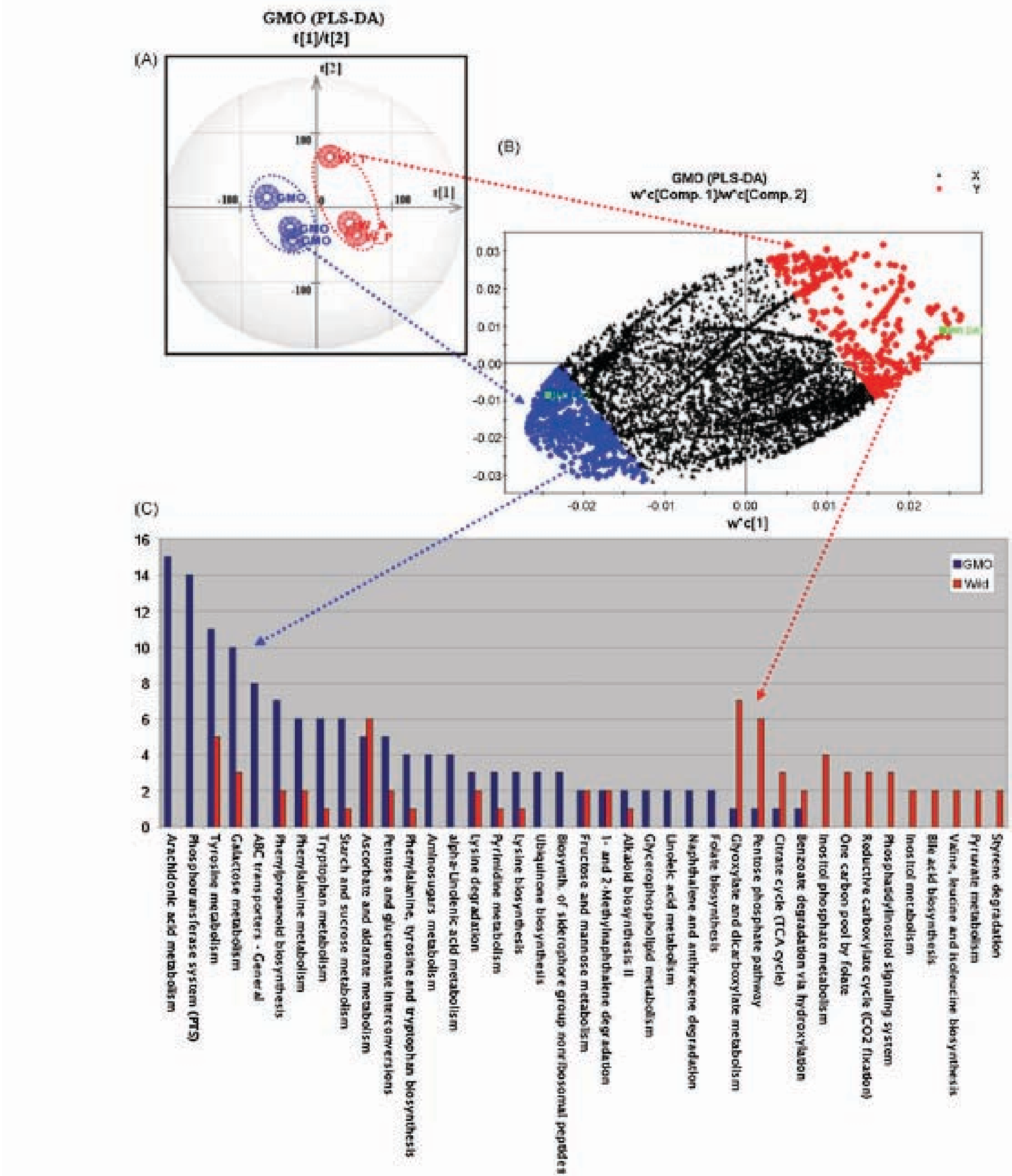
Cesare Manetti*, Cristiano Bianchetti, Lorena Casciani, Cecilia Castro, Maria Enrica Di Cocco, Alfredo Miccheli, Mario Motto and Filippo Conti

Table 3. *ANOVA results for selected signals from control (c) and transgenic (t) maize seed spectra*

Metabolite (signal)	*F*-value[a]	Order
Acetate (βCH_3)	0.3	
Ala (βCH_3)	14.6**	t <c
α-Glucose (C1H)	12.6**	t >c
Asn (βCH_2)	18.6***	t <c
β-Glucose (C1H)	17.2**	t >c
Choline [$N(CH_3)_3$]	105.6***	t <c
Dimethylamine (CH_3)	4.0	
Ferulic acid (HF)	4.5	
Formate (CH)	0.2	
GABA (αCH_2)	28.8***	t >c
Gln (βCH_2)	18.5***	t >c
Glu (γCH_2)	1.5	
His (C2H, ring)	9.2**	t <c
Ile (γCH_3)	2.4	
Melibiose (Gal1H)	6.6*	t >c
Pyruvate (CH_2)	3.4	
Succinate (α-βCH_2)	44.5***	t >c
Sucrose (F1H)	7.1*	t >c
Thr (γCH_3)	0.3	
Trigonelline (HA)	0.3	
Tyr (C2, H6, ring)	1.9	
Val (CH'_3)	0.5	

[a]*,**,***, Significant at the .05, 0.01, and 0.001 probability levels, respectively.

One of the many examples of differences in metabolism between transgenic and non transgenic plants (MON810 in this case).In most cases only known toxic substances are considered and not the utritional level and quality of the food. Moreover analyses *do not use really isogenic lines as controls*



An example of a good experiment on metabolome differences between GMO and the control

Nl gr 1/3

wild type

Nl gr 2/4

LEVEL 3: PHYSIOLOGY: *AN EXAMPLE OF UNINTENDED EFFECTS OF GENETIC TRANSFORMATION OF NICOTIANA LANGSDORFFI PLANTS*

N.lansgdorffii wild type compared with two lines from the same transformation event with the rat glucocorticoid receptor gene supposedly not interfering with _Nicotiana_ hormonal metabolism. Transgenic plants had strikingly different phytohormone levels, were partially sterile in the second generation, late flowerig, in a state of "genetic stress" as shown by the permanent activation of defence processes (high contents of abscisic acid, shikimic acid, polyphenols, salicilic acid), were resistant to Cr and Cd, to poly-ethylene-glycol, etc. -

LEVEL 4: THE PLANT ECOSYSTEM

Foliar application of glyphosate

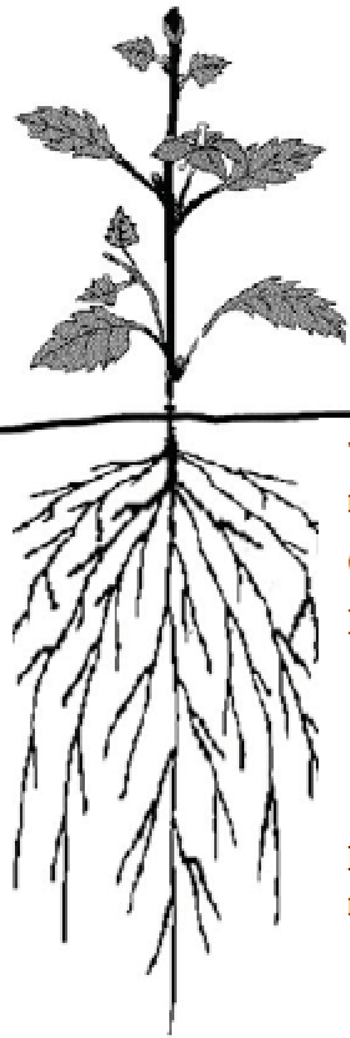
Systemic movement throughout the plant

Chelation of micronutrients

Intensified drought stress

Accumulation of glyphosate in meristematic tissues (shoot, reproductive, and root tips).

Translocation of glyphosate from shoot to root and subsequent release into the rhizosphere



Glyphosate accumulates in soil
(not biodegraded - co-metabolism)
Glyphosate desorbed from soil by P

Glyphosate toxicity to:
- N-fixing microbes
- Bacterial shikimate pathway
- Mycorrhiza
- Mn & Fe reducing organisms
- Biological control organisms
- Earthworms
- PGPR organisms

Toxicity to root tips by glyphosate or its toxic metabolites (e.g. AMPA)

Compromise of plant defense mechanisms

Promotion of:
- Soilborne plant pathogens (*Fusarium*, *Pythium*, *Rhizoctonia*, etc.)
- Nutrient oxidizers (Mn, Fe, N)
- Microbial nutrient sinks (K, Mg)

Reduced availability or uptake of essential nutrients (Cu, Fe, K, Mg, Mn, N, Ni)

Schematic of glyphosate interactions in soil

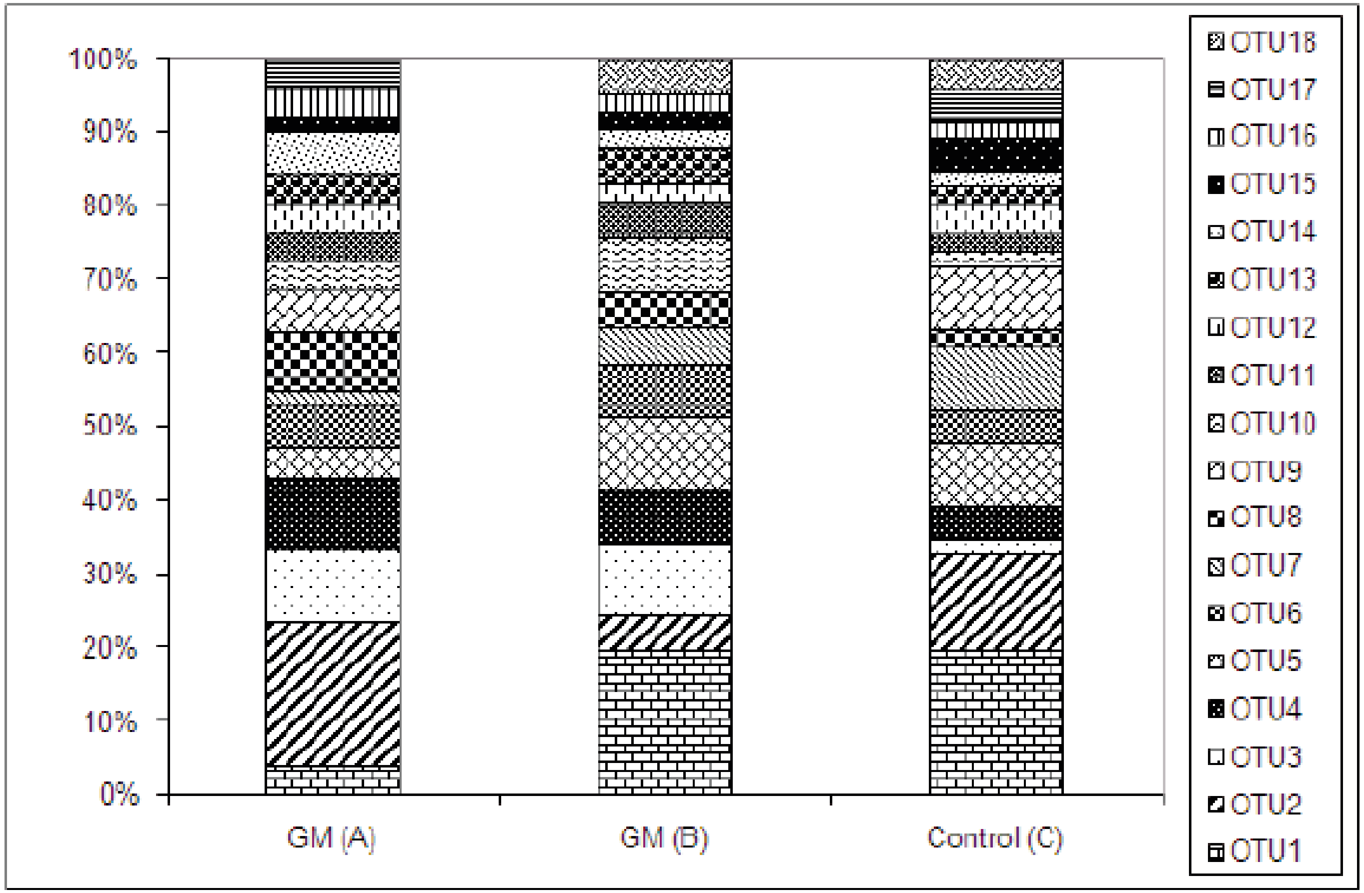


Figure 6. Relative distribution of the most frequent ARDRA pattern of cloned bacterial 16S rDNA fragments derived from the rhizosphere of transgenic (GM treatment A, GM treatment B) and control pine trees (C) from the summer sampling 2005. OTU = operational taxonomic unit.

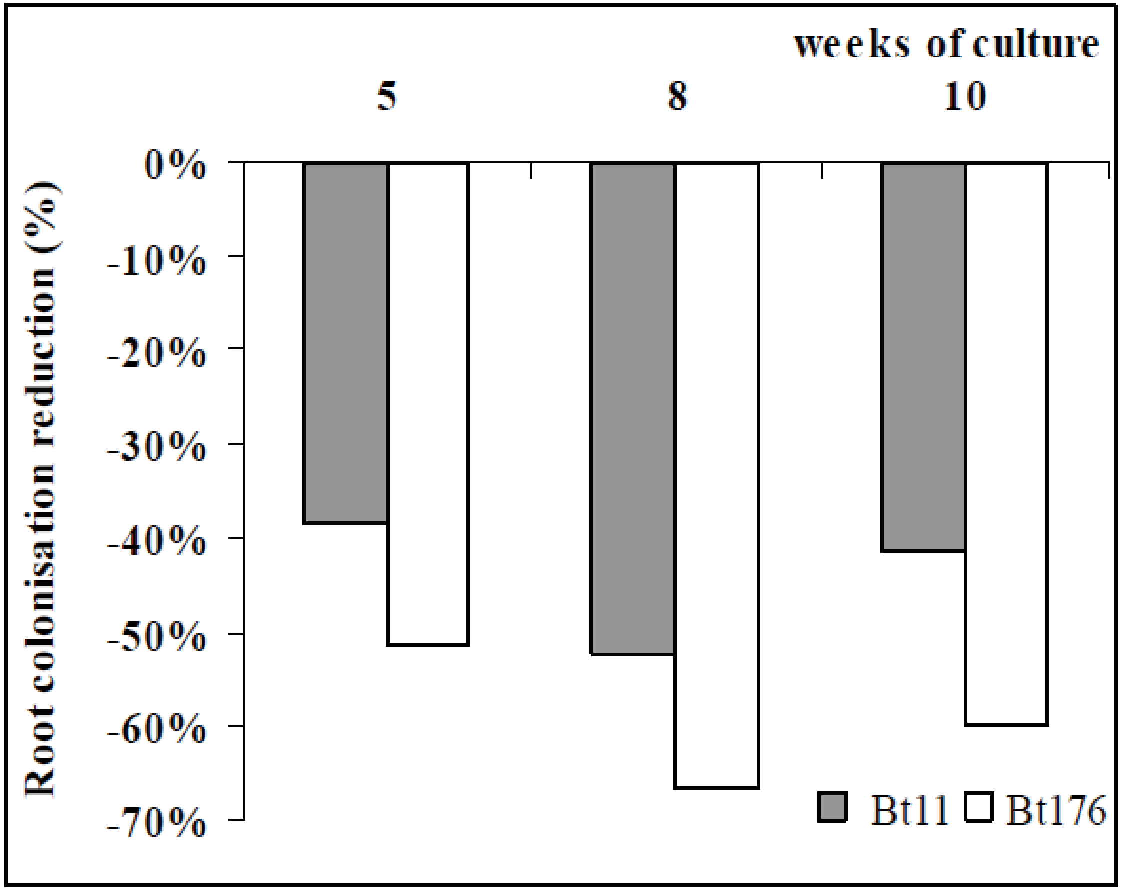


Figure 2. Reduction of colonisation by the AM fungus *Glomus mosseae* in roots of transgenic corn plants, with respect to control.

REDUCTION OF COLONISATION BY MYCORRHIZAE OF Bt TRANSGENIC CORN

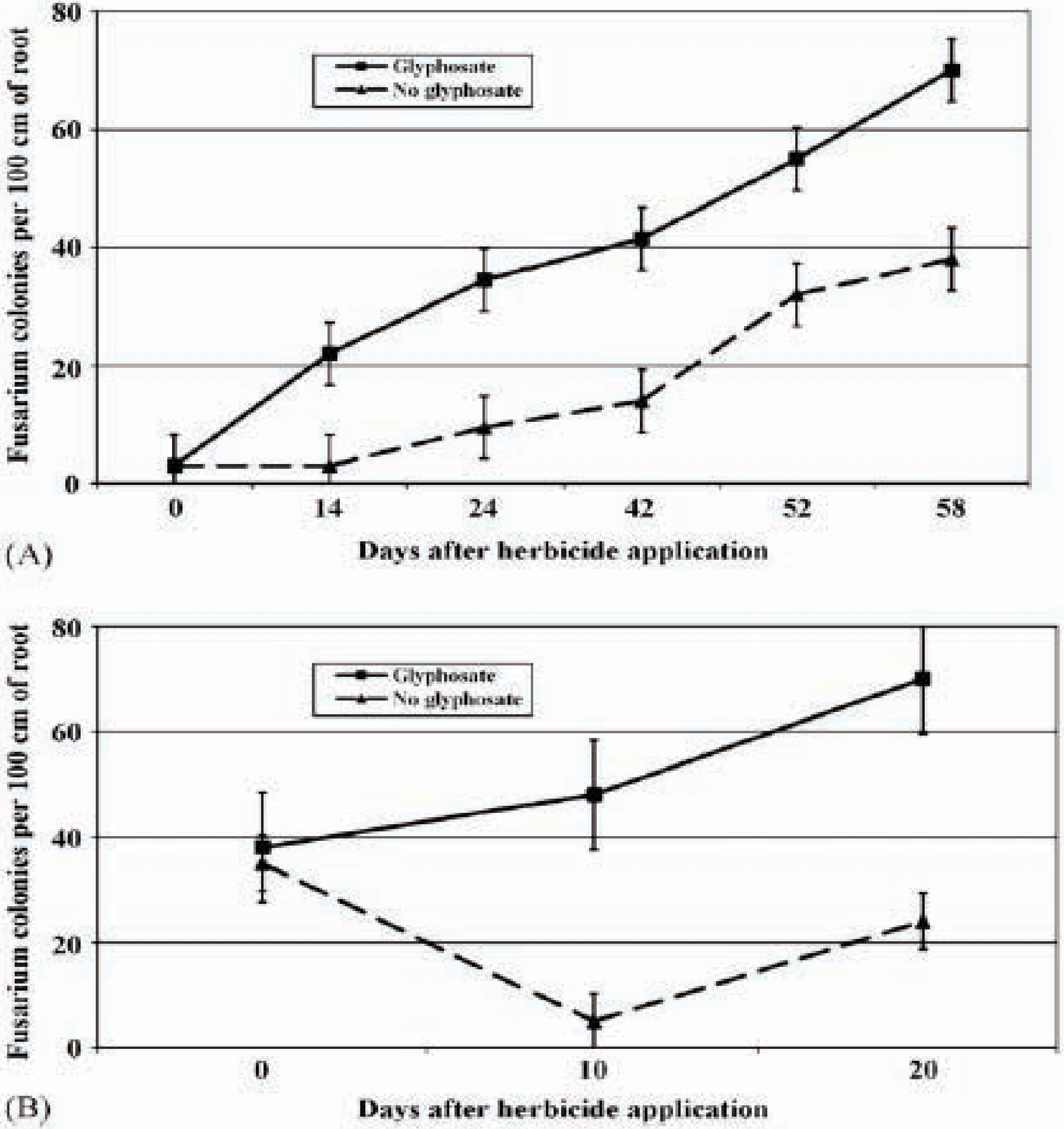


Fig. 1. Relationship of glyphosate application with root colonization of (A) glyphosate-resistant soybean ('Pioneer 94B01') and (B) glyphosate-resistant maize ('DeKalb DKC60') by *Fusarium* spp. Data in (A) based on Kremer (2003); data in (B) based on Means (2004). In both graphs, significant differences (*$P<0.05$) between glyphosate and no glyphosate treatments within dates are indicated by the vertical bars representing Fisher's protected LSD.

INCREASE OF COLONISATION OF SOYBEAN AND CORN BY THE PATHOGEN FUSARIUM INDUCED BY GLYPHOSATE

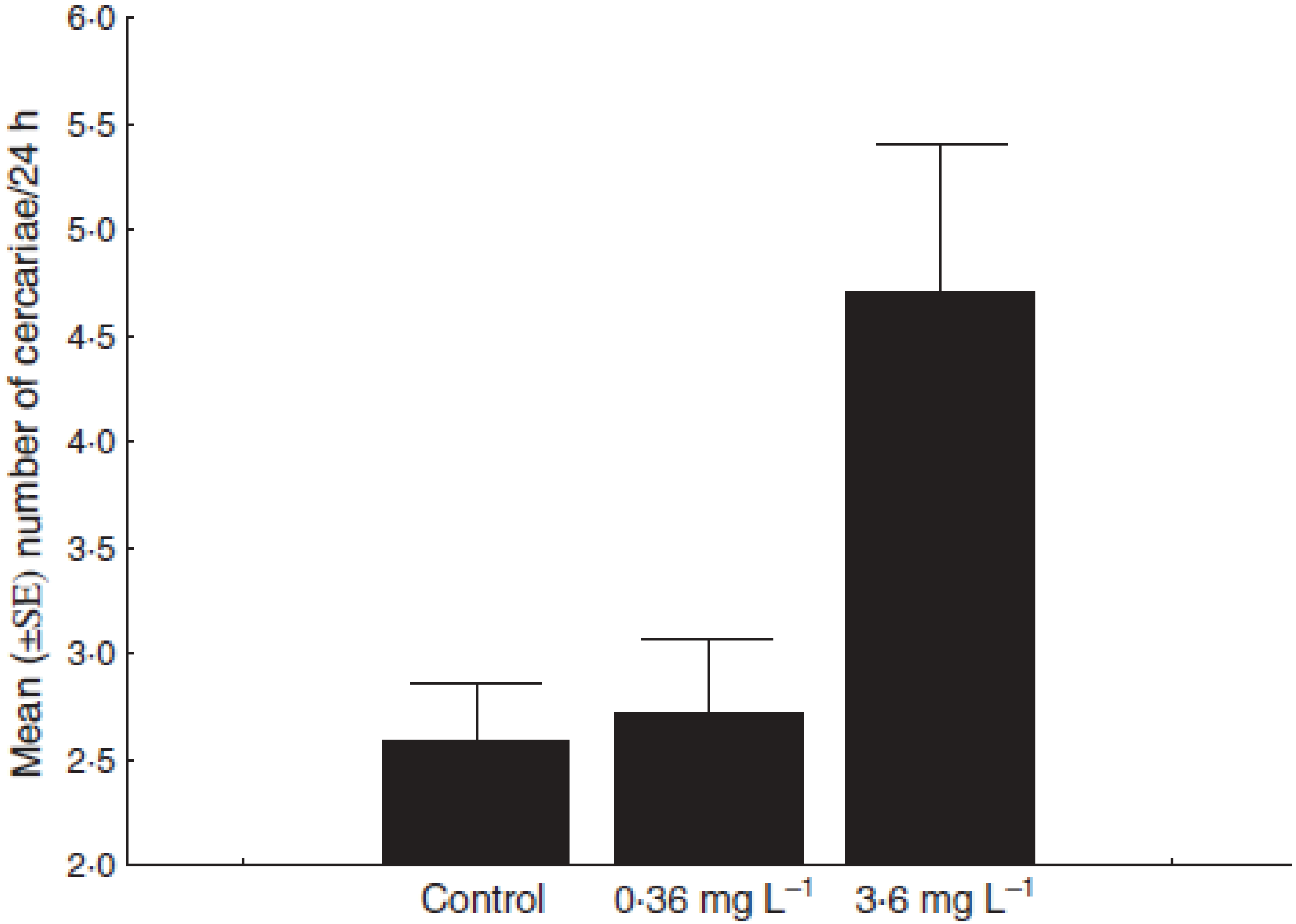


Fig. 2. Differences among treatment groups in *per-capita* shedding rates of *Telogaster opisthorchis* cercariae by *Potamopyrgus antipodarum*.

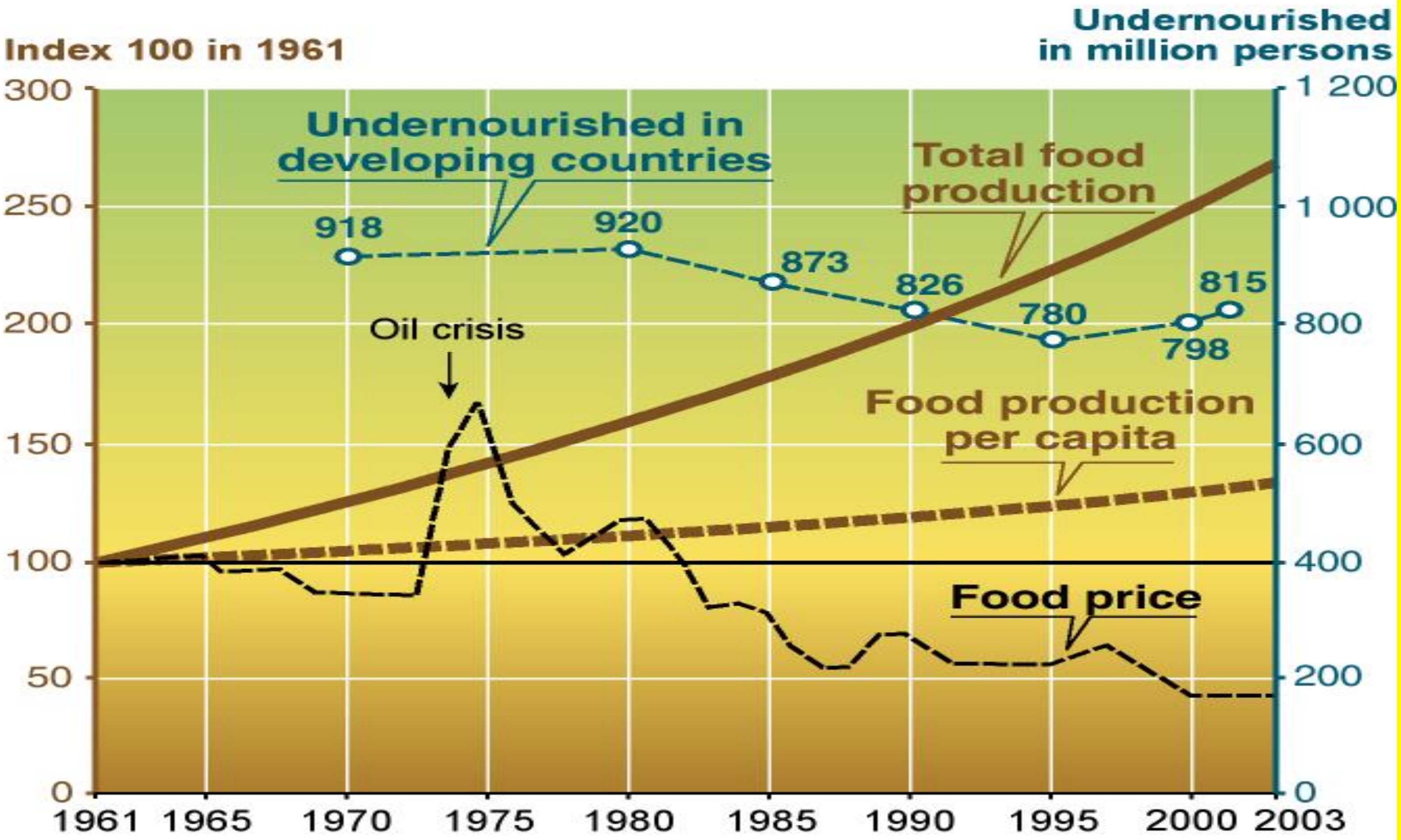
Table 1. Insufficiencies of currently used tests, criteria and interpretations; proposed improvements for GMOs health risks assessment. We reviewed here the current protocols used by industry and regulatory committees in commercialized agricultural GMOs. The feeding trials described in column 1 were performed in order to obtain GMOs commercialization, via regulatory agencies. The improvements proposed (column 2) will adapt these tests to modern knowledge in toxicology, in order to avoid the main consequences of overlooked risks (column 3).

Critical parameters and interpretations	Present regulatory assessment	Improvements proposed	Main consequences if improvements not applied
Number of animals / group	10 measured on 20 / group	At least 20 rats for 3 months, 10 or more for 24 months / group	Low statistical power
Number of controls versus treatments	Too many reference or control groups (320)/ 80 GMO-treated only	Avoid to multiply completely different control groups	Risk of concealing statistical effects
Species	Rat only (in mammals with blood analyses)	Rat and other(s) species such as Mice / Rabbit	Results too much species-specific
Replication of toxicological test	Only once	At least two	Reproducibility, Reliability not proven
Length	Subchronic (3 months)	Chronic (24 months) + developmental + transgenerational	Missing long term, fetal or transgenerational effects
Doses	2 doses	3 doses	Missing dose response relationship
Type of treatment	GMO	GMOs with/without associated pesticides	Confusion between mutagenesis / pesticides effects
Food composition	Substantial equivalence	More detailed composition with specific pesticides residues and metabolites, adjuvants	Missing potential contaminants and combined effects
Norms followed	OECD 408 strictly or less	OECD 408-453 with other details	Lack of hormonal sex specific data for instance
Number of blood analyses	2 measures only after 5 and 14 weeks	At least 3 the first trimester	Missing punctual phenomena
Biological interpretations Dose-effects	"Dose-related": proportional effects only taken into account with two doses !	Non linear effects to be studied (U or J curves)	Risk to avoid endocrine, carcinogenic, immune long-term effects…
Biological interpretations Sex specificity	Effects studied only if occurring in both sexes	Sex specific effects to be studied	Risk to avoid endocrine-specific effects
Biochemical modifications linked to histopathology	Necessary	Not always possible in 3 months	Risk of false negative results
Amplitude of effects studied	Effects inside of undefined historical norm of the species not studied	Any statistical difference with controls to be studied	Risk of false negative results
Final biological conclusion for an effect	Should be plausible for the regulatory committee	Necessity of more objective criteria: ex. lengthening of the test	Major risk of subjective interpretation

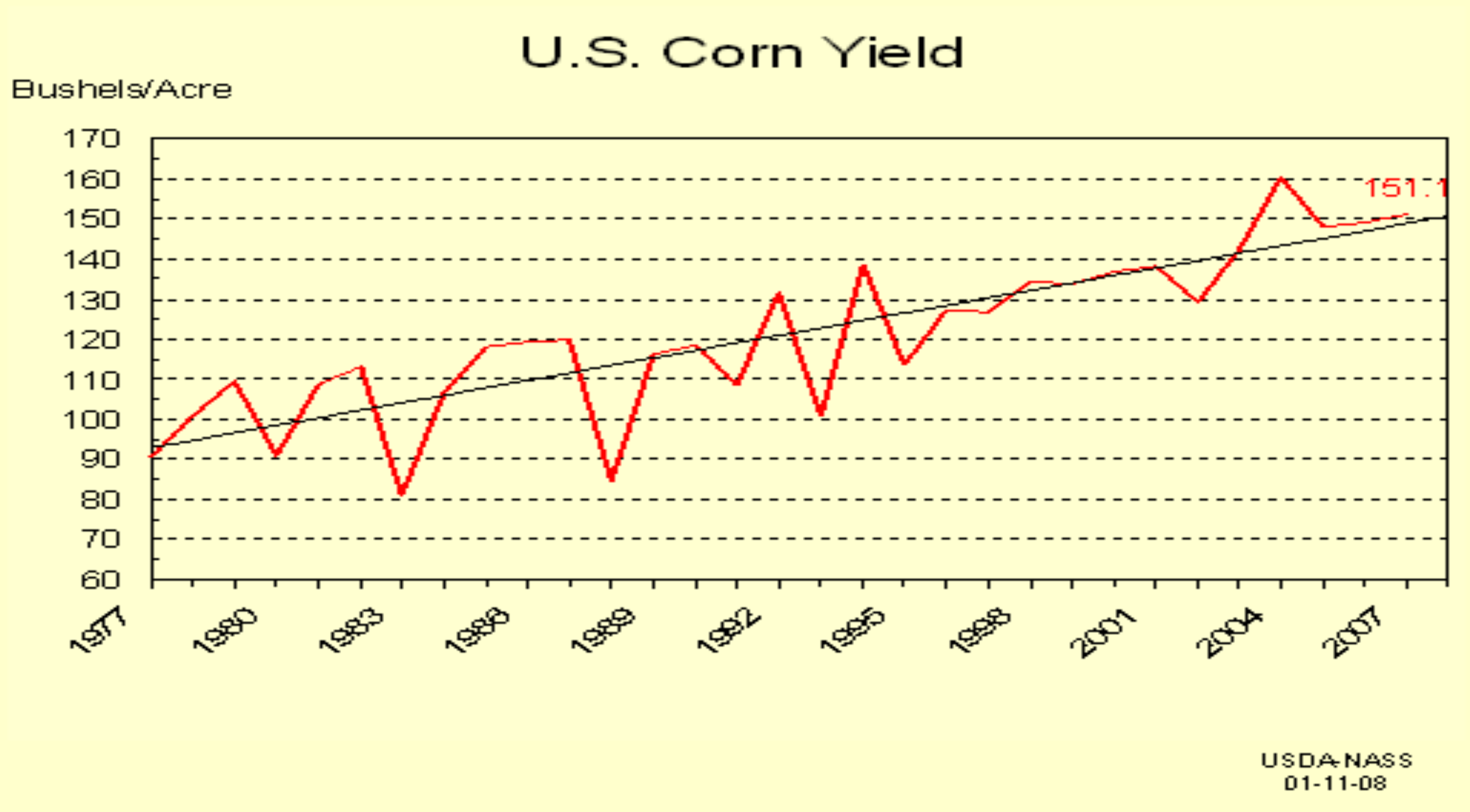
LEVEL 5: AGRICULTURES

The ideology of the optimal cultivars to be grown in all environments and agricultures, increased the costs of chemicals and energy, lowered prices and induced the loss of 75% of extant biodiversity (data by FAO). The early successes on famine of the green revolution stopped in 1995 and nowadays undernourished people are more than one billion.

What about GMOs?



Sources: FAOSTATS, SOFI, Millennium Ecosystem Assessment



The result of the DNA centric ideology has been a food bankrupt. Since the first created GMO in 1983 only four species (soybean, maize, cotton, canola) changed for only two characters (resistance to single insects and to herbicides) have been introduced with success in the market. Moreover, they are an utter failure in terms of yield, quality, and revenues for farmers as seen also in the USDA web site.

Table 1: comparison between bolls and fibre of non-Bt. and Bt. cotton

Item	Non-Bt.	Bt.
Number of bolls/plant	95 (70 - 120)	50 (25 - 75)
Boll size	6 - 8 gm	3.5 - 5 gm
Fibre length	34.5 mm	30.5 mm
Cotton Quality (grade)	A & B	B & C

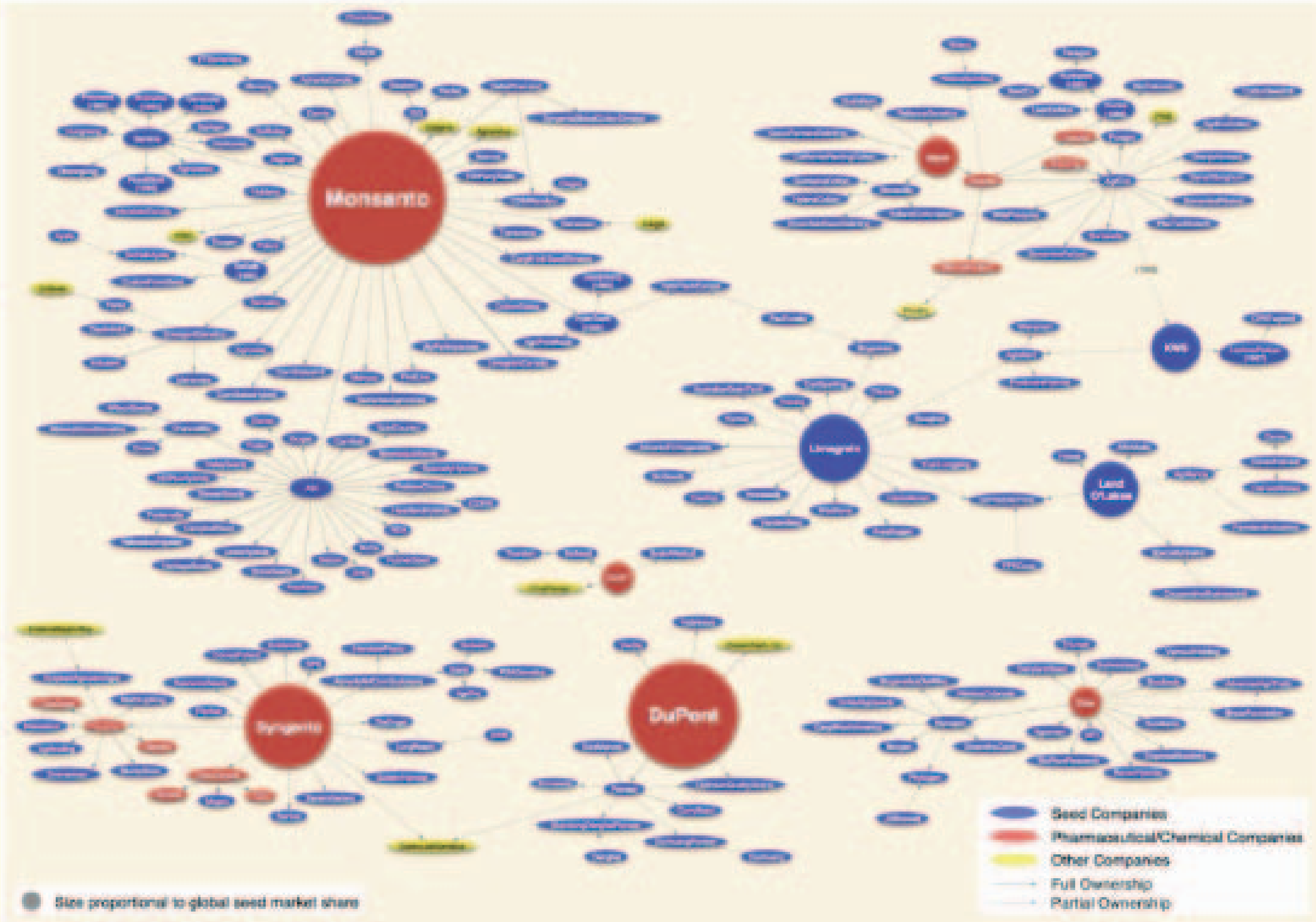
A comparison of bolls and fibre in non-Bt. and Bt. cotton in Table 1, showed that the number of bolls per plant was higher in the non-Bt. cotton variety. Whereas the non-Bt. variety averaged 95 bolls per plant in the Bt. variety the average was only 50 bolls. Fibre length was also longer in the non-Bt varieties, which had better grade cotton. Non-Bt. cotton was graded as A and B quality whereas Bt. cotton was graded as B and C. Moreover it is not resistant to pink worm

All this notwithstanding 134 millions hectares are cultivated with GMOs. GMOs are the most relevant example of economy degeneration as they are not competitive in the market for their quality or production but their success depends from the financial strength of few multinational companies controlling the whole agricultural food chain from the seeds to chemicals to products

Sahai

LEVEL 6: MARKETS AND SOCIETY

Figure 2. Seed industry structure, 1996–2008.



The "three GMO sisters" and the others were at the beginning chemical and pharmaceutical industries merging with or controlling the major seed industries, food dealers and have the monopoly of patents in their fields of interest. Nowadays *their incomes come from royalties, stock exchange gains, deals with governments.*

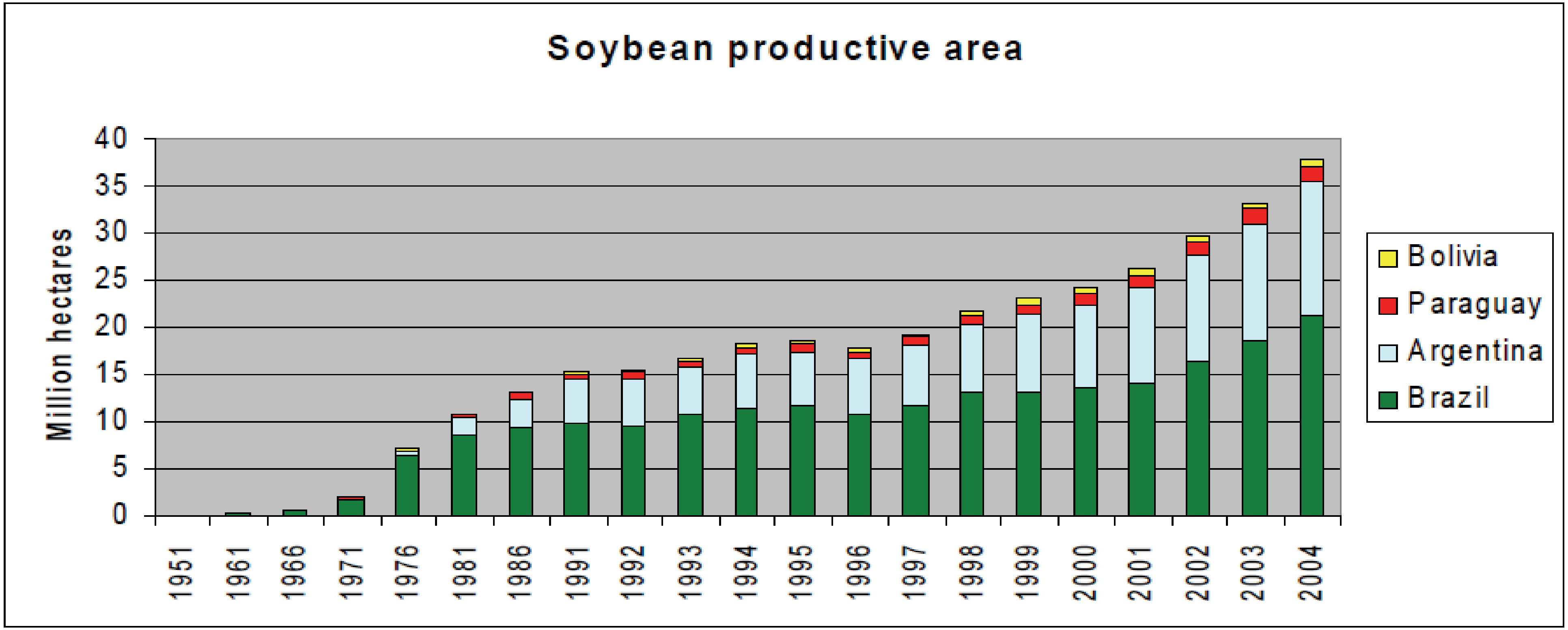


Figure 2.1

The growth of soy planting in South America 1950/51-2003/04. Five year intervals until 1990. Sources: FAO, CONAB, ISTA Mielke, Agriculture Ministries Argentina and Paraguay.

The case of soybean production in South America. **The increase of soybean production has accelerated after the introduction of GMOs. Small food producing farms have been either bought at low prices or occupied with force, particularly in Paraguay but also in the other Nations and substituted with large areas were only soybean is grown and immediately sold to developed Countries as animal feed. Farmers expelled were either utilised as low cost manpower or populated the favelas, losing their local cultivars ad their languages.**

Rate of growth of the main Argentinean crops

	CROP	1988/89- 1995/1996	1996/97-2003/2004
AVERAGE RATE OF GROWTH	Soybeans	4.3%	11.8%
	Wheat	7.0%	- 2.3%
	Maize	2.2%	- 0.1%
	Sunflower	7.4%	- 4.0%
	Seed Cotton	13.5%	- 7.9%
	Barley	10.7%	6.0%
	Potatoes	- 0.4%	- 3.3%
	Rice, Paddy	12.3%	1.1%
	Millet	9.4%	-19.1%

In South America therefore food production has been decreasing and market control by few has favoured speculation on food prices which increased world-wide with an unprecedented speed . The extension of this process to other countries and particularly to agricultures of small farms competing for quality and based on high prices would destroy them. This is the case of Europe and for this reason the recent EU recommendation (23.July-2010) may be a good tool to stopo the disaster.

Table 1: Changing Farm Numbers

Province		Census year 1988	2002	2008	02/88 (%)	08/02 (%)
Santa Fe	Number of Firms	36862	28103	26551	76	94
	Size (hectares)	300	400	406	133	101
Entre Rios	Number of Firms	27132	21577	17711	80	82
	Size (hectares)	228	294	316	129	108
La Pampa	Number of Firms	8631	7775	7502	90	96
	Size (hectares)	1444	1638	1556	113	95
Cordoba	Number of Firms	40061	26226	25910	65	99
	Size (hectares)	343	467	437	136	94
Buenos Aires	Number of Firms	75479	51116	31711	68	62
	Size (hectares)	361	505	550	140	109

Source: Instituto Nacional de Estadísticas y Censos INDEC), Censo Nacional Agropecuario 1988, 2002 and 2008

The reduction of the number of farms and increase in farm size induced by the change from local to industrial agricultures in Argentina areas

Graph 2: Capital-Labor substitution

("partido/departamento" data of Buenos Aires and Córdoba)

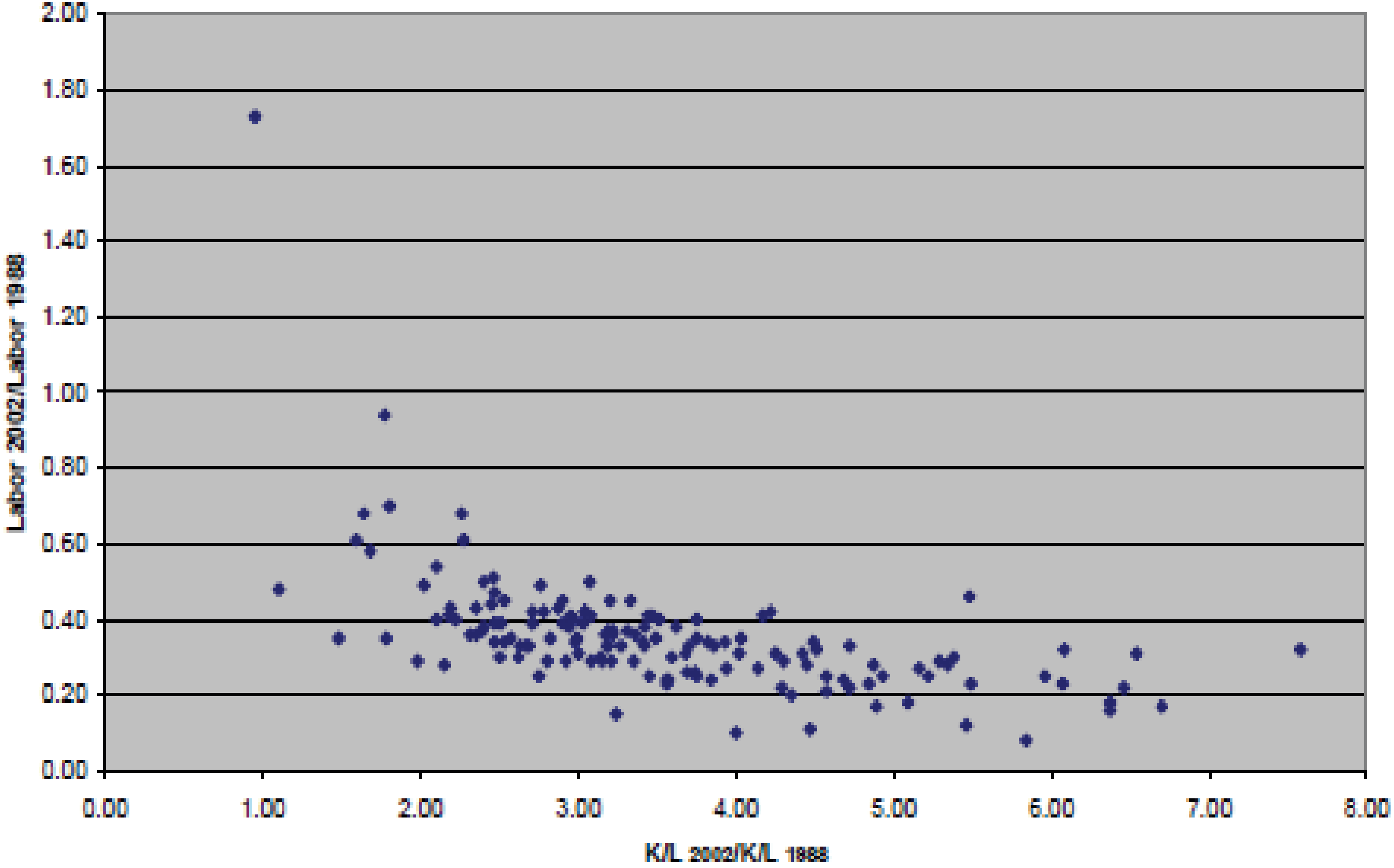


Figure 3.5

Conversion per habitat type in Argentina, Bolivia, Brazil and Paraguay.

Country	Forest type	Estimated conversion 2004-2020
		(x 1,000 ha)
Argentina	Atlantic Forest	300
	Chaco	4,850
	Yungas	200
Bolivia	Chiquitano Forest	550
	Chaco	550
Brazil	Amazon Transitional and Rainforest	3,600
	Cerrado	9,600
Paraguay	Atlantic Forest	1,000
	Chaco	900
Total		**21,550**

Table 3.1

Estimated conversion 2004-2020 of major forest habitats in soy production countries

The increasing industrial agriculture often also implies the conversion in arable land for soybean cultivation of forests therefore having a negative impact on all the present world crises, namely the financial, environmental, food ones