UNIVERSITY OF CRAIOVA
FACULTY OF AGRICULTUREL

The efficacity of total weeds control at maize crop by using genetic-modified hybrids with Liberty Link and Clearfield resistance genes, on lessive reddish-brown soil in central part of Oltenia

Conducător științific
Dr. ing. MIHAI NICOLESCU
Cercetător științific principal gradul I

Doctorand
Ing. SORIN OȚELEA

CRAIOVA
2004

Introduction

Damages due by weeds in agricultural crop of Roumania, are at high level in the last ten years, especially because of wrong technologies determinated by economical and social conditions accordiugly with transition period.

In such situation are necessary studies and experiments for development of ,,total weed combat" theorie, but also for find new fight ways against so called ,,green pollution" phenomena.

Maize as one of the three principal cereal crops of aur cnntry benefits in present by the mast recently conquest of genetics and breeding. Whole area, arannd three billions hectare can be saw with F1 hybrids a proper affert already existing from roumanian cretions and also from foreign companies witle good achievement in domain.

Genetic-modified maize hybrids, totl resistance for herbicides are progressive elements, which interested fctors from decisional to practice level had not to ignore it.

All these made us to perfomed experiments, which are the aim of this doctoral thesis, during three years, with themes adapted in each year, at biological material: genetic-modified hybrids and proper herbicides.

Experimental conditions and material used

The soil where we initiated demonstration plot with genetic-modified (GMO) CLARICA maize hybrid in 1999 placed on Getyc Piemount, is lessive reddish-brown affected by pseudogleysation and podzolisation with acid reaction (ph=5,2-5,5) less in nitrogen, slight applied with phosphorus and good in potassium, with 1,5-2% humus content, claylaam texture, and low water permeability. Land placed on second terrace at fin with Amaradia confluence where initiated experiments in 2000 and 2001 is alluvial soil, medium humificate with a sandy-loam texture, slight cid reaction (ph=5,8) poor in nitrogen, and medium supplied with phosphorus nd potassium, more permeable for water comparated with tableland.

Value of soil moisture characteristic (field water capacity, wilting point, active moisture range) give soils around Agricultural Research-Development Station (SCDA)- Simnic, properties that rise drought effert during plant growth season. Enviranmental conditions in experimented years (following tables) during maize vegetation period show that: 1999 by point of view of rainfall amount ws favourable for maize crop, the exceed against multiannualy normale in Iune and july blanced the lack in May faavauring growth and plant development. Also the value of temperature clohy to multiannualy mean made possible passing through meanthy maize fenophase in normal conditions.

Tabelul 3.2.1

Climatic data for maize grawing season during agricultural year 1998-1999 at S.C.D.A. SIMNIC

		Cool season	April	May	June	July	August	September
Rainfall mm (amount)	Rainfall days number	-	10	8	6	8	4	5
	First decade	-	12,0	10,5	12,5	8,5	4,0	122,5
	Second decade	-	10,5	18,0	61,0	44,0	0,0	0,0
	Thirth decade	-	26,0	9,5	23,5	44,5	17,0	0,0
	Total decade	200,0	58,5	38,0	97,0	97,0	21,6	122,5
	Normal value per 60 years	234,8	42,8	61,7	63,8	54,6	43,6	38,6
	Difference	-34,8	+15,7	-23,7	+33,2	+42,4	-22,6	+83,9
Temperature values ^{0}C	Monthby mean	-	12,0	15,8	21,1	22,9	23,4	20,6
	Normal value per 68 years	-	11,4	16,8	20,4	22,6	22,0	19,8
	Difference		+0,6	-1,0	+0,7	+0,3	+1,4	+0,8

Described like a dry year-2000, with lake of rainfall in May, June, july and August. The effect was increase by the atmospheric drught due to high temperatures in summer months with 29 drought days (temperatures above 35^0C) and 24 hot nights (temperature above 20^0C).

Such conditions demande irrigation with 1600 m^3 water / ha, divided in four waters.

Tabelul 3.2.2

Climatic data for maize grawing season during agricultural year 1999-2000 at S.C.D.A. SIMNIC

		Cool season	April	May	June	July	August	September
Rainfall mm (amount)	Rainfall days number	-	8	4	2	2	2	5
	First decade	-	10,5	5,0	6,5	2,0	2,0	32,0
	Second decade	-	6,0	4,5	-	51,0	-	41,0
	Thirth decade	-	33,0	12,0	2,0	-	3,0	12,0
	Total decade	175,7	49,5	21,5	8,5	53,0	6,0	84,0
	Normal value per 60 years	234,8	42,8	61,7	63,8	54,6	43,6	38,6
	Difference	-59,1	+6,7	-40,2	-55,3	-0,4	-37,6	+45,4
Temperature values ^{0}C	Monthby mean	-	16,5	18,7	21,7	22,9	22,7	20,1
	Normal value per 68 years	-	11,4	16,8	20,4	22,6	22,0	18,7
	Difference	-	+5,1	+1,9	+1,3	+0,3	+0,7	+1,4
	Hot days number	-	-	-	7	10	12	-
	Hot nights number	-	-	-	8	7	9	-

Year 2001 begin with a severe lack of rainfall during cool-season (octobre-31 March) balauced in prt by the rainfall exceed from April. But decided for maize crop were rainfall during June and July that twofold quantitive against normal value registred per many years. Generally temperature values were clasly to multiannual mean for each month escepting August where lack of rainfall was accompanied by persisting drought. In this situation it had to irrigate watering 400 m^3 each, during 02-14 August.

Tabelul 3.2.3

Climatic data for maize grawing season during agricultural year 2000-2001 at S.C.D.A. SIMNIC

		Cool season	April	May	June	July	August	September
Rainfall mm (amount)	Rainfall days number	-	5	7	10	8	0	3
	First decade	-	0,0	11,0	64,0	71,0	0	15,0
	Second decade	-	39,0	6,0	55,5	28,0	0	24,5
	Thirth decade	-	47,0	21,0	8,5	11,0	0	0,0
	Total decade	105,5	86,0	38,0	127,0	110,0	0,0	39,5
	Normal value per 60	234,8	42,8	61,7	63,8	54,6	43,6	38,6

	years							
	Difference	-129,3	+43,2	-23,7	+63,2	+55,4	-43,6	+0,9
Temperature values ^{0}C	Monthby mean	-	10,4	16,4	19,4	23,4	24,8	18,7
	Normal value per 68 years	-	11,4	16,8	20,4	22,6	22,0	18,7
	Difference		-1,0	-0,4	-1,0	+0,8	+2,8	0,0

For this doctoral thesis started from materials that we can procure (Claricaa and Evelina hybrids in normal and genetic-modified (trg.) variant with liberty Link and Escort total herbicides). For man-modified variant of these two hybrids was used FRONTIER herbicide in preemergence treatment, completed with CAMBIO postemergence treatment at CLARICA hybrid and with PRIMEXTRA GOLD postemerge treatment at Evelina N. hybrid.

Total herbicides at postemergence treatment were applied at maize 3-4 leaves stage (at single treatment situation) and at 7-8 leaves stage in variants where the treatment was repeated once again.

In conclusion we will show that LIBERTY LINK maize hybrid (LL) tolerant at mmonia gluphosinat has a gene indentified by the AGR EVA company.

Action mechanisme of LIBERTY LINK herbicide is to inhibate glutamine synteses enzyme which transform ammonia in glutamine inside cell, so the effect is rapidly ammonia accumulation that kill plant tissue and inhibate photo synthesis.

CLEARFILD maize hybrid has a gene isolated from normal maize twhich give it resistance against imidazoline (active element of total herbicides : ESCORT, PIVOT, LIGHTNING and CONTOUR).

When resistance gene exist in both parents (inbred lincs) the hybrid is notice IR (imidazolin resistence).

Contact action of imidazolin is complet with its soil perssistance that will assure a control also for further emerge weeds. The offerts for CLEARFIELD commercial hybrids is assure by PIONEER DU PONT company.

Results and discutions

The analysis of data obtained during 1999, from three variant of CLARICA hybrid (normal, Bt + LL, and LL) pointed out alike be haviour regarding accompli she yield and also for main components of it.

So, the included of Liberty Link and Bt resistance genes against total herbicides and PIRAUSTA sp. pest attack, does not change hereditary characteristics of CLARICA hybrids. Obtained yield carried out better efficaciosly of treatment in two phases (V3 variant) with 2 l/ha LIBERTY rate each: 58,0 q/ha obtained yield against 43,7 q/ha yield at V2 variant trated once with 3 l/h LIBERTY LINK herbicide.

The some situation is present at V5 against V4 where was used CLARICA LL (table 4.2.1.). regarding to Pirausta sp. resistence due to Bt gene, this was oriously at Claric Bt + LL variant, assuring a near total protection against pest, fregnenthy attack in this case being 2-3 % comprated with 43-48 % registed at Clarica LL variant.

Tabelul 4.2.1

Rezultatele obţinute în lotul demonstrativ cu hibridul de porumb Clarica transgenic, la S.C.D.A Şimnic, 1999

Specificare	V1	V2	V3	V4	V5	V6
Prod. boabe q/ha	56,3	47,0	58,0	43,2	56,9	27,4
Înălţ. plantei cm	206	210	198	203	200	170
Grt. medie şt. grame	212	208	213	200	220	110
Ştiul. La 100 plante	107	104	110	106	100	91
MMB (g) la 15% U	211	220	217	222	219	192
% boabe pe ştiulete	81	82	81	80	81	72

% U la recoltare	27,8	26,9	28,0	27,3	29,6	27,7
Atacul de *Ostrinia* Frecvenţă/ intensitate	48%/8	2%/2	3%/2	43%/8	40%/7	42%/8

In 2000 byologicl material (Clrica LL and Claric N as well as Evelina IR and Evelina hybrids) with specific herbicides (Liberty Link and Escort) allowed through going studies with comparation of results obtained with classic herbicides as well as total herbicides at those two hybrids normal and modified (trg). In this case the level of yield assure by total herbicide uses at Clarica trg. And Everina trg. is alike yield level level obtained with classic herbiside.

Damage high level due to these two total herbicides at normal hybrids variant, pointed out the measures that are necessary to be taker when are used total herbicide at normal maize crop an other species which are near by.

The level of yield lasses due to total herbicides under normal maize is present in table 5.24. by perceutage yield losse. Thus at Claarica N variant trated with Liberty L damge level was 63,0% and at Evelina N variant treated with ESCORT was 66,4 %.

Tabelul 5.2.4

Damage grade due to total herbicides treatment applied at normal maize hybrids (non-genetic modified)

Hibridul	Producţia boabe		Grad dăunare %	Erbicidul
	q/ha	%		
Clarica transgenic	82,9	100,0	63,0	Librety L. 2+2 l/ha
Clarica normal	30,7	37,0		
Evelina transgenic	88,2	100,0	66,4	Escort 4 l/ha
Evelina normal	29,6	33,6		

The impact of Liberty L gene present at Clarica trg. and Clearfield gene at Evelina trg. hybrid, was studied by measuring and determinatiol in experimental plaats, at morphological caracteres (table 5.2.5.) as well as at fenological traits (tble 5.2.5.) finding that resistance genes against total herbicides da not modify studied parameters.

Tabelul 5.2.5

The impact of Liberty L and Clearfield genes under morfological characteres at maize hybrids

Hibridul	Înălţime pl./ins. (cm)	Indice foliar	Nr. şt. la 100 pl.	Grt. ştiul (g)	% boabe pe ştiul.
Clarica normal	207/124	2,72	112	159	82
Clarica transgenic	210/121	2,88	110	161	81
Evelina normal	220/130	3,07	104	168	81
Evelina transgenic	222/132	2,98	108	170	82

Chemical composition of grain at studied components: fat and protein content is also un-modified cause by the present of resistance genes (table 5.2.7.). Protein content is also the some, the ratio between main mino acids and their total being close in value at eache hybrids in normal and modified variant (table 5.2.7.).

Tabelul 5.2.7

Chemical composition of Clarica and Evelina Kernel hybrids in normal and genetic – modified variants (% from total)

Specificare	Clarica		Evelina	
	normal	transgenic	normal	transgenic
Grăsimi	4,01	3,89	3,80	3,65
Proteină brută	11,12	11,32	11,25	11,76
Din care:				
Total aminoacizi	9,24	9,56	9,47	10,49
Aminoacizi esenţiali	4,91	5,03	4,74	5,18
AAE / TAA	53,14	52,61	50,05	49,38
LYS / TAA	4,73	4,92	4,86	4,49

The lack of any influence under morphological characters, under traits related with grawing plase and under biochemical behavior has a genetic explanation in connect with informations volum included into ,,pool gene,, plnt which is limited only to ,,interested gene,, for resistance at total herbicides (figure 5.2.8.). In case of hybridization (classic transfer methad) inside new cultivares are transferated many genes that will must to eliminate after by selection. But if the present of Liberty Link and Clearfield genes have no effect above morphological and technological traits, the herbicides applied for weeds control reduced the high of plants, leaf index, ear weight and finally decreases the yield. The same negative effet for plants grawing and their development and yield lasses werw registred in the ease of classic herbicides use at Clarica and Evelina normal variants.

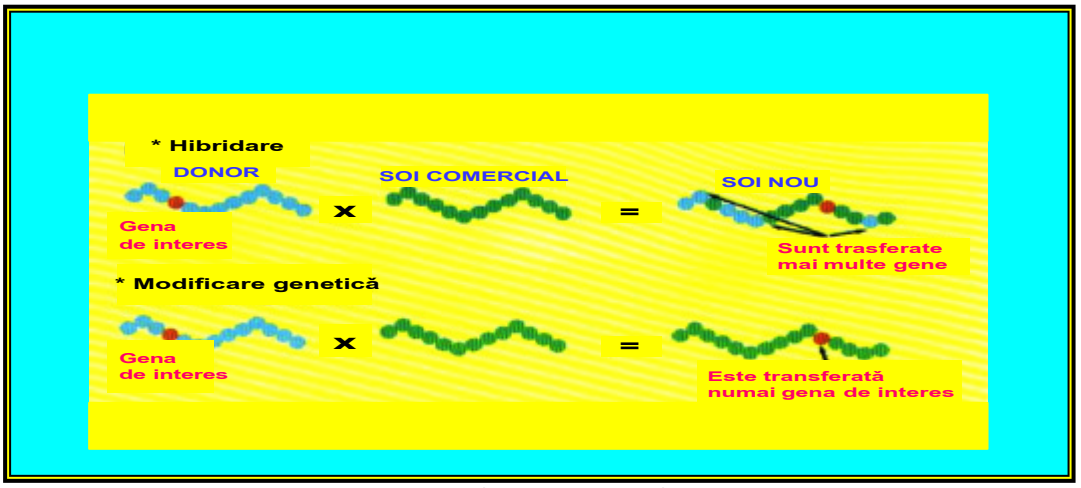

Fig. 5.2.8. The advantages of genetic-modify process in breeding: a high precision and rapidly (after Marcela Badea, 2000)

Tendency diminution of grain yield in all herbicided variants is obvious by using a non – herbicided variants, clean with sepeated weeding. This phenomena is accepted by users of herbicides because the losser are low and the of mannualy weeding are difficult. In 2001 was studied the effect of herbicide rates and timp of apply under yields, weed control efficiency and the weeds level in plot. Damage grade in perceutage of yield losses at two modified lybrids increase with the rate of used herbicide. Biger rates, 65 % above recommandated limit applied later increase very significantly damage level of yield to 44,2 % respectively 57,8 % (table 6.2.2.).

The efficiency of weeds control expressed by percentage killed weeds per plot, rise at higher rates of herbicides but without a yield spores caused by a fitotoxic effect into plants. (table 6.2.2.). Weeds grade presented gravimetric like green weeds weight / m^2 reduces obviously with higher rates but at late application this parameter increases because the weeds are advanced in growth and more resistances to „contact,, action of herbicides.

Also at later treatmens maize plants diminut the spread of herbicides on weeds that are shorter in ligh. (table 6.2.2.).

Tabelul 6.2.2.

The effect of rates (D) and time of apply (ep) at total herbicides under yield losses, weeds control efficiency and weeds grade in plot during 2000 at SCDA Simnic

Tratament	Prod. boabe			Gradul de dăunare %	Eficacitate combatere %	Nivel de înburuien. g/mp
	q/ha	%	S			
5 praşile man.	93,9	100,0	mt	-	100	180
4,0 l/ha LL	87,7	93,4	-	6,6	82	340
6,5 l/ha LL	70,6	75,2	000	24,4	98	249
6,5 l/ha LL	52,4	55,8	000	44,2	70	428
Nepr. neerb.	29,2	31,1	000	68,9	0	1056
C L A R I C A L L						
5 praşile man.	95,6	100,0	Mt	-	100	188
4,0 l/ha Escort	83,3	87,1	00	12,8	80	326
6,5 l/ha Escort	65,2	68,2	000	31,8	96	228
6,5 l/ha Escort	40,4	42,2	000	57,8	67	498
Nepr. neerb.	29,1	30,4	000	69,6	0	1116
E V E L I N A I R						

Clarica LL DL 5% = 7,1% **Evelina I R DL 5% = 8,8%**

1% = 9,5% **1% = 1,7%**

0,1 % = 12,5 % **0,1 % = 15,4 %**

The effect of rates and time application under grain yield was also reflected by blooming delayed with 6-8 days at higher rates in optimum as well as in delayed treatmens. (table 6.2.4.).

Tabelul 6.2.4.

Blooming fenological phases at two maize hybrids, made in 2001 – conditions at SCDA Simnic

Varianta	Clarica LL				Evelina I R			
	Data însp.	Data mătăs.	Dif. zile	%U la rec.	Data însp.	Data mătăs.	Dif. zile	%U la rec.
5 x P	9 VII	12 VII	x	24,5	12 VII	14 VII	x	26,6
D1 ep I	12 VII	14 VII	2	24,0	15 VII	17 VII	3	27,5
D2 ep I	14 VII	16 VII	4	25,7	18 VII	20 VII	6	29,5
D2 ep II	16 VII	18 VII	6	26,3	19 VII	22 VII	7-8	27,9
NN	9 VII	12 VII	0	23,4	12 VII	15 VII	0	25,5

The analysies of grain yield elements at this two genetic-modified hybrids carried out closely positive correlation between yield and ear weight and also between yield and prolificity. Because of the toxic effect inte plants due herbicides was no a pasetive correlation between yield and control efficacity in experimental plot. Negative correlation between yield and weeds grade was supressed till the limit of signification, also because fitotoxic plants effect at higher rates which reduced weeds grade but grain yield too.

Conclusions and recommendations

Finlly the presentation of main recommendations, based on obtained results, in field experiments, with two genetic-modified maize hybrids comparated with normally variant (non-modified) give us the chance fo printed out some important aspects:

1)First request for using total herbicides, is avoid the spread of substance above other agricol species ,,growth near genetic-modified maize aseq. Acording with our observations and users informations receved from company which give them this products, if is negligented direction and air intensy from modified maize area to normal maize area or other species, the damage recording can be 70-80 % from yield.

2)Second main demande is releated with the importance of products concentration in commercial substance, which at Clarica LL is 2+2 l/ha when the herbicides treatment is post-emergency in two phases or 4 l/ha only at 3-4 leanes maize stage. For Escort herbicide recommandated rate is 4 l/ha ance at 3-4 leares maize stage in experimented conditions.

Lay – treatment, at 7 -8 leares stage and with high of plants around 80 cm, lead to lower yield when also plants present clear symptoms of toxicity. The rise of herbicides rate with 65 % abouve correct rate lead to important yield losses, no malter time apply.

3)Regarding to herbicides spread method is important the grade with orifices for spray to be placed behind plow. The grade placed before plow, as we made in demonstration plot in 1999, lead to lower weeds control on the trace plow area. At lay-treatment, when maize plants high is 80 cm is necessary a special grade with orifices at weeds high. Oterways a part of herbicide will falls on maize leres.

4)The efficacity of technological packet: genetic modified hybrid-specific herbicides – is esteem by seed production compny like a great improment in market conquest for their own products.

Jugeing this by point of biodiversity conservations, at scale of varieties and hybrids for main crops to apread on large area a limited number of modified hybrids with their specifical herbicides can be a seal risk.thus, is necessary in our country where ase cultivated approximately 3 million hectare with maize, to by test a large sort of genetic-modified hybrids, which will cover different conditions that exist, throung their diversify.

5)The mast efficiency uses of herbicides in generally and restriction that canceru less agresive technologies against envirament, make necessary researchs for associate the non-pollution total herbicides (lack remenense) with systemic herbicides. This scheme type can assure a total protection af maize crop but also clean very strog weeded area.

6)Agricultural specialists and also users of genetic-modified hybrids mize resistant at total herbicides is important to make a real information whicle will avoid misinterpretation from news papers, mde by unfit person (National NPaper 27.oct.2004).

7)At the end, we want to point out one again unopportuuity reserve from some domain against genetic-modified organism with a eloquent and directy speech of Prof. dr. Marcela Elena Badea: ,,OMG are in the middle of strong ,,idea fight,, that fret developed countries and restless the poor one, lead to discution in business fold, as wele as in politic and administrative area in media but also in street where people is called at demonstration where often vehemently in speech is direct proportional with unknowledge of subject.