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IWGO

Proceedings

XIX CONFERENCE
of the International Working Group on *Ostrinia nubilalis* and other maize pests

Guimarães, Portugal

August 30 - September 5, 1997

OILB

ORGANISATION INTERNATIONALE
DE LUTTE BIOLOGIQUE CONTRE LES
ANIMAUX ET LES PLANTES NUSIBLES

IOBC

INTERNATIONAL ORGANIZATION
FOR BIOLOGICAL CONTROL
OF NOXIOUS ANIMALS AND PLANTS

IWGO

INTERNATIONAL WORKING GROUP ON *OSTRINIA* AND OTHER MAIZE PESTS

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CAFÉS DELTA

CONTENTS

INTRODUCTION i
LIST OF PARTICIPANTS ii

Harald K. Berger
The XIXth IWGO Meeting in Guimarães; Portugal 1

Ostrinia nubilalis Hb

P.Anglade and A.Panouillé
Maize synthetic populations sources of resistance and tolerance to ECB damage on late stages of plant development 5

Sylvie Derridj, Sameh Mehri, Frédéric Marion-Poll
Corn leaf surface cations and *Ostrinia nubilalis* oviposition 13

Ivezic´, M., Raspudic´, E. and Milinarevic´,M
The European corn borer (*Ostrinia nubilalis* Hüb.) on corn in Croatia 23

Al. Bărbulescu
Data on emergence of the second generation of the European corn borer (*Ostrinia nubilalis* Hb.) in Romania 31

I. Rosca and Al.Barbulescu
Study of European corn borer (*Ostrinia nubilalis* Hb.) flight and dispersal, in Romania, part of insect pest management system 41

Sesamia nonagrioides Lef

A. Butron, R. A. Malvar, P. Velasco, P. Revilla, A. Ordas
Antibiosis and tolerance of maize to the pink stem borer (*Sesamia nonagrioides* Lef.) **53**

P. Anglade, L. Peypelut and J. C. Vible
Observations on overwintering larval population of the pink maize stalk borer *Sesamia nonagrioides* Lef., in southwestern France: sampling for population density, influence of temperature on larval mortality **67**

J. A. Tsitsipis, A. A. Fantinou and M. P. Alexandri
The corn stalk borer, *Sesamia nonagrioides*, modern control considerations **79**

Ostrinia and *Sesamia*

Maria M. R. Pereira, António Mexia
Field evaluation of population development of *Sesamia nonagrioides* Lef. And *Ostrinia nubilalis* Hüb., at Alentejo (Portugal) **97**

A. Özpinar, S. Kornosor
The studies on *Ostrinia nubilalis* Hübner (*Lep.:Pyralidae*) and *Sesamia nonagrioides* (Gahan) (*Lep.:Noctuidae*) in Turkey **103**

Mário Gonçalves
Maize pests in Portugal and their control **113**

Diabrotica virgifera virgifera LeConte

Tatjana B. Keresi, Dusan Camprag, Rodosav R. Sekulic, Pero M. Strbac
Comparison of trapping methods for monitoring of *Diabrotica virgifera virgifera* LeConte adults **131**

Ivan L. Sivcev
Harmfulness and control of the western corn rootworm in Yugoslavia **141**

Peter Baufeld and Siegfried Enzian
Establishment potential of the western corn rootworm (*Diabrotica virgifera*) in Germany **153**

Gábor Princzinger, Zoltán Ilovai
Appearance and spread of the western corn rootworm *(Diabrotica virgifera virgifera)* in central-eastern Europe 163

Other pests

Lorenzo Furlan, Miklós Tóth, István Ujváry
The suitability of Sex pheromone traps for implementing IPM strategies against *Agriotes* populations (*Coleoptera: Elateridae*) 173

Luís Gerson L. Reis
Plant-parasitic nematodes associated with maize in Portugal 183

Dumitru Mustea
The corn leaf aphid *Rhopalosiphum maidis* Fitch. 191

Quantification and economical evaluation

Maria R. Pereira, Fernanda Amaro, António Mexia
Losses caused by the corn borers *Sesamia nonagrioides* (Lef.) and *Ostrinia nubilalis* (Hüb.) in maize – A contribution for their quantification 197

Franja Baca, Julka Baca and Nebojsa Momirovic
Importance of the second generation of ECB (*Ostrinia nubilalis* Hbn.) in the production of maize as a second crop 207

João Portugal, Maria M. R. Pereira
Evaluation of economic threshold and tolerance theshold for *Sesamia nonagrioides* (Lef.) and *Ostrinia nubilalis* (Hub.) in corn 217

Barnabás Nagy, Ferenc Szentkirályi
Changes in the pest status within maize insect assemblages in the Carpathian basin 223

Biological control - *Trichogramma* and *Ephestia kuehniella*

Gavrilita Lidia
The migratory capacity of *Trichogramma dendrolimi* in cabbage and maize fields 237

Felicia Muresan and D. Mustea
Some results regarding the influence of the growing substrate of substitution host – *Ephestia kuehniella* Zell. and of *Trichogramma spp.* **245**

Sevcan Coskuntuncel, Serpil Kornosor
The effectiveness of inundative release of *Trichogramma evanescens* Westwood (*Hymenoptera; Trichogrammatidae*) against *Ostrinia nubilalis* Hubner (*Lepidoptera; Pyralidae*) in Turkey **253**

Franja Baca, Dragutin Hadzistevic´, Zeljko Kaitovic´
Presence of Lydella Thompsoni Hert. On ECB (*Ostrinia nubilalis* Hbn.) larvae in IWGO trials at harvest **265**

S. H. Mohamed
Effect of some chemical and microbial insecticides, and planting dates of maize on large sugar-cane worm infestation and the associated predators **275**

Transgeny – *Bacillus thuringiensis (Bt)*

C. Richard Edwards
An overview and evaluation of transgenic *Bt* corn for European corn borer control in the USA **285**

L. C. Lewis
Alternative methods to assesss effectiveness of *Bacillus thuringiensis* against *Ostrinia nubilalis (Lepidoptera: Pyralidae)* in corn **291**

Philosophy

Silas Pego
Resistance or tolerance? Philosophy, may be the answer **303**

INTRODUCTION

The **Portuguese Ministry of Agriculture, Rural Development and Fisheries**, through its **Northwest Regional Services** (DRAEDM) in collaboration with our **National Institute for Agronomic Research** (INIA-EAN), organized and hosted the XIX INTERNATIONAL IWGO CONFERENCE which took place, for the first time in its history, in Portugal.

Our involvement and enthusiasm was rewarded with the pleasure to welcome such a distinguished body of scientists in a specialized field of research related with a crop that really matters to the Portuguese economy and culture. Four main reasons can explain and justify our deep involvement in this scientific event.

First, because the Portuguese Northwest Region in which this Conference took place, represents the traditional maize area of our country where a kind of agricultural revolution took place during the XVII/XVIII centuries. The new plant of maize, as it slowly replaced wheat, was the great responsible for that revolution.

Second, because DRAEDM actually is in charge of two national institutions closely linked with maize: the Maize Breeding Centre (NUMI) which is the only official Portuguese research institution for maize, and the Portuguese Plant Gene Bank (BPGV) which, besides a large spectrum of other seeds, possesses as its main collection the germplasm of maize. And this could be, by itself, enough to be involved in such a scientific event.

Third, because for our maize farmers the two most important biologic enemies are *Sesamia nonagrioides* and *Ostrinia nubilalis* which constituted the historical basis for the IWGO organization.

Finally, as the fourth reason, because a new pest is emerging in Europe, coming from East to West and representing a serious economical menace to all European farmers. In our case, even as the westerner country in Europe, we wanted to be aware of this new moving insect and be up to date with all scientific innovations to control or sustain this pest - *Diabrotica virgifera virgifera* - through either taking advantage of tolerant germplasms or by the new advances related with transgeny as it is the case of the new transgenic seeds of maize with Bt (*Bacillus thuringiensis*) genes.

In fact, being aware that our BPGV possesses one of the richest European maize germplasm collections, we welcomed this Conference as a scientific event that could contribute to trigger our national potentiality in this field as a possible source of germplasmic raw materials that, once studied and characterized, can offer a scientific and practical solution to solve real problems at the farmers' level.

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THE XIX th IWGO MEETING IN GUIMARÃES; PORTUGAL

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The XIXth Meeting of IWGO in Guimarães is certainly a highlight within the almost 30 year history of IWGO. Never before had so many participants taken part in an IWGO - meeting, never before were so many countries represented. About 60 participants from 23 countries and all continents (except Australia) came to Guimarães Portugal, to take part in this meeting.

The explanation for this exceptional participation had obviously more than one reason: first - and I don't hesitate to say it - is the place of the meeting. Portugal is doubtless a country well worth visiting. Hardly known by most Europeans (and Americans) and certainly one of the most interesting countries in Europe. The second reason is a shift in the subjects IWGO has been dealing with during the last years. The original idea of IWGO was to exchange inbred lines within the group and test these lines for resistance against the most important maize pest throughout the world, the European Corn Borer (*Ostrinia nubilalis* Hbn.). The results of this breeding program were available for all member countries. Three synthetic lines resistant to ECB have been developed and released (IWGO 1, 2, and 3, both late and early). Most of the results of this testing program have been published during the last years. By this time the group had grown considerably, the interests became more diverse, e.g. a pheromone project was established by France (INRA) and other pests also became of interest .

Since its establishment in 1968 IWGO (International Working Group on *Ostrinia*) has been very active. Many meetings took place and several publications were issued: "Report of the International Project on *Ostrinia nubilalis* Phase I Results 1969 and 1970" (Budapest, 1973), "Report of the International Project on *Ostrinia nubilalis* Phase II Results" (Budapest, 1975), "Report of the International Project on *Ostrinia nubilalis* Phase III Results" (Budapest, 1976). Anglade, P., J. Stockel & IWGO co-operators: "Intraspecific sex pheromone variability in the European corn borer". Since

1980 IWGO publishes a biannual, IWGO-NEWSLETTER. The NEWSLETTER issues mainly the summaries of papers of previous meetings, but also a large number of original papers have also been published. The NEWSLETTER is also a medium to uphold contact within the group and to distribute information about the members. Until now,16 volumes, mostly of two issues per annum have been released.

Throughout IWGO - history many meetings were held: 1969: Ist Vienna, Austria; 1970: IInd Zemun/Belgrade, Yugoslavia; 1971: IIIrd Bordeaux, France; 1972: IVth Martonvasar, Hungary; 1973: Vth Zagreb, Yugoslavia; 1974: VIth St. Paul, Minnesota, USA; 1975: VIIth St. Petersburg, USSR; 1976: VIIIth Madrid, Spain; 1977: IXth Wroczlaw, Poland; 1978: Xth Bergamo, Italy; 1980: XIth Vienna, Austria; 1982: XIIth Pistany, CSSR; 1984: XIIIth Colmar, France; 1986: XIVth Beijing, China; 1988: XVth Varna, Bulgaria; 1991: XVIth Martonvasar, Hungary; 1993: XVIIth Volos, Greece and 1995: XVIIIth Turda, Romania. Meetings of the technical and publication committee in Martonvasar, Hungary (1973), in Bordeaux, France (1978), in Zemun Polje, Yugoslavia (1980), in Martonvasar, Hungary (1983) and in Bordeaux, France (1992) prepared publications and evaluated the results gained in the respective countries.

After several meetings proceedings were issued to inform all interested scientists not having been able to attend about the meetings: "Proceedings of the 13th Workshop of the International Working Group on *Ostrinia* IOBC/OILB" (Colmar, 1984), "Proceedings of the XIVth Symposium of the International Working Group on *Ostrinia"* (Beijing, 1986), "Proceedings of the XVth Symposium of the International Working Group on *Ostrinia* " (Varna, 1988); “The proceedings of the XVIIth meeting in Volos”, Greece“ (1995) and, “The proceedings of the XVIIIth meeting in Turda”, Romania (1996). But times changed. Although retaining the abbreviation, IWGO, the group changed its name to “International Working Group on *Ostrinia* and other Maize Pests”. The group is now dealing with all aspects concerning corn pests throughout the world. Therefore the number of scientists interested in this working group increased, last but not least because maize is certainly one of the most wide spread crops in the world. It is grown in many countries on all continents. And the same main pests in maize occur everywhere where maize is grown.

The third reason for this outstanding event in IWGO history could be the appearance of *Diabrotica virgifera virgifera* in Europe. Not only IWGO but also EPPO - the European and Mediterranean Plant Protection

Organisation – and FAO - the Food and Agricultural Organisation of the United Nations showed interest in the occurrence and the spread of this pest. The appearance of this new pest in Europe and the difficulties connected with free trade in Europe brought EPPO on the plan. A pest risk assessment was set up and quarantine regulations were issued. FAO had great interest in eradicating or at least to reducing the speed of expansion out of this pest. One of the largest difficulties in the fight against this new pest was its first appearance in former Yugoslavia. In the meantime *Diabrotica* covers large areas in Serbia, where more than 250.000 ha are already infested, and has reached wide areas in Croatia, the southern part of Hungary and the western boundaries of Romania. The countries Bulgaria, Austria, and Bosnia and Herczegowina are threatened. But due to the close connections between the infested countries and other European countries *Diabrotica* is a threat for all maize growing countries in Europe, where about 14 million hectares of maize are grown.

But also other subjects became more and more important for plant protection in maize. B.t.- corn is just one of the topics which excited great interest in Europe and the States. The European Corn Borer (*Ostrinia nubilalis*) found its way in the daily press, and people who did not even know that there are pests threatening maize learned a lot about this pest. But also papers on other maize pests such as *Ostrinia furnacalis*, *Sesamia nonagroides*, aphids and others, were presented at these meetings and showed the need for IWGO and its biannually congresses.

IWGO is certainly one of the oldest Working Groups within the Global IOBC. The group was founded in 1968 during the XIIIth International Congress of Entomology in Moscow, but the roots reach back to the US - regional project on *Ostrinia* already begun in 1951. The group was established under the aspect of this US regional project. The founders of the group in 1968 were D. HADZISTEVIC (Yugoslavia), who had the original idea of founding a group of international co-operation, H.C. CHIANG (USA), who brought the ideas of the US projects into the group and was its first president, I.D. SHAPIRO from Russia (USSR), T. PERJU (Romania), C. KANIA (Poland) and B. DOLINKA (Hungary), all well-known researchers or maize-breeders. The group was originally organised in such a way, that each member country was represented by one "member", all other participants from the same country were "associate members".

Researchers taking part in the meetings and coming from the country where the meeting took place were "guest members". The second president after the retirement of Prof. CHIANG in 1983 was Pierre ANGLADE from Bordeaux, France. Since 1993 I am convenor of the group.

IWGO is today a well established large international working group which now deals with all matters of maize pests and pest resistance. The group is open to all scientists with interest international co-operation on these subjects.

MAIZE SYNTHETIC POPULATIONS SOURCES OF RESISTANCE AND TOLERANCE TO ECB DAMAGE ON LATE STAGES OF PLANT DEVELOPMENT

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ABSTRACT

In Europe, both the second ECB generation in southern areas and the only one generation in the north coincide with post-tasseling stages of plant development. Sources of resistance and tolerance in the late stages of maize are needed in breeding programmes. Three original synthetic populations ECB2 Flint, ECB2 Dent, ECB Whole life tolerant were developed. They include lines resistant or partially resistant to sheath-collar feeding and tolerant lines to late borer infestations generally with good combining ability for yield. The aim of the study was to evaluate the C0 cycle of the 3 synthetics for yield ability and prevention of yield losses caused by the borer.

The study was conducted on populations and crosses to testers in an experimental network for agronomic traits and in a split-plot design with artificial ECB infestation for evaluation of yield losses due to the borer.

Artificial infestations on leaves at full silking stage resulted in an intermediate level of visual tolerance rating for the three populations and in average yield losses around 12-18 % instead of 26 and 9 % for susceptible and resistant checks, respectively. However, average grain yield ability in absence of the borer was around 15 % less than the commercial cultivar used as control.

Nevertheless, the material can be improved by crossing to new available tolerant lines with good combining ability for yield. Then, a multitrait recurrent interpopulation selection might be possible for the ECB2 F and ECB2 D populations in order to improve their performances.

Key words: Maize pests, *Ostrinia nubilalis*, synthetic populations, tolerance ratings.

INTRODUCTION

In Southern Europe, European corn borer (ECB), *Ostrinia nubilalis* Hbn. normally has 2 generations per year. Second generation larvae feed on full silk stage of plant development. Furthermore, in northern Europe where the borer has only one generation per year, a large part of the borer larvae population feed primarily during anthesis on pollen and sheath-collar tissue. For these reasons, sources of resistance and tolerance to the ECB during the late stages of plant development are needed in Europe for both early and late plant material. Breeding programmes for sheath-collar resistance (SCR) rely on limited number of lines (B52, B86, DE 811) or populations (MO-2 ECB2, BS9 (CB) C4). However, partial SCR was detected in an IWGO programme carried on in Iowa.

Eighty lines exchanged among IWGO members were heavily infested during anthesis for a total of 12 egg-masses (e.m.) per plant then rated in classes 1-9 for SCR and compared to checks B52 highly resistant, B86 resistant, WF9 and W182E susceptible. If 85 % of the material appeared susceptible, 9 lines were rated 5-6 (intermediate) and 2 flint type lines EA 3130 (Spain), JI 769 (China) were rated 3-4 (resistant) (Guthrie, 1984).

Meanwhile, more than 200 inbred lines known as tolerant to late whorl ECB infestation were tested again in France on full silk stage of plant development (1985-1991). The plants were lightly infested (2 e.m. per plant) rated in classes 1-9 for tolerance and compared to checks B52 sheath-collar feeding resistant, F564 tolerant and W182E susceptible. Around 20 lines have exhibited fairly good tolerance rates. Moreover, these lines were tested for SCR in late infestations at 6 e.m. per plant. Some of them have shown an intermediate SCR rate as F664, F888 (France), TVA 2048 (Slovakia) while the others were rated susceptible.

Consequently, crosses were made in view to combine characters of SCR expressed as antibiosis to young larvae and characters of tolerance expressed as damage reduction on the plant, previously detected in IWGO and French breeding programmes. Two synthetics flint and dent, respectively, were developed (ECB2 F and ECB2 D). Like resistance, tolerance was shown to be primarily additive in gene action (Kaan *et al.*, 1983). The efficacy of a multitrait recurrent selection for tolerance and yield ability in a synthetic population was recently shown (Anglade *et al.*, 1996).

On the other hand, Russel and Guthrie (1982) have released the BS9 (CB) C4 synthetic developed by intermating lines resistant either to the first or to the second ECB generation and selected for resistance to ECB for the whole life of the plant. In a similar way, an experimental synthetic population was developed in France from US germplasm. Twelve lines issued from crosses between CI31A (ECB1 resistant), B52 and MO-2 ECB (ECB2 resistant) were obtained in nurseries by selection for earliness and tolerance to both early (late whorl stage) and late (full silk stage) ECB infestations. They were intermated in view to develop an original synthetic population resistant and tolerant to ECB for the whole life of the plant (ECB WLT).

The objectives of this study were:

1) To evaluate, before recurrent selection, the three C0 synthetics for tolerance and agronomic traits.
2) To assess the worth of their general tolerance to prevent yield reductions due to the borer.

MATERIAL AND METHODS

Materials

A detailed description of the basic materials used in the development of the synthetics ECB2 F (flint type) and ECB2 D (dent type) was presented in Table 1. These lines were selected on the basis of data collected in field tests under artificial ECB infestations during several years among a lot of lines known either for SCR or for tolerance. They cover a large range of flowering earliness from-5 to + 13 days around W182 E. Their tolerance ratings TR were expressed by the mean average differences to the mean TR of the checks B52 and F564. Their SCR was estimated on the basis of the Iowa and France tests.

Experimental methods

The main study was conducted at the Maize Experimental Unit, INRA France at Saint Martin de Hinx (SMH) in 1996. The experimental design was a split-plot in which infested and uninfested treatments were the whole plots, 12 entries were the subplots and each treatment was replicated six times. The 12 entries consisted of :

1) The three synthetic populations.
2) The three synthetics crossed to 2 ECB susceptible testers: MO17 (Lancaster) and F618 (Stiff Stalk Synt.).
3) Three check hybrids : B73 x MO17 (susceptible), B52 x MO17 and DE 811 x MBS 847 (resistant x intermediate tolerant).

Usual agronomic practices were followed (sowing date, plant density, irrigation). Two infestations, of two egg-masses each, were made at 8 day interval, after tasseling, pinning the eggs on the leaves. Data were collected for silking dates, preharvest stalk lodging (%), visual rating of ECB damage

(Anglade and Hadzistevic, 1970) grain weight adjusted to 15 % grain moisture later converted to megagrams per hectare. More informations on the method are avaible in a recent publication (Anglade *et al,* 1996). Moreover, the same entries were included in a larger experimental network and were evaluated for agronomic traits in 4 locations in southern and central France and compared to the appropriate commercial cultivar, Mondain.

Table 1 - Characteristics of the components of the synthetic populations ECB2 Flint and ECB2 Dent.

Inbred lines	Origins	Pedigrees	Silking days	Tolerance ratings	SCR groups
Synthetic ECB2 Flint					
TVA 2048	Trnava, Slovakia		- 5	- 0.4	S
EA 3130	INIA, Spain		+ 5	- 0.1	R *
ARG BF	INTA, Argentina		+ 6	+ 0.1	S
LO 42	Bergamo, Italy		+ 6	+ 0.3	S
F 564	INRA, France	F7 x F64	- 1	+ 0.3	I
JI 769	Jilin, China		+13	+ 0.6	R *
F 650	INRA, France	F192 x 0h43	+ 3	+ 0.7	S
EAF 36	INRA, France	EA 2087 x F2	- 3	+ 0.8	I
Synthetic ECB2 Dent					
B 52	USA		+11	- 0.3	HR *
DE 811	USA		+ 9	- 0.2	R *
I 147	INRA, France	W64A x F546 xF546	- 2	- 0.2	S
F664	INRA, France	B52 x W705A	+ 5	- 0.1	I
MBS 797	USA		0	- 0.1	S
F888	INRA, France	ETO (M) C9	+ 6	+ 0.3	I
B86	USA		+ 9	+ 0.3	R *
FP23	INRA, France	SSS2 x IHP	+4	+ 0.4	S
FP Oh 225	INRA, France	F478 x 0h432	+ 1	+ 0.8	S
Hao Bai	Beijing, China		+ 2	+ 0.9	S
Susceptible check W182 E			0	+ 2.6	S

Silking = differences in days to W182 E ; Tolerance ratings = differences to the mean TR of the checks F564 and B52; Sheath-collar resistance SCR = HR = highly resistant, R = resistant, I = intermediate, S = susceptible. Asterisk concerns data from W.D. Guthrie.

Analysis

Variance analyses were made for the different data. Correlation and regression coefficients were estimated between several data. The plot means of visual ratings of ECB damage corrected for their significant regression on silking date were used as tolerance rating (TR). The yield losses (YL) were calculated as the differences of yield between control (uninfested) and infested plots and converted to % of control. Moreover, the increase in tolerance of each entry compared to the susceptible check was expressed by the ratio:(YL % of susceptible check - % YL of each entry) on YL % of susceptible check (Fig. 1) which corresponds to a decrease of yield reduction due to the borer.

RESULTS

Agronomic traits

In the experimental network the well-known hybrid B73 x MO17 used as susceptible check had shown a mean yield ability about 13 Mg ha^{-1} with a 24 % mean lodging. Grain yields for the test crosses of the synthetics were about 2.3 – 3.0 Mg ha^{-1} (17 % - 22 %) less than B73 x MO17 with a lower level of stalk lodging (16 %) and with a 2-3 % lower grain moisture.

The differences of yield observed between the mean of test crosses and the population were 3.8, 2.1 an 3.3 Mg ha^{-1} in ECB2 F, ECB2 D and ECBWLT, respectively, which correspond to 56, 22 and 40 % heterosis effects (Table 2).

Plant tolerance ratings

In the SMH experiment (Table 2), the relatively high level of artificial infestation joined to favourable conditions to larval development might explain the mean TR data. Nevertheless, the mean TR of the synthetics were rated intermediate:3.3, 3.3, 3.7 in ECB2F, ECB2D and ECBWLT, respectively, among the resistant and susceptible checks: 2.5 and 4.6, respectively.

Prevention of yield losses

The correlation coefficient between YL and TR, $r_{(10)} = +0.86$, was significant. On the contrary, no correlation was found between YL and either silking date or yield ability.

The YL % were 6.7 and 26.2 % for B52 x MO17 and B73 x MO17, respectively. On the same tester MO17, the YL % were 11.7, 16.2 and 18.1 % for ECB2 F, ECB2 D and ECB WLT, respectively (Table 2).

Table 2 -Tolerance ratings and other agronomic traits for the 3 synthetic populations and, their crosses to testers compared to resistant (R) and to susceptible (S) checks and to a commercial cultivar.

Synthetic populations	**SMH Experiment**				**Network**		
Crosses to testers Checks	Silking dates (no of days) (1)	Grain yield uninf. plots (Mg ha^{-1})	Tolerance ratings TR	Yield losses inf. plots %	Stalk lodging %	Grain moisture %	Grain yield uninf.plots (Mg ha^{-1})
ECB2 F Population	76	6.46	3.1	14.2	18	30.3	6.75
x MO17	80	8.91	3.4	11.7	22	31.4	10.59
x F 618	77	9.72	3.3	18.3	9	31.1	10.52
ECB2 D Population	79	9.85	3.2	12.8	12	30.1	9.64
x MO17	80	11.58	3.0	16.2	20	30.6	12.03
x F618	78	10.48	3.7	20.7	10	30.9	11.40
ECB WLT Population	80	7.69	3.7	23.8	25	30.8	8.13
x MO17	81	9.21	4.0	18.1	20	31.3	11.31
x F618	78	11.12	3.4	19.2	12	31.3	11.49
R B52 x MO17	86	7.36	2.4	6.7	-	-	-
R DE811 x MBS 847	82	11.42	2.6	10.8	7	31.5	12.78
S B73 x MO17	84	11.38	4.6	26.2	24	33.8	13.60
MONDAIN	-	-	-	-	9	30.0	13.10
Means	80	9.60	3.0	16.6	15	31.1	10.95

(1) - Silking dates: number of days from planting time (May, 9 th)

The decrease of yield reduction was correlated with the visual tolerance ratings (r_{10} = - 0.87) (Fig. 1). These percentages of decrease in yield reduction were around 60 - 75 % in the resistant checks and 44,37,22 % in the ECB2 F, ECB2 D, ECB WLT respectively.

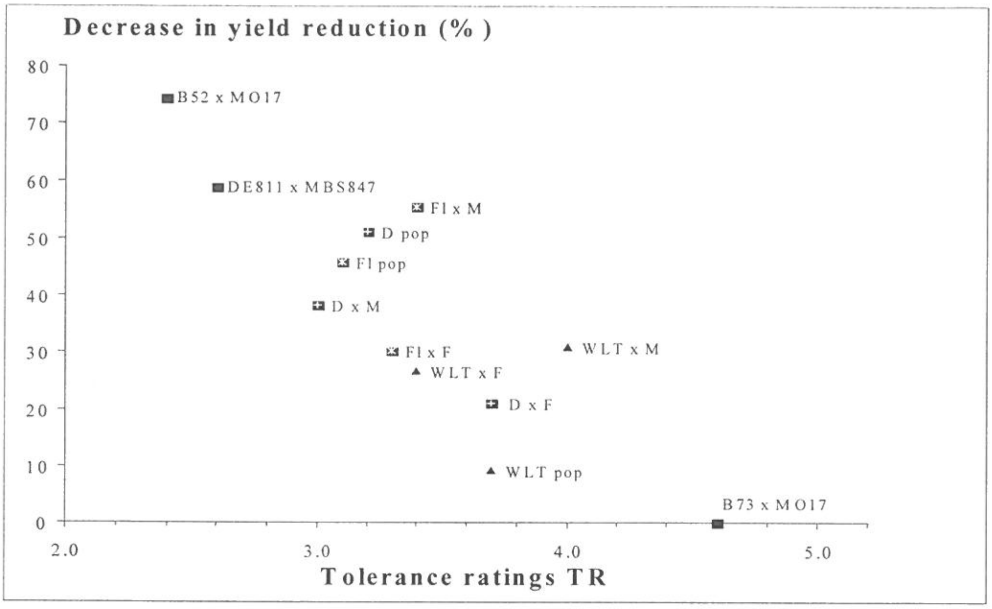

Fig. 1 - Relationship between x = tolerance ratings and y = decrease (%) of yield reduction due to the borer compared with the yield losses (YL %) observed for the susceptible check B73 x MO17.
Fl, D, WLT pop design the ECB2 F, ECB2 D, ECBWLT populations, respectively. Letters M aud F design the MO17 and F618 tester lines for the crosses to the three populations.

CONCLUSION

The aim of the project was to develop original synthetic populations tolerant to ECB damage on late growth stages of maize and adapted to European conditions of maize crops.

The first step of the project was fulfilled: three original synthetics were developed. Emphasis may be put on a flint synthetic ECB2 F made with lines issued from IWGO exchanges rated either sheath-collar feeding resistant in Iowa tests or tolerant in late stages of maize growth in French experiments. A dent synthetic ECB2 D was obtained by intermating the classical sheath-collar feeding resistant US lines and some tolerant INRA lines. The C0 cycles of these two synthetics have shown an intermediate degree of tolerance to ECB infestations in late stages of plant development both for visual damage and for reduction of yield losses due to the borer. A synthetic tolerant for the whole life of the plant ECBWLT using a limited number of sources of resistance to the two US generations of the borer have shown lower performances than the two others both for tolerance and yield ability.

The yield ability of test crosses in absence of the borer for the two ECB 2 synthetics was about 15 % less than the commercial cultivar used as control. Consequently, they had to be improved by introducing a lot of tolerant lines with good combining ability for yield. Some new INRA lines (Panouillé *et al.,* 1997) will be used for this purpose.

The improved C0 cycle versions of the two complementary ECBF and ECBD synthetics might be used in a multitrait recurrent interpopulation selection in view to get improved sources of ECB tolerance in late stages of plant development.

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CORN LEAF SURFACE CATIONS AND *OSTRINIA NUBILALIS* OVIPOSITION

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ABSTRACT

The leaf surface represents a boundary between the plant and its environment. Throughout insect life, events like host or site selection for ovipositing or feeding, involve contact stimuli. Biochemical stimuli are rather predominant in contact stimuli. Cuticle and wax components are part of them, but we have demonstrated by spraying water on leaf surfaces, that other metabolites originating from the leaf tissues are present in minute but detectable quantities. They could thus provide insects useful information about the plant species specificity and plant physiological state.

In a series of previous experiments, we have found evidence that *Ostrinia nubilalis* females can detect simple chemicals at the surface of the leaves. Chemicals such as sugars or aminoacids modify the oviposition behaviour. Although these chemicals are hydrophilic substances, we were able to find them in detectable amounts at the surface of the leaves. In this study, we have examined the presence of salts at the surface of the leaves, and investigated their role on the perception and on the behaviour.

Key words: Maize pests, *Ostrinia nubilalis*, leaf cations.

INTRODUCTION

The leaf surface represents a boundary between the plant and its environment. Throughout insect life, events like host or site selection for ovipositing or feeding, involve contact stimuli, among which biochemical components are rather predominant. Cuticle and wax components are part of them (Udayagiri and Mason, 1997) but we have demonstrated by spraying water on the leaf surfaces, that other metabolites originating from the leaf tissues are also present in minute but detectable quantities. They can thus provide insect useful information about the plant species specificities and plant physiology.

Chemicals such as sugars, amino acids, or organic acids were found (ng per cm2) at the surface of the leaves. They influence the *Ostrinia nubilalis* (Hbn.) oviposition behaviour. In a series of previous experiments, we found evidences that females can detect them by sesilla on legs and ovipositor. In this study, we examined the presence of cations at the surface of the leaves, and investigated their role on the insect behaviour and perception.

MATERIAL AND METHODS

The complete protocol included the following series of operations: observations of oviposition of *O. nubilalis* females on whole corn plants maintained in cages, collection of corn leaf surface chemicals by spraying water on the leaf surface, analysis of cations Na^{+}, Ca^{++}, K^{+} on the two leaf sides from different positions and at different corn growth stages, sensory recordings from taste sensilla located on female legs, behavioural tests of oviposition on artificial substrates.

Insect

O.nubilalis adult maintained on an artificial diet but periodically infused with insects of which the origin were larvae collected in the field in autumn in St. Martin de Hinx, were obtained from I.N.R.A. le Magneraud. Pupae received from le Magneraud and adults until bioassays with plants were maintained in an environmental chamber at 25° C with a photoperiod of 16L:8D and 80 % relative humidity. After two days of egg laying on artificial substrate (filter paper) females were released in greenhouse compartments.

Plants

Corn (*Zea mays* L.) plants (Dea corn hybrid) were grown hydroponically with a nutrient solution in greenhouses. Sowings were done from February to May. Each plant was in a 7.4 l volume plastic pot filled with perlite and earth (2v,1v). 16L:8D photoperiod was maintained by additional light, temperature means were 15°C during the dark and 25°C during light and day, means of relative humidity were varying from 90 % to 50 %. Experiments were conducted at two plant growth stages : mid whorl (I) and late whorl (II).

Plant-insect assays and collection of leaf surface cations

Six individual corn plants of each growth stage were given to insects in greenhouse compartements divided in 6 cages of 200 cm l x 200 cm L x 300 cm h by viel. Twenty females and 20 males were released in each cage two hours before sunset during the month of June. After two nights the number of egg-masses and eggs per egg-mass were noted as well as their localisation on plant (leaf position and leaf side).

Four plants similar to the 6 plants given to the insects were sampled for chemical analysis at the same time as the insect release. Each plant constitutes one replicate. At each position leaves were cut and then sprayed (Derridj 1996) with pure water, upper and lower surfaces separately. Washings of several leaves were gathered in three groups: position 1: 4, 5, 6 th leaves, position 2 : 7, 8, 9 th leaves, position 3 : 10, 11, 12 th leaves counted from the plant bottom.

Oviposition on artificial substrate

Filter papers were impregnated with a salt solution with concentrations found on position 3 Stage I, NaCl: 48.3ng per cm^2+ $CaCl_2$: 67.6 ng per cm^2+ KCl: 17.5 ng per cm2. They were prepared 1 hour before giving them to insects. They were given in two-choice bioassays with control supports impregnated with ultrapure water 15 mn before the start of scotophase, during 3 hours. Twenty individual females in cages were examined. They had already oviposited during 1 day and were followed on 3 days. Results are presented by the discriminant index D.I which is calculated by : N egg-masses on salts - N egg-masses on control /N egg-masses on the two supports x100 .

Chemical analysis

Leaf washing samples were sent to INRA SRIV at Antibes (France) where the cations Na^{++}; Ca^{++}, and K^{+} were analysed by capillary ionic analysis (CIA.).The separation mechanism by capillary zone electrophoresis is based on differences in charge to mass ratio (Compton et al 1988).

Statistical analysis

A non-parametric Monte-Carlo test was used to analyse results of oviposition preference (Vaillant and Derridj 1992). Quantities of cations collected and numbers of eggs per egg mass were compared by the Student **t** test. Standard errors are represented on figures with means.

Sensory recordings

Contact chemosensilla located on the last segment of adult tarsae (sensilla CRa and CRb: Marion-Poll et al., 1992), were recorded using a DC taste amplifier (Marion-Poll and Van der Pers, 1996). An adult was briefly narcotized with CO2 and mounted ventral side up in order to expose the ventral side of its legs. One capillary electrode filled with saline served as an indifferent electrode and was inserted into a leg joint. Another electrode filled with the test solutions used to stimulate taste hairs. The electrical signal was amplified, stored on a PC computer and further analyzed using a specialized program (Awave: Marion-Poll, 1996).

RESULTS

Insect oviposition

The great majority of egg-masses were oviposited on the under leaf sides whatever the leaf position and the plant growth stage. On each whole plant the mean number of eggs deposited on the upper leaf side was 50 against 207 on the under leaf side at the early whorl stage, and 61 against 263 respectively at the late whorl stage. Distribution of eggs on the plant showed an increase from the bottom to the top of the plant. The younger leaves were prefered for oviposition at both growth stages I and II (Figures 1 and 2).

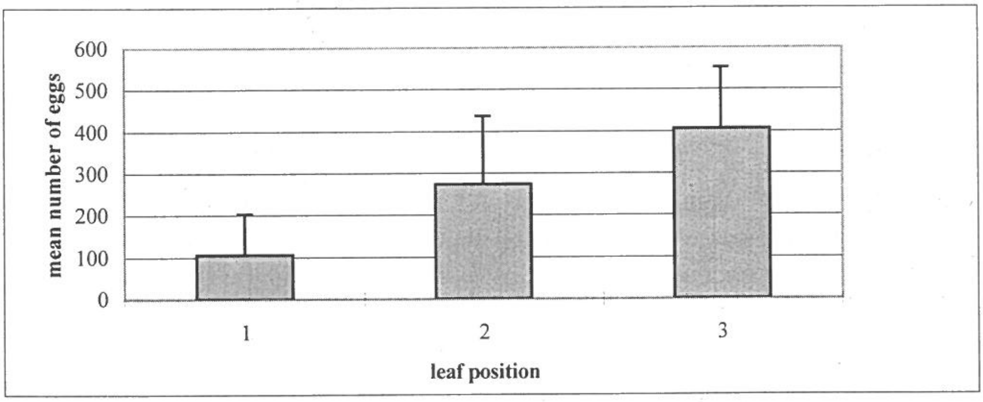

Figure 1 – Distribution of *O. nubilalis* eggs on corn under leaf sides at the mid whorl stage (position 1: 4, 5, 6 th leaf, position 2: 7, 8,9 th leaf, position 3:10, 11, 12 th).

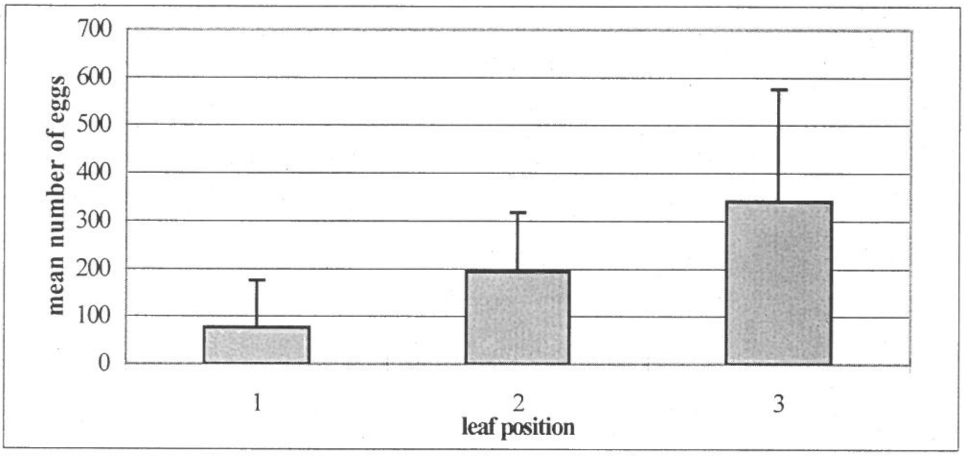

Figure 2 – Distribution of *O. nubilalis* eggs on corn under leaf sides at the late whorl stage (position 1: 4,5,6 th leaf, position 2: 4, 5,6 th leaf, position 3:10,11,12 th).

At the stage I for the positions 1, 2 and 3 respectively the number of eggs per egg-mass was 23 ± 5.8, 23.15 ± 4.4, and 29.64 ± 5. At position 3 the number of eggs per egg-mass was higher. At the stage II the numbers of eggs per egg mass were similar for 1, 2, 3 leaf positions respectively 39.12 ± 12.6, 41.5± 9.64, 42.85± 8.44.

Cations in the leaf washings

Plants were generally richer in cations on the upper leaf sides whatever the leaf position and the plant growth stage. At the stage I quantities of each cation Na^+, Ca^{++}, and K^+ were respectively on the upper leaf side 409, 294, and 68 ng per cm2 against 74, 148, 41 on the under leaf side. At stage II values were respectively 162, 204, 24 on the upper leaf side against 80, 125, and 34 on the under leaf side.

The comparison between leaf positions on the under leaf sides showed that the elder leaves from the bottom (position 1) were generally richer in the three cations than the other two leaf position (2 and 3). Within these two last positions there was no visible difference. At the stage II results were nearly similar to stage I except for Na^+ for which the position 1 was richer than position 3 but similar to position 2 (Figures 3 and 4).

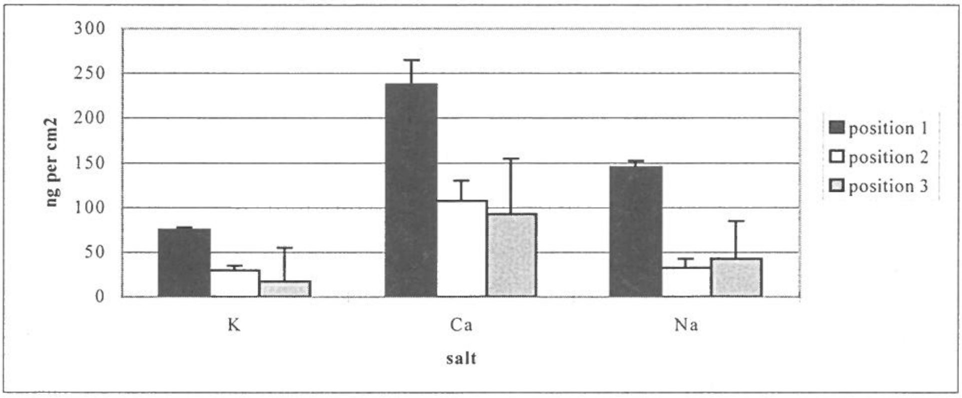

Figure 3 – Quantities of salts collected at the corn under leaf side at the mid whorl stage (position 1:4,5,6 th leaf, position 2: 7, 8, 9 th, position 3:10, 11, 12 th).

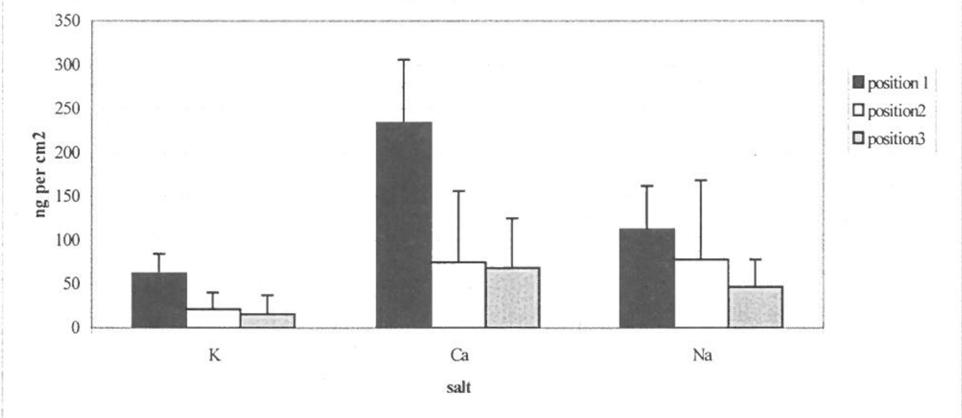

Figure 4 – Quantities of salts collected at the under maize leaf side at the late whorl stage (position 1:4, 5, 6 th leaf, position 2: 7, 8, 9 th, position 3:10,11,12 th).

Oviposition and cations

Comparing the egg distribution on plants and the cation compositions, the more K^+ and Ca^{++} quantities were collected on the leaf surfaces the less numbers of egg-masses were laid. The best correlation was obtained with Ca^{++} quantities: Log. (egg masses) = -2.43 Log (Ca^{++}) + 17.12, $r^2 = 0.68$. In dual choice females laid more egg-masses on the salty substrate (Figure 5).

The number of eggs per egg-mass was 10.8 on salty substrate against 15.8 on control and at the end the number of eggs laid by females on the 2 substrates were similar.

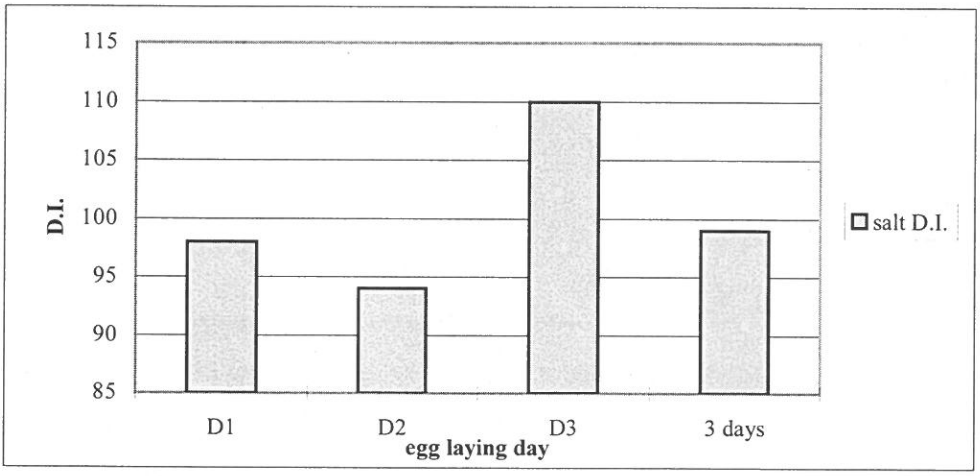

Figure 5 - *Ostrinia nubilalis* oviposition on two artificial supports a and b impregnated respectively with pure water and with salts (Na, Ca, K chlorure) expressed by the discriminant index calculated on the number of egg masses (a - b) / bx100.

Sensory hairs

Each leg of adult *O. nubilalis* bears 2 rows of 5 to 10 strong taste hairs located on the ventral surface of the last segment of the legs. In addition to these ventral hairs denoted CRb, on each side of the leg are found CRa hairs, which have a slightly different morphology (Marion-Poll et al., 1992). On CRb hairs (Figure 6), the recordings performed indicate that NaCl is a strong stimulant for one sensory cell, KCl stimulates two neurons whereas CaCl2 is not perceived at low concentrations and inhibitory at higher concentrations.

CRa hairs have a slightly different profile as concerns the neurons excited by these salts, but CaCl2 shows also an inhibitory activity. These recordings indicate that salts are well perceived and possibly discriminated by *O. nubilalis* adult females when touching a plant surface with their legs.

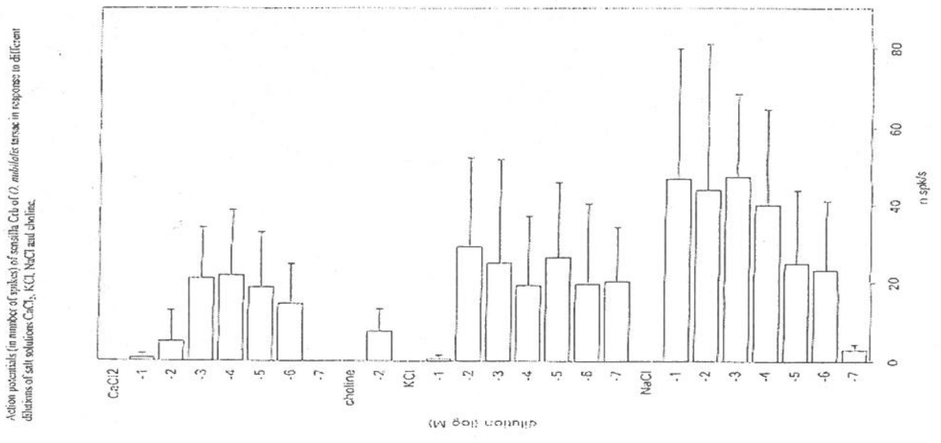

Figure 6 – Action potentials (in number of spikes) of sensilla Crb of *O. nubilalis* tarsae in response to different dilutions of salt solution $CaCl_2$, KCL, NaCl and choline.

DISCUSSION - CONCLUSION

Cations are present on the leaf surfaces. The upper leaf side is more exposed to deposits coming from the atmosphere. That can explain high quantities of Na^+ (from 70 to 600 ng per cm2) we observed on the upper leaf side. Na^+ couldbe produced by cooling climatisation conditions which used sodium salts as water-softener. At the under leaf side, cations are more representative of the plant physiology. Their presence is related to the leaf age. The quantities of cations vary from 25 to 200 ng per cm2. Na^+ quantities and Ca^{++} vary more than K^+ ones.

The females have sensilla on tarsae and on the ovipositor which are stimulated by the 3 cations (personal communication from Dr Städler E.).

The ectrophysiological recordings indicate that salt concentrations can result in stimulation or inhibition according to their levels.

On plants the relation between *O. nubilalis* oviposition and the cations collected on the leaf surfaces is negative. The upper leaf side and the older leaves were avoided.

The concentration of cations used on artificial support induced laying of more egg-masses without any change in the number of eggs. Their effect when isolated is different from the one which was observed on plants and other compounds like the soluble carbohydrates which act on the laying of eggs and not on the number of eggs per egg-mass. This conduct to further examine oviposition responses of *O. nubilalis* including cations with other activ compounds as those already known.

ACKNOWLEDGMENTS

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THE EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* Hüb.) ON CORN IN CROATIA

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ABSTRACT

The corn, one of the most dominant crops in Croatia, from 1992 - 1996 was cultivated every year on 365,854 ha, with corn seed production of 1.670.000. tons (4,59 t/ha).

In Croatia, the European Corn Borer (*Ostrinia nubilalis* Hübner) is a major pest on corn, having two generations per year. The highest crop losses are due to the stem and ear attacks. The average attack of 37,08% of ECB was determined during 25 years (1971-1996), in the eastern part of Croatia.

Until 1985 the attack of ECB was under 50% and from 1992 to 1996 it was 60,55%. In the last few years, the intensity of the attack is very high and it is very difficult to find out tolerant hybrids for this pest. It was noticed, that FAO groups 300, 400, 500 and 600 are infected more than 50% by ECB. The average attack of ECB from 1992 - 1996 per FAO groups is as follows: FAO 100 - 34,25%; FAO 200 - 45,83%; FAO 300 - 56,25%; FAO 400 - 66,01%; FAO 500 - 65,14%; FAO 600 - 71,76% and FAO 700 - 55,57%. Only agrotechnical measures are used in our country to control ECB. In 1995, the trials were carried out by biological preparation Biobit XL and the attack was reduced by 41%. This kind of measures should be continued in the future together with integrated pest management.

Key words: *Insecta, Pyralidae, Ostrinia nubilalis* Hübner, European corn borer, corn hybrids, Slavonia and Baranya, Croatia

INTRODUCTION

Corn is one of the most important cereal crops in Croatia. Total production was 2.37 million tons (1980-1989) from an area of 507,000 ha: an average yield of 4.68 tons per ha, and total production was 1.67 million tons (1992-1996) from an area of 365,854 ha: an average yield of 4.59 tonnes per ha. Family farms account for 80 percent of production. In general, family farms achieve much lower yields than ex-social sector enterprises, because of lower levels of mechanization and lower input use. Among the large number of corn pests, there is always a high intensity of attack of the European Corn Borer (*Ostrinia nubilalis* Hübner) which is one of the most economically important pests in Croatia.

The Corn borer has one to two generations, in our conditions. The number of generations depends on climatic factors and on the host plant. According to Wressell (1952) multigenerations are highest, so the second generations had 71% in comparison with the first generation. Intensity of attack varied from year to year on the territory of Baranya and Slavonia. According to Valenèiæ et al. (1986) the corn borer attack was 32.95% (18 - 56 %) during four years on seed corn production. Larva and tunnels were along the whole stalk, but the largest number was under the ear (Ivezić et al. 1993, 1994) . During 1988 the scientists investigated the effect of biological preparation (Bactospein, *Bacillus thurigiensis Berlinier*) on the larva stage of the corn borer, and reduction of attack by 9% was determined (Valenčić et al. 1988).

Our investigation was carried out from 1992 to 1996 at Pannonia region, the agricultural hearthland of Croatia. This region lies between the river Drava to the North and river Sava to the South. The aim of this investigation was to discover the intensity of attack of the European corn borer on the different corn hybrids of all FAO groups.

MATERIAL AND METHODS

The investigation was being carried out on corn hybrids on the territory of Slavonia and Baranya from 1992 to 1996. The trials were carried out at the fields of ex-social sector enterprise "Belje" PIK on the territory of Valpovo region, with mostly domestic hybrids from FAO groups 100 to 700.

In 1991 we could not do our investigation because of the aggression on Croatia. The territory of Baranya was occupied, and this area belongs to “Belje” PIK enterprise.

Every year, before harvesting, corn stalks were dissected on 50 plants of every hybrid (totally 212 variants with 122 different corn hybrids). At dissection of plant, larvae and tunnels were registered above the ear, on the ear and under the ear at every examined variant. Intensity of attack was calculated in percentage per hybrid, and number of larvae and tunnels per plant.

In 1995, the trials were carried out on the second corn crop (Styra) by biological preparation Biobit XL (*Bacillus thuringiensis* Berlinier var. kurstaki).

Climatic conditions were recorded for every year from 1992 to 1996.

RESULTS AND DISCUSSION

The European corn borer as a pest was continuously present from 1992 to 1996 in our field experiment. Figure 1 shows intensity of attack of corn borer per year, during 5 years of investigation. Average attack for those 5 years was 60,55%. The lowest attack was 1996 with 36% and the highest in 1995 with 81%.

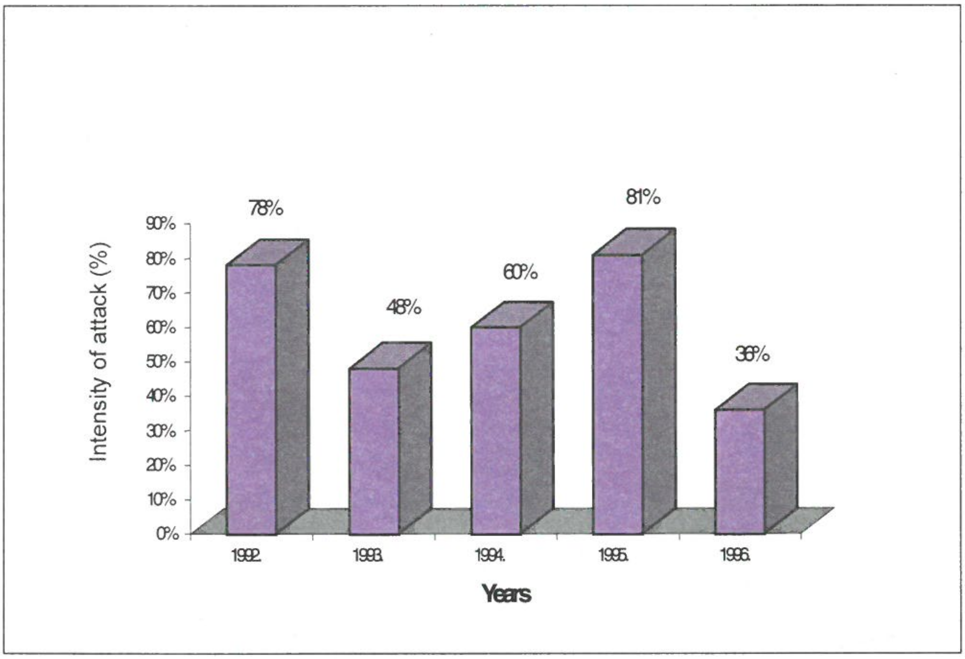

Figure 1 – Intensity of attack of ECB from 1992 - 1996

Number of larvae and tunnels per corn plant are shown in Figures 2 and 3. The highest number of larvae was in 1992 with 0,53 and in 1996 with 0,45 per plant.

Number of tunnels per plant was the highest in 1992 with 2.01 and in 1996 with 1.90. Average number of tunnels was 1,45 and 0,38 larvae per plant, during the interval 1992 -1996.

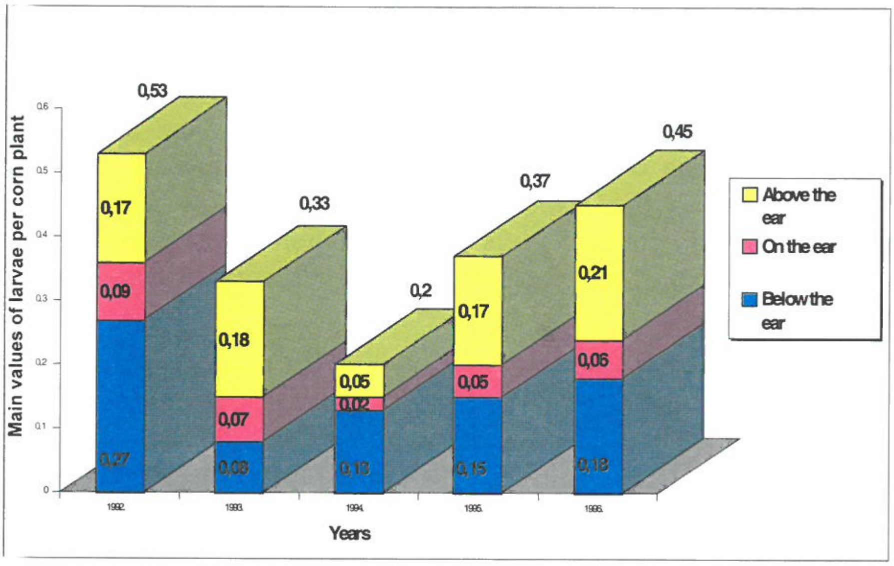

Figure 2 – Number of larvae per corn plant from 1992 - 1996

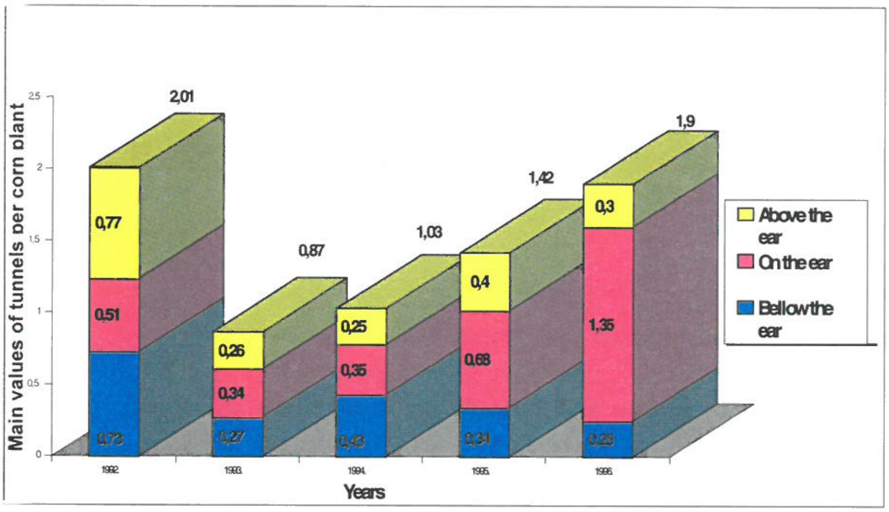

Figure 3 – Number of tunnels per corn plant from 1992 - 1996

Intensity of attack in 1996 was 36 % but the highest number of tunnels (1.35) was on the ear, so, the yields were low (Figure 4).

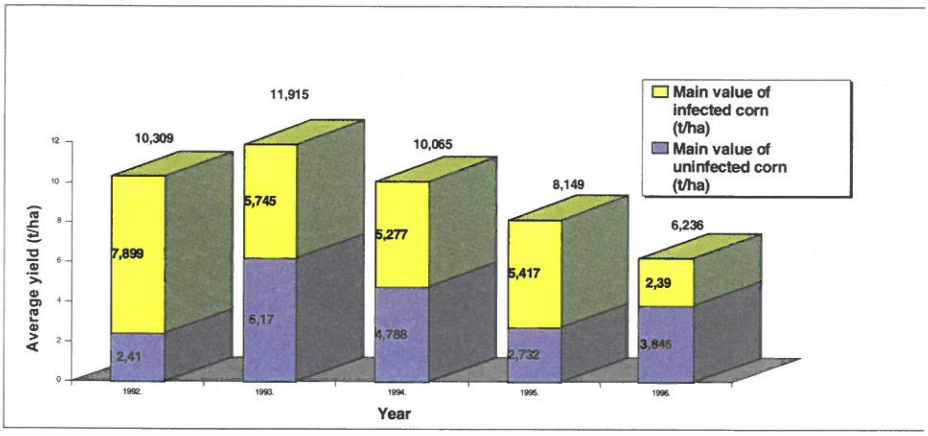

Figure 4 - Main value of corn yield from 1992 – 1996

The result shows (Figure 5) that the average attack was 34,25% (FAO group 100), 45,83% (FAO group 200), 56,25% (FAO group 300), 66,61% (FAO group 400), 65,41% (FAO group 500), 71,76% (FAO group 600), 55,57% (FAO group 700).

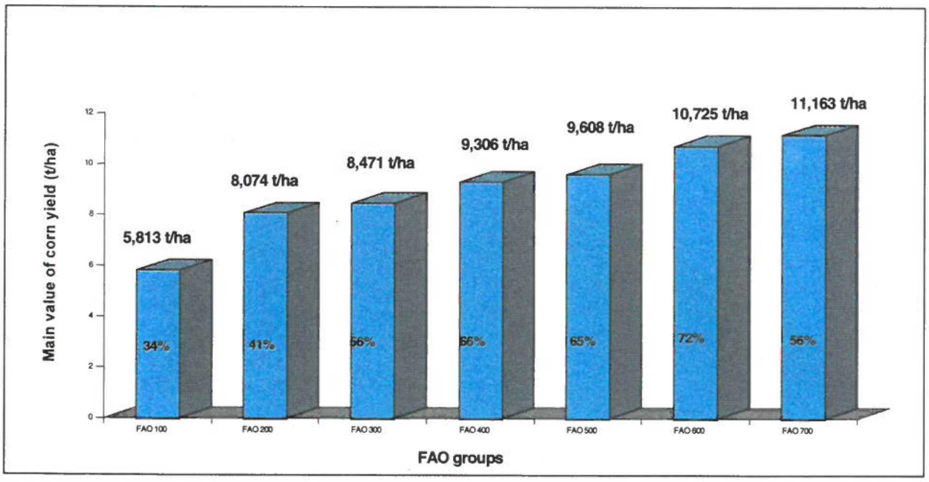

Figure 5 – Main value of corn yied per FAO groups in the period 1992-1996

Number of larvae and tunnels per FAO groups 100 to 700 is shown in Figures 6 and 7. It could be seen that the highest number of tunnels was at FAO groups 400, 500 and 600, and larvae followed this data.

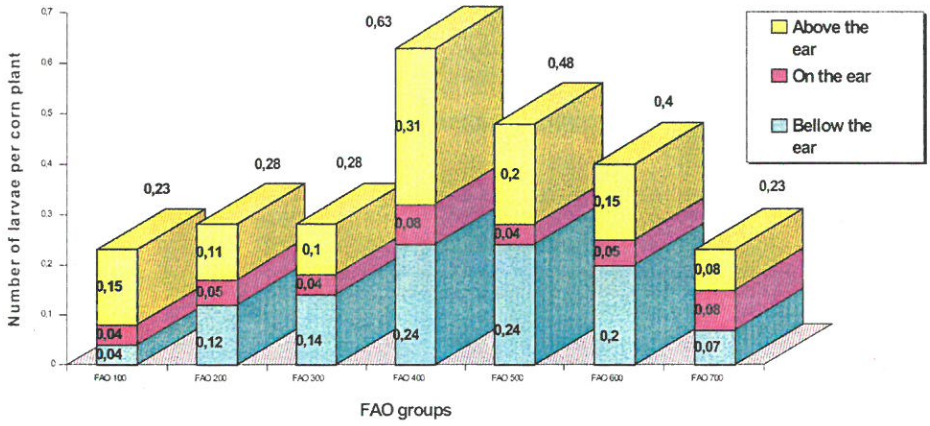

Figure 6 – Number and position of larvae per corn plant

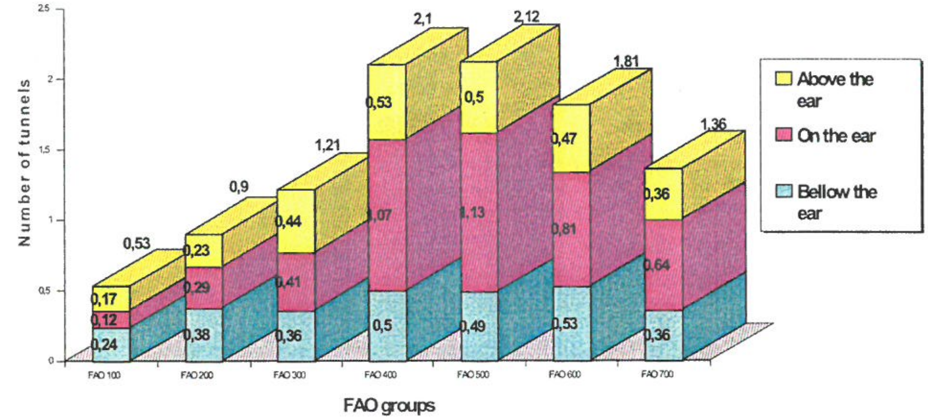

Figure 7 – Number and position of tunnels per corn plant

In 1995 ECB was controlled by Biobit XL and the pest was reduced by 59%. With the use of Biobit XL larvae was reduced by 79% and tunnels by 60% (Figure 8). According to this data the ECB shows increasing tendency on corn in Slavonia and Baranya. Control of this pest should be stressed at seed production through integrated pest management, specially with agrotechnical measures and by biological insecticides.

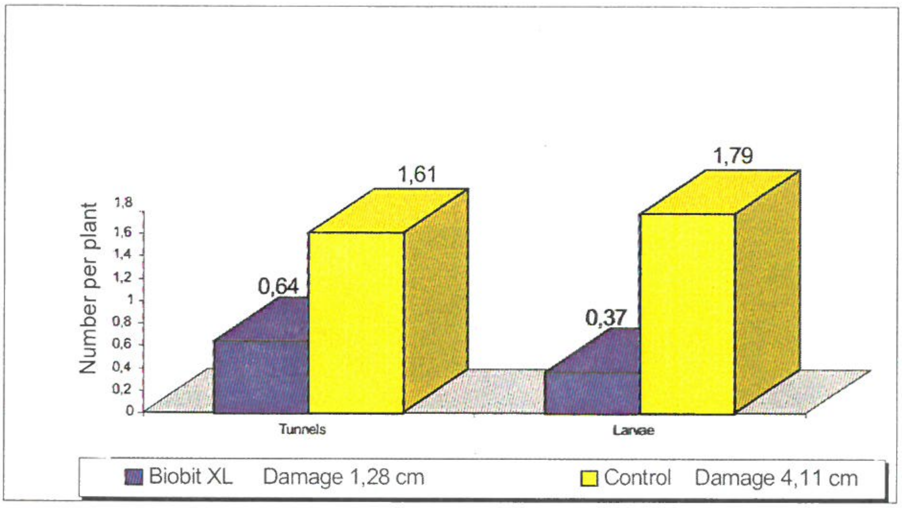

Figure 8 – Number of tunnels and larvae per corn plant (1995)

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DATA ON EMERGENCE OF THE SECOND GENERATION OF THE EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* Hb.) IN ROMANIA

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ABSTRACT

As in the southern zone neighbouring our country prevalence of occurrence and damages by the second generation of *O. nubilalis* was ascertained, it was considered necessary to assess the status of this generation in the country. Investigations have been conducted at Fundulea during 1957-1960 and 1975-1996. Thus, apparent extent of second moth generation has been evaluated by splitting maize stalks in autumn, before harvest, based on presence of pupal exuvia.

It was found that *O. nubilalis* develops a complete generation throughout this country, and a second one, partial and incomplete in the southern zones. The extent of moths emergence in the second generation recorded in general low values, reaching 30% when the insect grew in maize, but 100% in hemp.

In the South of country hemp grows at reduced extent, particularly as wild forms and does not greatly influence the total moths population; in this respect, a decisive role was played by maize, the most preferred plant by the borer. Percentage of second generation moths was not usually influenced by the way of plant infestation, natural or artificial. In the principal maize crop the percentage of the 2nd generation was higher, as maize sowing was earlier. In very sensitive maize inbred lines a trend was recorded for emergence of the 2nd generation moths to a higher percentage than in the resistant ones.

The maize principal crop planted at optimum seed-time has been attacked nearly exclusively by the first generation larvae of the borer. The principal maize crop sown late in spring was attacked both by the first generation larvae and to a higher extent by the 2nd generation larvae. Up to the present the 2nd generation larvae of *O. nubilalis* did not exhibit economic significance for the principal maize crop planted at optimum time.

Key words: Maize pests, *Ostrinia nubilalis* Hübner,

INTRODUCTION

Ostrinia nubilalis Hb. has a huge dispersion area in nearly all world zones, particularly in the Northern Hemisphere, being mentioned as pest in Europe, Western Asia, Northwest Africa and North America.

The occurrence of the European Corn Borer in a wide range of environmental conditions, from tropical to temperate zones, is explained, as

Caffrey and Worthley (1927) stated, by the fact that this insect withstands very different environmental conditions, such as from droughty steppes in the southern European part of the former USSR (average annual temperature 7°C, annual rainfall 330 mm) to those in Guam Island (average annual temperature 27°C, annual rainfall 2,500 mm). Facing such environmental conditions, the life-cycle of *Ostrinia nubilalis* shows wide variety, induced by both ecological and genetic factors.

The annual life-cycle includes an overwintering diapause, irrespective of the generations number. Though this diapause involves a genetic component (Arbuthnot, 1944; Beck and Apple, 1969; Sparks, 1965; Sparks et al., 1966; Showers et al., 1975), reaction to diapause is achieved only when larvae are submitted to certain photophases and temperatures during their development (Beck and Honek, 1960). According to Hudson et al. (1989), photophase, temperature and heredity are the main factors affecting diapause, and these interact, in order to determine the annual life-cycle of the borer. Diapause is caused by short days and low temperatures. In this manner, in northern latitudes where vegetation season is relatively short, *Ostrinia nubilalis* populations tend to be univoltine, and conversely, in zones with longer vegetation season two or more generations can evolve. It results that, due to conditions of temperature and day length, which largely differ in the whole distribution area, ECB adapted to local environmental conditions, by a process of natural selection.

It is worth mentioning that there is not a sharp separation between two neighboring zones exhibiting different life-cycles. In the transition area, e.g. with one generation and other with two generations, a variable percentage of first generation larvae enter diapause, while the other larvae pupate and moths emerge, these resulting in the second, partial generation. In such cases larvae hatching late do not always succeed to end their evolution before winter and prematurely die. The second generation is thus incomplete (Guennelon, 1972). From this standpoint, the southern part of our country can be considered as a transition zone where, besides one full generation, a second, incomplete one can occur. In the rest of the country the insect life-cycle performs only in an unique generation (Paulian et al., 1962).

Having in view recent data (Baca et al., 1993) revealing dominance in apparition and damages by the ECB second generation in Serbia, Yougoslavia, southern zone neighbouring our country, it was felt necessary to present the status of this generation in Romania.

MATERIAL AND METHODS

Trials have been performed at the Research Institute for Cereals and Industrial Plans, Fundulea, starting in 1975, when a special programme has been initiated on maize breeding for resistance to *Ostrinia nubilalis*. In view of the existence of severe attack by this pest, all tests on maize resistance to insect have been performed on artificial infestation, thus affording possibility to differentiate numerous maize forms after the attack by ECB.

In every maize line or hybrid usually 8 - 10 plants have been infested, each with 4 - 8 egg-batches, placed in leaf verticillus, some 1-2 weeks prior to panicle emergence. It is to mention that infestation took place mainly by the end of June and early half of July, thus corresponding to mass oviposition of natural ECB populations (Paulian et al., 1962).

In Autumn, before harvest, the number of full-grown larvae and pupal exuvia have been recorded by splitting stalks, thus establishing the extent of moths emergence providing the second insect generation.

RESULTS AND DISCUSSION

Occurrence of the second, partial and incomplete generation of *Ostrinia nubilalis* in our country was revealed for the first time by the author of the present report, and recorded in the paper by Paulian et al. (1962).

Studies effected on the life-cycle of this species during 1958-1960 under different ecological conditions led to establishing the number of annual generations. As it results from Table 1, in the South of this country, at Fundulea, two generations evolved, while only one in Transilvania, at Turda. More precocious moths emergence at Fundulea allowed part of larvae to finish development and pupate, thus resulting the second generation. The Table 1 shows earlier moth emergence from larvae fed on hemp plants. Paulian et al. (1962) mentioned the occurrence at Fundulea of overlapping between the flight of first and second generation. As a result of flight evolution of the 2nd moth generation, egg-batches have been found on maize plants throughout August, particularly in late crops intended for silage. During September, larvae of 2nd generation entered diapause, after some 20 days of feeding. Thus, it resulted that larvae of 2nd generation also closed their development before cold start, so that both first and second generation larvae started overwintering.

Part of larvae hatched later, do not reach maturity and die from frost.

Table 1 - Evolution of *Ostrinia nubilalis* adults during 1958-1960

Period	Moths %					
	Fundulea			Turda		
	1958	1959	1960	1958	1959	1960
Generation I						
11 - 20.05	-	0.1	-	-	-	-
21 - 31.05	0.8	0.2	-	-	-	-
1 - 10.06	0.8	0.1	1.1	-	-	-
11 - 20.06	4.8	0.7	3.0	-	-	0.6
21 - 30.06	44.0	41.0	27.9	1.9	3.0	8.1
1 - 10.07	45.3	50.0	45.1	41.4	39.0	29.7
11 - 20.07	4.0	7.0	22.9	51.8	46.5	43.2
21 - 31.07	0.2	0.1	-	4.7	11.5	15.4
1 - 10.08	-	-	-	-	-	2.9
Generation II						
21 - 31.07	0.7	0.9	63.4*	-	-	-
1 - 10.08	59.0	62.0	36.5	-	-	-
11 - 20.08	40.0	37.0	-	-	-	-
21 - 31.08	0.3	0.1	-	-	-	-
1 - 10.09	-	-	-	-	-	-

* hemp

In Table 2 rate of 2nd generation moth emergence is exposed. It can be seen that in the Southern part of this country, part of first generation larvae pupated to an extent reaching in 1957-1960 up to 30% in maize and 10% in hemp, being a possibility for evolution of the second generation of this pest.

Referring to hemp, it is to note its reduced presence, particularly of a wild form, having not significant influence on the total of moth population.

Table 2 - Pupation percentage of *Ostrinia nubilalis* - larvae, **Generation I** to give **Generation II**, during 1957 – 1960

Locality	Pupae %	
	Maize	Hemp
Fundulea	6 - 13	70 - 100
Other localities from south	1.3 - 30	-
Turda	0	-

When analysing *Ostrinia nubilalis* pupation in the first generation during the last 26 years (1975-1996), it can be noted (Table 3) that this process was detected every year, its extent varying from 0.01 to 17.62%.

A trend for increased extent of 2nd generation moth emergence was remarked in the recent years at Fundulea.

Table 3 - Pupation percentage of *O. nubilalis* - larvae, **Generation I** to give **Generation II**, during 1975 – 1996

Year	**Total**		**Moths**	**Year**	**Total**		**Moths**
	larvae and pupal exuvia	**pupal exuvia**	**%**		**larvae and pupal exuvia**	**pupal exuvia**	**%**
1975	1989	11	0.55	1987	140	9	6.42
1976	5740	1	0.01	1988	318	22	6.91
1977	1835	17	0.93	1989	3449	180	5.22
1978	2520	5	0.20	1990	2967	265	8.93
1979	5865	26	0.44	1991	599	19	3.17
1980	5833	18	0.30	1992	5364	67	1.24
1981	3337	31	0.93	1993	14811	499	3.37
1982	3138	1	0.03	1994	11577	540	4.66
1983	13064	139	1.06	1995	5235	560	10.69
1984	4270	4	0.09	1996	21484	3787	17.52
1985	2170	99	4.56		**115732**	**6303**	**5.45**
1986	27	3	11.11				

Data on the two last years, analysed according to the mode of infestation of maize plants, show that pupation and adult emergence processes in the 2nd generation performed usually to similar extents both under unique natural infestation and with additional artificial infestation (Tables 4, 5).

That means that the artificial infestation performed in the peak oviposition by the natural moth population, evolution of hatched larvae recorded for artificial infestation did not influence the pupation percentage. It results that the environment factors had decisive role in starting, at the same rate, the pupation process, though for artificial infestation egg-batches originated in an artificially grown populations under continuous flow for several successive generations have been used.

Table 4 - Pupation percentage of *O. nubilalis* - larvae, **Generation I**, depending on the kind of plant infestation, Fundulea

Year	Infestation	Total		Moths %
		larvae and pupal exuvia	pupal exuvia	
1995	artificial + natural	2652	459	17.30
	natural	1768	340	19.23
1996	artificial + natural	6387	893	14.00
	natural	1744	260	14.90

Taking into account data on pupation percentage of first generation larvae, as depending on maize seed-time (Table 5), existence of a much higher percentage of a moths is remarked for an earlier seed-time and artificial infestation, even during the peak of oviposition by the natural population. Data in this Table support the statement that pupation process takes place to a higher extent as infestation and larval evolution occur earlier.

Table 5 - Pupation percentage of *O. nubilalis* - larvae, **Generation I**, depending on seed-time and period of artificial infestation of plants, (Fundulea, 1996).

Seed time	Infestation	Date of infestation	Total		Moths %
			larvae and pupal exuvia	pupal exuvia	
5 May	artificial and natural	24.05 - 5.07	2559	557	21.76
	natural	-	692	122	17.63
27 May	artificial and natural	12 - 26.07	5016	226	4.55
	natural	-	1771	86	4.85

When examining the pupation rate of the first generation larvae in maize inbred lines with extreme values regarding reaction to ECB attack, usually a tendency exists for higher percentage pupation of larvae derived from very sensitive lines to the pest attack (Table 6) compared to the resistant ones.

Table 6 - Pupation percentage of *O. nubilalis* - larvae, **Generation I** to give **Generation II** in maize lines with extreme values of reaction to pest attack, Fundulea

Year	Resistant lines			Very sensitive lines		
	Total		Moths	Total		Moths
	Larvae and pupal exuvia	pupal exuvia	%	larvae and pupal exuvia	pupal exuvia	%
1975	229	2	0.87	332	3	0.90
1976	453	0	0	2142	0	0
1977	226	2	0.88	1013	9	0.89
1978	241	0	0	1394	1	0.07
1979	435	1	0.22	2983	29	0.97
1980	532	2	0.37	4229	5	0.12
1981	156	5	3.20	1346	38	2.82
1982	3120	1	0.03	10560	2	0.02
1983	1747	13	0.74	7762	90	1.16
1984	680	0	0	3500	12	0.34
1985	115	1	0.86	1060	32	3.02
1987	170	8	4.70	930	70	7.52
1988	30	4	13.33	1460	250	17.12
1989	354	15	4.23	2949	194	6.58
1990	279	6	2.15	1543	184	11.92
1991	160	4	2.50	424	4	0.94
1992	454	4	0.88	2890	46	1.59
1993	1160	20	1.72	11150	437	3.92
1994	1460	32	2.19	18505	480	2.59
1995	214	8	3.74	1928	218	11.30
1996	150	20	13.33	3221	478	14.84
	12365	**148**	**1.20**	**81321**	**2582**	**3.17**
Minimum value			**0**			**0**
Maximum value			**13.33**			**17.12**
Average value			**1.20**			**3.17**

Referring to the attack induced in maize by the borer, the fact is well-known that larvae in the first generation evolve exclusively on maize crop, so called "principal", usually sown in April and early-May. The earlier maize sowing, the higher frequency of attacked plants is. Data in Table 7 show that the attack by the borer was more marked in late sowing, by the end of June, this denoting that, besides infestation with first generation eggs, a higher proportion of infestation occurred through the eggs of second generation moths. Analysis of results on the attack induced in maize as principal, double or successive crop (Table 8) reveals the attack was totaly different: in the main crop the attack was performed exclusively by the larvae of the first generation, while in successive crops was caused exclusively by the second generation larvae.

Table 7 - Frequency of plants attacked by *Ostrinia nubilalis* depending on seed-time in 1991

Seed - time	Attacked plants (%)
3.05	10.6
7.06	7.9
25.06	28.0

Table 8 -Frequency of plants attacked by *Ostrinia nubilalis* depending on pest generation in 1991

Crop	Sowing date	Plants attacked (%)	
		Generation I	Generation II
Principal	10 - 20.04	31 - 70	-
Successive	10.07	-	0 - 12

These results, as well as a series of other unpublished data, outline the real occurrence of a second generation under the conditions prevailing in the South of this country. The fact that when splitting maize stalks in experiments with assessment of maize resistance to this pest, late maize forms were prevalently remarked, an insignificant presence of low-size larvae was noticed, confirming occurrence on the second generation, however, due to maize phenophase unfavourable to oviposition and development of the second generation, the maize principal crops sown at optimum seed-time usually is not attack by the larvae of this generation.

As to maize sown in late spring or in successive culture, providing favourable phonologically phases for development of ECB second generation, it is to mention that in the southern part of this country this is not an usual practice, having very reduced share. For this reason the second ECB generation, though present in the South is actually devoid of economic significance.

It results from the above facts that a second generation of *O. nubilalis* exists in the southern part of this country, however to reduced extent, without showing economic significance, unlike in Serbia, where this generation prevailed in the recent years both by emergence and damages in the main maize crop (Boca et al., 1993).

A case similar to that in the southern part of our country was recorded by Nagy et al. (1993) in Hungary, stating that, though a second flight of *O. nubilalis* moths is present, this pest does not usually develop a second, complete generation.

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STUDY OF EUROPEAN CORN BORER (*OSTRINIA NUBILALIS* Hb.) FLIGHT AND DISPERSAL, IN ROMANIA, PART OF INSECT PEST MANAGEMENT SYSTEM

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ABSTRACT

The paper presents results obtained in Romania during 1990-1996 in field trials with pheromone formulations developed for European Corn Borer (*Ostrinia nubilalis* Hb.). There is a relatively good enough formulation of Z and E sexual synthetic pheromone, this formulation being no so efficient and specific.

It is stated that in Romania Z pherotype is predominant all over the country. Pheromone traps can be used to draw up flight curves of *O. nubilalis* males. Control of ECB by mass trapping of males or male disorientation has failed in small corn field surrounded by forest.

Pheromone trap is helpfulness in a future integrated control system of pest by determination of releasing time of males with inherited sterility.

In order to develop an insect pest management, a special attention was paid to the investigations on the ECB dynamics and the estimation of the natural population by means of pheromone traps and markers. It was used synthetic sex pheromone like a monitor pest flight.

Key words: European Corn Borer (*Ostrinia nubilalis* Hb.), pheromone, flight distance, F-1 sterility, Romania

INTRODUCTION

The European corn borer is considered in Romania the most important pest of maize crop after panicle apparition, being spread throughout the cropping zones in the country. Damages caused by this pest as grain can reach sometimes up to 40% (Paulian et all., 1961). Multiannual data indicated averages of 44% plants attacked, 1.1 larvae/plant, 23,180 larvae/hectare and 550 kg./ha. yield loss or 7.5% (Paulian et all.,1976). The pest develops one generation per year, except for zones in the South, where partial second generation occurs. The population of this second generation is less than 20% of the first generation and it is without any economical importance. Its importance results only in maintaining a high level of pest populations. However, the pest also feeds on hemp and sorghum crops as well as on various species of wild flora.

Due to its outstanding economic significance for maize crop in Romania, extensive research has been effected particularly on its chemical (Voinescu and Barbulescu, 1986), biological control (Galani et all., 1979; Rosca and Barbulescu, 1983; Rosca et all., 1983, 1984) and on the development of hybrids resistant to its attack (Barbulescu, 1981; Barbulescu and Sarca, 1983; Barbulescu and Cosmin, 1987). During recent years, particular attention was paid to the study of synthetic sex pheromone (Rosca et all., 1985, 1990, 1991) and since 1988 to the investigations on male sterilization by radiation (Barbulescu and Rosca, 1993; Rosca and Barbulescu, 1989, 1990, 1993).

As a consequence of preliminary results recorded during 1982 -1987, 4 promising pheromone variants were found and from these we chose two, namely: E_5 (Z 11-14OAc + E 11-14OAc at a ratio of 97/3) and I (Z 11- Oac + E 11 - 14 Oac at a ratio of 3/97 + tetradecenyl acetate) (Rosca et al. 1991).

RESULTS AND DISCUTION

The results in Table 1 reveal the relatively high number of *O. nubilalis* males that were captured throughout the period under study, this number varying according to year and pheromone variant used. It is to stress that it was undoubtedly for the first time that in Romania both the pherotype of this species, CIS (Z) and TRANS (E) exist. The biggest number of males captured/trap [E_5 (Z 11-14OAc + E 11-14 OAc at a ratio of 97/3)], 35.25 was registered in 1994.

Table 1 - Number of *Ostrinia nubilalis* Hb. males captured / trap / year – FUNDULEA

	YEAR						
Pherotype	1990	1991	1992	1993	1994	1995	1996
E	1,75	1,25	0,75	2,75	1,5	0,5	0
Z	8,25	14,75	23,25	29,5	35,25	17,2	10,5

Specificity of pheromone formulations are not optimal, since other micro-lepidoptera like in forested areas *Tortrix viridana* L. is also frequently caught and *Etiella zinkenella* Tr. and *Emelia trabealis* Scop. in the other zones, however these species can be easily separated from the target species.

In Romania there is a network for forecast and warning for diseases and pests, and ECB is one of the pests that are supervised. The actual system of forecast and warning consists in watching, for a minimum of 3 fields in each district, the frequency of attacked plants by cutting the corn stems and, in fields in which there were more than 20,000 larvae/ha, it is considered that in the next year it will be possible to appear an attack of the pest.

Pheromone traps can be used to draw up flight curves of *O. nubilalis* males in the first generation and also in the second one in the years and localities where this appears (Figure 1).

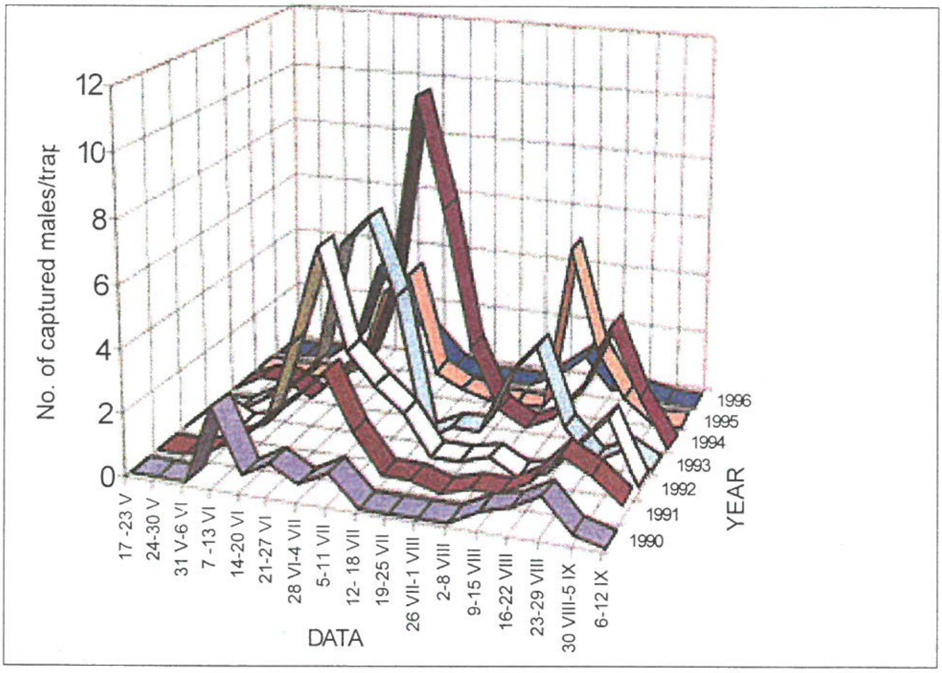

Figure 1 - Flight dynamic of species *Ostrinia nubilalis* Hb. CIS pherotype (FUNDULEA 1990-1996)

Seeking first for increased pheromone efficiency, the pheromone lures were changed every 2 weeks, because the number of captured males decreased with lure ageing.

Our results underline that the second generation generally occurs in Romania at the end of August or early in September and practically is devoid of significance, since on one hand, it is numerically low, and on the other hand, during this period the maize crop is going to maturation (Figure 2).

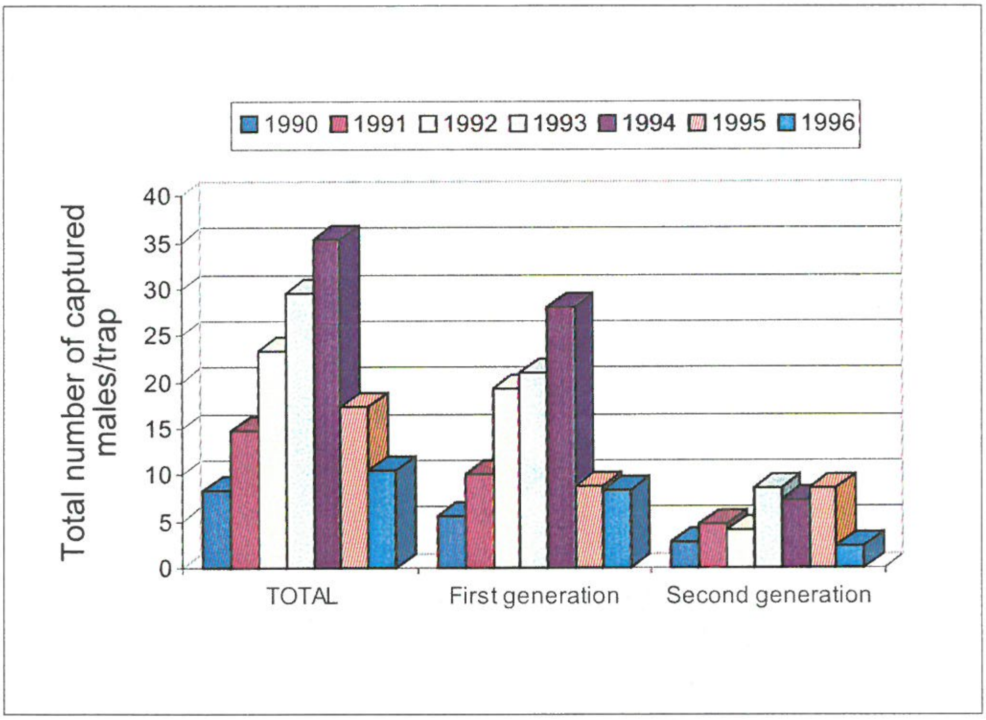

Figure 2 - Number of captured *Ostrinia nubilalis* Hb. males, depending on the pest generation

In order to find a possibility for an IPM system introducing, trials using pheromone for pest control were carried out in small areas surrounded by forest (Table 2).

Table 2 - Effect of using pheromone in small isolated fields [FUNDULEA]

	Attacked stems (%)			No. larvae/ attacked plant			Level of population (%)		
Variant	1993	1994	1995	1993	1994	1995	1993	1994	1995
Mass trapping of males (A sticky trap at 25/25 m)	28.3	28.0	12.6	1.12	3.1	0.75	+ 7.8	- 8.5	+ 45.8
Check	21.0	31.5	10.8	1.14	3.01	0.6	-	-	-
Male disorientation (A Z pheromone lure at 10/10 m)	22.5	46.5	18.3	0.8	2.9	1.3	- 43.7	- 5.6	+ 12.7
Check	26.0	42.0	21.1	1.23	3.4	1.0	-	-	-

Mass capture of ECB males was made by 16 sticky traps/ha (25/25m). The lure *CIS* (Z) was changed weekly and the total number of males captured was 231 in 1993, 325 in 1994 and 147 in 1995. It was registered an increasing of pest populations in 1993, 1995 and a slight decreasing in 1994. Mating disruption (male disorientation) was made by 100 *CIS* (Z) lure/ha which were changed weekly during the first flight of moth (15 June-30 July).

Control of ECB by mass trapping of males or even by male disorientation has failed in small corn field surrounded by forest. The control of the pest was uncertain in some years when an arising of pest population was registered. Although it seems that there was a decreasing of pest population in the field in 1993 and 1994 and a slight increasing in 1995, the price and the uncertain results turned prohibitive the continuity of our trials.

In order to develop an insect pest management, a special attention was paid to the investigations on the ECB dynamics and the estimation of the natural population by means of pheromone traps and markers. It was used synthetic sex pheromone like a monitor for pest flight.

The innate ability of insects to predispose themselves to movement on atmospheric transport systems is a successful mechanism to assure the survival of the species. It is obvious that information on the flight of ECB is highly important and that knowledge of the dispersion and flight range of any insect in all its aspects is meagre indeed and for this reason a great part of our research was directed toward to establish the distance to which a moth may fly. The distance insects will fly when distributed in Nature is very important to establish the flight range. Few things were known about the flight distance of ECB. Jermy and Nagy considered that in Nature, the adults could migrate on long distances.

Dye marking method is usually made with an emulsification mixture of Calco oil red N-1700 [150 mg/kg diet (0.015%)] and sunflower oil, mixed homogeneously in the agar of the semisynthetic diet for ECB (Barbulescu and Rosca, 1993). The larvae and moths were marked after eating the diet.

Releasing and recapture of marked moths was used in order to compare the behavior of F-1 males with normal males, released in the field and recaptured in pheromone traps situated at 25,100 and 200 m away in 1993, at 100, 200 and 300 m in 1994, at 300, 600, 900, 1200 and 1500 m in 1995 only for normal males and 1000, 2000, 3000, 4000 and 5000 m in 1996 only for normal males far from the releasing site in N, S, E and W direction. The released marked moths have to be checked individually for the red color of abdomen.

Due to the fact that the recapture in the traps of marked moths were almost at the same level [5.88% in 1993, 3.88% in 1994, 0.23% in 1995 and 0.25% in 1996 at control and 4.45% in 1993 and 3.66% in 1994 at males with inherited sterility in F-1] and the pattern of distribution of captures has shown that dispersal capacity of moths was the same (flying of moths being no more than 300 m/day during that time of experimental conditions), it seems that there is no difference between F-1 and control moths. Response of both variants were the same regarding the synthetic sexual pheromone [*CIS Z* (E-5)] and for this reason during 1995 and 1996 the experiments were made only with control marked males.

Experiments from 1995 and 1996 have shown that released tagged ECB moths have been recaptured in pheromone traps at a distance no more than 3000 m from the point of release, and probably this is the distance for which the moths are capable to fly (Figure 3).

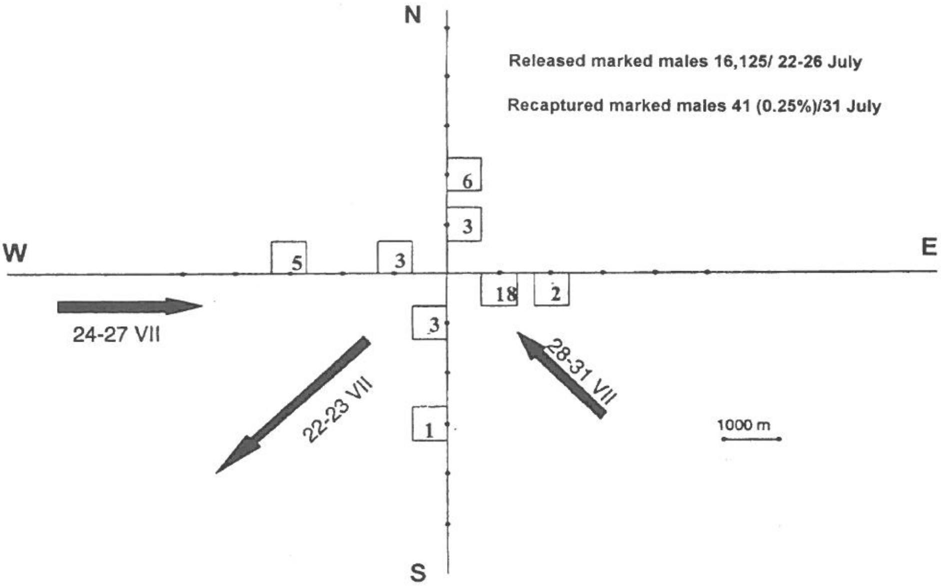

Fig. 3 **BEHAVIOUR OF Ostrinia nubilalis Hb. IN FIELD FUNDULEA - 1996**

Estimates of European corn borer populations in different Romanian's areas are difficult as we have said above. Tagging large numbers of individual moths and employing a marking/recapture method for estimating the density is a method by which a lot of troubles can be solved.

The population density is calculated using the formula of Jenkins (1962):

$$\text{Total population} = \frac{\text{Number marked released X total number recaptured}}{\text{number marked recaptured}}$$

In Table 3 we have tried to estimate the population of ECB in that particular area at one period of time, even in some cases the moment of moths flight has almost gone, like in 1995 and 1996.

Table 3 - Evaluation of *Ostrinia nubilalis* Hb. population by releasing of "Calco red dye" males marked technique

RELEASING DATA	Number of Released marked males	Number of released and recaptured males	Total of captured males	Surveyed Area (ha)	Density of Natural pest males (ha)
12 VI-1993	714	42	63	10,5	34
21 VI-1994	876	34	49	9,0	43,8
5 VIII-1995	15132	35	45	225	13,22
21 VII-1996	16125	41	42	2500	1,36

In order to find another possibility for an IPM system introducing, in the same small area surrounded by forest, during 1993-1996, there where trials using the release of F-1 sterile males for control of the pest.

Unfortunately due to missing in proper time biological material with F-1 inherited sterility, releasing of 3480 males with F-1 inherited sterility and 3610 normal males in control field in 1993 was toward the end of ECB flight in 9 July, respectively 3039 and 2580 in 21 July 1994. Even in this case during the years it was registered a decreasing of pest population (Table 4).

Table 4 - Effect of using males with inherited F-1 in small isolated fields [FUNDULEA]

	Attacked stems (%)				No. larvae/ attacked plant				Level of population (%)			
Variant	1993	1994	1995	1996	1993	1994	1995	1996	1993	1994	1995	1996
Males with inherited sterility	14.0	13.3	60.7	2.25	0.85	0.8	1.46	0.8	+ 24.0	- 67.4	- 54.0	-90
Check	13.2	21.5	80.7	18.25	1.38	1.51	2.17	2.1	-	-	-	-

Population of larvae has decreased after treatment with released males with F-1 inherited sterility in 90% in 4 years of treatment.

Judging from results presented in Table 4, it seems that using inherited sterility of ECB could be a component of an integrated control system, in connection only with releasing of *Trichogramma* spp. and microbial pesticides, because using pheromone in mass trapping or in males disorientation has no effects in experimental conditions.

CONCLUSIONS

1. Pheromone traps can be used to draw up flight curves of *O. nubilalis* males.
2. Control of ECB by mass trapping of males or male disorientation has failed in small corn fields surrounded by forest.
3. Experiments from 1995 and 1996 have shown that released tagged ECB moths have been recaptured in pheromone traps at a distance no more than 3,000 m from the point of release, and probably this is the distance for which the moths are capable to fly, this means that a male may fly no more than 300 m/day.
4. Inherited sterility could be a major component of an integrated control system and results confirm data obtained in previous years. During 4 years, in a small area encircled by forest it was possible to reduce the pest population in 90%.

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ANTIBIOSIS AND TOLERANCE OF MAIZE TO THE PINK STEM BORER (*SESAMIA NONAGRIOIDES* Lef.).

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ABSTRACT

The pink stem borer (*Sesamia nonagrioides* Lef.) is the most important pest of maize in the Northwest of Spain. Several reports about resistance to *Sesamia nonagrioides* of inbreds and populations of maize have been made since the sixties, but there are not studies about tolerance to pink stem borer attacks. The objective of this work was the evaluation of resistance and tolerance of ten inbred lines and the 10-parent diallel among these inbreds. Hybrids and inbred lines were tested in adjacent trials over two years in the Misión Biológica de Galicia (Northwest of Spain). A randomized complete block design with four replications was used for the inbred trials. A simple lattice 10 x 10 was used for evaluating the diallel crosses along with 10 check hybrids. Five plants per plot were infested at silking with a mass of about 40 eggs of *Sesamia nonagrioides* per plant.

Stem and ear damage traits were recorded at harvest. Yield of infested plants and yield of no infested plants per plot were computed to calculate the percent of yield loss caused by the pink stem borer attack. A principal component analysis for each year for stem damage traits and another for ear damage traits were made for evaluating all the stem and ear damage traits together, respectively. The first principal component for stem damage traits and for ear damage traits could be considered as a stem and ear resistance index. The regressions of yield loss on the first principal components of ear and stem damage traits, respectively, were computed for each trial.

The quadrants created by the intersections of the line for the mean of first principal component and the regression line were used to separate the genotypes into four groups: resistant and tolerant, resistant but not tolerant, tolerant but not resistant and neither resistant nor tolerant.

Inbreds A661, EP31, A509, Z77016 and F7 showed stem resistance. EP31 and PB60 exhibited tolerance to stem damage. A661 and EP31 standed out by their ear resistance. PB60 was also tolerant to ear damage. The stem resistance of hybrids was similar to the mean stem resistance of their inbred parents. However, the tolerance and ear resistance of hybrids were not, in general, predicted by the mean performance of their parents. PB60 x F7 showed ear and stem resistance and tolerance.

We conclude that the two mechanisms of defense to the pink stem borer attack, (resistance and tolerance), were found among inbred lines and hybrids. The performance of hybrids to stem attack could be predicted from the performance of the inbred parents, but hybrids should be directly tested to evaluate tolerance. The hybrid tolerance could depend on the tolerance of the inbred parents and, also, on the specific hybrid combination. A multitrait selection scheme using resistant traits and yield could improve the defence mechanisms against pink stem borer.

Key words : *Sesamia nonagrioides*, tolerance, resistance.

INTRODUCTION

The most important maize pest in North America is the European corn borer and the situation is similar in Central Europe. However, in the South of Europe the pink stem borer (*Sesamia nonagrioides* Lef.) also causes significant damage to maize. It is the main borer in the Northwest of Spain specially in locations near the coast (Cordero *et al.* 1996).

Plants have a great diversity of strategies and mechanisms of defense against pests. The mechanisms of defense may be classified into three groups, namely antixenosis, resistance and tolerance (Parlevliet 1981). Antixenosis reduces the probability of contactbetween potential consumers and plants. The non-preference type of mechanism was described in some genotypes against the European corn borer (Barry and Darrah 1978). Dicke and Guthrie (1988) proposed that late maturity corn is more attractive than early one to the moths of the second generation of European corn borer. The same results were found for the second generation of *Sesamia nonagrioides* (Malvar *et al.* 1993).

Antibiosis or resistance is the ability of the plant to reduce the growth and/or development of the larvae after contact has been initiated. The search for maize genotypes resistant to the European corn borer has been in progress for over 60 years in inbreds lines (Guthrie and Dicke 1972, Hudon and Chiang 1985, and Hudon and Chiang 1991) and populations (Reid *et al.* 1991). Sources of resistance to the pink stem borer have also been detected within populations and among inbred lines (Anglade and Bertin 1968, Malvar *et al.* 1993, Cartea *et al.* 1994, and Malvar *et al.* 1995).

Tolerance is the mechanism by which plants reduce the extent of damage per larva present. Tolerance does not reduce levels of infection in contrast with resistance or antixenosis. It is very difficult to detect the differences in tolerance among plant genotypes because it is necessary to determine the amount of yield reduction per unit of infection. This means that yield under protected conditions has to be compared with the yield of an infected crop. This is a complicated work with large experimental errors so few studies has been done about true tolerance (Inks *et al.* 1993).

There is an increasing use of tolerance rather than resistant genotypes in pest management programs. The use of resistant varieties produces selection pressure on the insects and there is some possibility of breakage of the resistance.

The tolerance effect is on the plant itself without affecting the populations of insects. The presence of the two types of defense mechanisms in plants would allow not only to reduce insect abundance on the plants, but also the recovery from the damage caused by the few surviving larvae. Kaman and Mihm (1995) found some tolerant and resistant varieties against fall armyworm, sugarcane borer and southwestern corn borer. Both types of defense mechanisms were detected against *Chilo partellus* (Kumar 1994).

The objective of this study was to estimate the antibiosis and the tolerance against *Sesamia nonagrioides* in ten inbred lines of maize and their single crosses in a diallel to evaluate their potential use for a pest breeding program.

MATERIALS AND METHODS

Ten inbreds with different degrees of resistance to stem tunneling by *Sesamia nonagrioides* (A509, A661, A637, CM105, EP28, EP31, EP42, F7, PB60 and Z77016) were selected as parents of a diallel. In 1994, the 10 inbred lines were crossed to produce a complete diallel set with reciprocals.

The 90 F_1 single crosses were evaluated along with 10 checks in a 10 x 10 simple lattice design. The experiment was carried out in 1995 and 1996 in Pontevedra, in the Northwest of Spain (42°25'N, 4°57'W 20 m above sea level). Each two-row experimental plot consisted of 15 hills with two kernels per hill. The rows were spaced 0.80 m apart and the hills were spaced 0.21 m apart. Hills were thinned to one plant after emergence, obtaining a final plant density of approximately 60 000 plants/ha.

The 10 inbred parents were evaluated in a randomized complete block design with four replications. The experimental plot was identical to the hybrid trial except that 13 kernels were planted in each row in the inbred trial. Both trials (hybrids and inbreds) were separated by border rows to limit competition but they were managed under the same conditions.

At silking, in all trials, five plants per plot were artificially infested with a mass of about 40 eggs of *Sesamia nonagrioides*. The infestation technique has been described by Anglade (1961), but the eggs were laid between the shank of the main ear and the stem instead of laying them on the third leaf below the main ear. Egg masses were obtained according to Eizaguirre's rearing method (Eizaguirre, 1989). The other plants in the plot grew under natural infestation. In 1996, in the hybrid trial one row per plot was protected with granular insecticide. In the inbred trial insecticide was not used because it could affect plant development.

At harvest time the ears were harvested and the stems of artificial infested plants in each plot were dissected. In the stem, data were recorded on number of entry holes, number and length of tunnels, and number of larvae of *Sesamia nonagrioides*. The general appearance of the ear was rated on a 5 point scale from 1 (ear without damage) to 5 (wholly damaged ear). Data on the number and length of tunnels per ear, number of larvae of *Sesamia* in the ear, the percentage of ears without damage, with damaged grain, with damaged cob, and with damage in both grain and cob were also taken.

At harvest ears from infested and uninfested plants in each plot were weighted and shelled. Grain weight was later adjusted at 14% moisture content. On the base of yield on the infested and uninfested plots, the percentage of yield loss was computed as:

$$\% \text{ Yield loss} = (1 - \text{yield of infested plot/yield of uninfested plot}) * 100$$

Hybrids and inbred lines were analyzed separately in each year. The reciprocal crosses were not considered because reciprocal effect was not important for resistant traits (unplublished data). Therefore, the mean for each F_1 was calculated and the check hybrids were also removed for the analysis. To analyze all damage traits together principal component analysis were used. The stem and ear damaged traits were analyzed separately. The first principal component is a linear combination of the original traits whose variance is the greatest of all possible combinations. Often the first principal component can be used in place of the original variables and it can be considered an index to measure the damage produced by *Sesamia nonagrioides*.

The regressions of yield loss on the first principal component for stem and ear damage traits were computed for pooled data of all genotypes on hybrid and inbred trials. Following the method of Ortega *et al.* (1980) the quadrants created by the intersections of the line for the mean of first principal component and the regression line were used to separate the genotypes into four groups: resistant and tolerant, resistant but not tolerant, tolerant but not resistant and neither resistant nor tolerant.

For each inbred line we computed the general combining ability (Falconer 1981) for first principal component of stem and ear damaged traits and for yield losses. For the same traits the correlation coefficients between general combining ability and the performance per se of the inbred lines were estimated.

RESULTS AND DISCUSSION

All stem damage traits were analyzed together using principal component analysis. An analysis was made for inbreds and hybrids for each year. The first principal component (PC1) explained 76% of total variation for 1995 and 55% in 1996 for the trials of inbreds. For hybrids the proportion of variation explained by PC1 was 63% and 70% in 1995 and 1996, respectively. The PC1 was the only component whose eigenvalue was higher than 1 for all analyses. The coefficients of PC1 were positive, therefore the PC1 could be considered as a stem index of susceptibility.

Resistant genotypes showed high negative values for this component. The coefficients of the original traits on the PC1 were about 0.50 for number of entry holes, tunnel length and number of larvae of *Sesamia nonagrioides*. The coefficient for number of tunnels was about 0.40 and it was removed in the analysis of the inbreds for 1996 because the coefficient was negative.

The inbred line CM105 was removed from the inbred analysis because it grew badly both years. The inbreds EP31, Z77016, A661, and specially A509 and F7, showed stem resistance in both years (PC1 < 0). A509 was also the most resistant inbred in a previous study (Butron *et al.* submitted). F7 and A509 showed the best general combining ability in both years (Table 2).

The correlation coefficient between inbred performance *per se* and general combining ability for stem resistance was 0.77. It means that the performance of hybrids for stem attack could be predicted from the performance of the inbred parents. This is in agreement with the idea that the additive effects are the most important for resistance to insects (Jennings *et al.* 1974, Soon-Kwon *et al* 1989, Ajala 1992).

General combining ability was more important than specific combining ability for traits of resistance to *Sesamia nonagrioides* (unpublished data). The most resistant hybrid combinations were A637 x F7 in 1995 and EP28 x F7 in 1996 (Table 1).

Table 1 -First principal component (PC1) for stem and ear damage traits and yield loss for inbreds and hybrids in 1995 and 1996.

Genotypes	1995			1996		
	PC1 Stem	PC1 Ear	Yield loss (%)	PC1 Stem	PC1 Ear	Yield loss (%)
A509	-1.73	-2.44	2.38	-1.44	0.13	26.07
A509 x A637	-1.44	-0.85	18.81	0.83	-4.63	3.02
A509 x A661	-2.14	-2.72	18.22	-2.43	-0.97	9.17
A509 x CM105	0.99	-0.71	14.90	-1.41	-2.06	21.21
A509 x EP28	-1.56	1.52	15.98	-1.40	-2.96	10.82
A509 x EP31	-1.71	-2.23	7.68	-0.71	-0.12	7.45
A509 x EP42	0.62	0.70	20.24	0.85	2.70	13.90
A509 x F7	-2.75	-2.56	19.57	-2.35	-1.16	4.17
A509 x PB60	-0.17	1.13	15.60	0.50	1.10	15.97
A509 x Z77016	-1.96	-0.07	13.30	-1.90	2.59	14.68
A637	1.50	1.12	7.83	0.12	-0.62	15.57
A637 x A661	2.95	0.95	20.20	1.78	2.01	20.40
A637 x CM105	0.31	3.03	41.70	-0.29	-2.79	13.42
A637 x EP28	-0.95	-0.02	7.42	-1.62	-1.31	13.49
A637 x EP31	-0.40	0.96	30.13	1.44	2.50	12.93
A637 x EP42	2.08	-3.19	7.42	2.93	0.34	-2.61
A637 x F7	-3.24	-0.56	15.27	-1.76	2.91	15.17
A637 x PB60	3.62	-0.42	6.98	0.68	1.95	27.12
A637 x Z77016	0.26	1.61	6.85	2.31	1.84	13.53
A661	-1.05	-2.90	12.28	-0.60	-2.87	7.86
A661 x CM105	-1.82	-4.01	11.45	0.50	-5.29	11.98
A661 x EP28	-0.73	-1.01	4.36	-0.49	-0.99	1.17
A661 x EP31	1.53	-1.12	26.57	0.05	-2.55	1.53
A661 x EP42	2.21	-0.19	29.78	4.60	1.81	25.82
A661 x F7	-0.24	-4.50	5.62	-0.90	1.05	9.40
A661 x PB60	-0.04	-0.44	19.59	-0.21	0.16	30.71
A661 x Z77016	1.38	1.46	23.88	-1.04	-0.52	10.92
CM105						
CM105 x EP28	-0.02	-0.31	14.43	-1.11	0.39	3.39
Cont.						

CM105 x EP31	-0.09	0.11	29.10	-0.20	-1.08	-11.83
CM105 x EP42	1.34	-3.27	20.51	2.33	2.40	26.57
CM105 x F7	-0.22	-2.87	7.28	-0.56	2.58	11.90
CM105 x PB60	0.25	1.16	21.12	-1.18	-0.66	25.31
CM105 x Z77016	-0.06	-1.38	25.82	-2.71	-1.37	11.16
EP28	0.69	2.42	19.46	0.17	-1.04	17.18
P28 x EP31	-0.20	3.53	23.16	0.84	-0.09	10.19
EP28 x EP42	0.80	-1.59	20.50	0.50	1.90	6.88
EP28 x F7	-1.91	0.77	4.94	-2.80	-1.78	5.19
EP28 x PB60	0.67	-0.33	7.91	-1.75	1.19	24.27
EP28 x Z77016	-1.07	1.79	17.20	-0.62	-0.00	9.82
EP31	-0.7	-2.9	11.06	0.0	-1.4	12.8
EP31 x F7	-0.50	1.65	4.36	0.93	-1.47	19.44
EP31 x PB60	1.91	1.26	23.80	2.45	-0.05	22.69
EP31 x Z77016	-0.95	1.21	12.72	2.84	1.28	19.67
EP42	3.60	2.26	28.00	2.98	-0.23	30.65
EP42 x F7	2.02	3.44	4.48	1.84	-0.85	0.16
EP42 x PB60	2.25	1.07	35.84	-0.72	0.74	12.50
EP42 x Z77016	1.63	0.18	26.07	0.37	1.06	6.19
F7	-1.77	0.67	-0.13	-1.11	3.13	24.87
F7 x PB60	-1.34	-1.46	3.57	-0.26	-1.03	-3.29
F7 x Z77016	-2.92	4.84	24.18	-1.88	1.32	8.08
PB60	0.39	0.56	-1.16	0.47	1.50	15.44
PB60 x Z77016	-0.51	2.82	37.31	-0.01	0.71	-1.59
Z77016	-0.94	1.23	21.63	-0.56	1.43	22.48
LSD (5%) for inbreds	**2.47**	**3.06**	**14.41**	**1.81**	**2.54**	**10.39**
LSD (5%) for hybrids	**2.29**	**2.89**	**13.54**	**2.36**	**2.71**	**13.04**

The hybrid A509 x F7 ranked second and third in 1995 and 1996 respectively. This hybrid could be used directly by farmers or used by breeders as source of new resistant inbreds.

Stem resistance was not correlated with ear resistance (Butron *et al.* submitted) so, the analysis were made independently. The PC1 for hybrids for ear damage traits explained 52% and 46% of total variation in 1995 and 1996, respectively. The PC1 had positive coefficients for all damage traits except percentage of ears without damage so a genotype with a high negative value for PC1 was considered to have ear resistance.

General appearance, proportion of ears with damaged grain, number of tunnels, and proportion of ears with damaged grain had the highest coefficients (about 0.40).

The lowest coefficient was 0.09 for number of larvae of *Sesamia*. The second component explained approximately 25% of the total variation but some coefficients for this component were negative and other positive, so it is difficult to explain its meaning with biological sense.

In the analysis of inbreds the variation explained by the PC1 was higher than in the analysis of hybrids (0.63% in 1995 and 0.70 in 1996). The number of larvae of *Sesamia*, percentage of ears with damaged cob and percentages of ear with damaged grain and cob were removed for principal component analysis of 1996 because their coefficients were positive and if they were included the PC1 could be not used as an index to measure the ear damage.

EP31 and A661 had ear resistance in both years. A509, that had showed resistance in a previous study (Butron *et al.* submitted) had a good performance in 1995 and bad in 1996 (Table 1).

The correlation coefficient between performance *per se* and general combining ability for PC1 was lower than the correlation coefficient for stem traits (r = 0.40). A reason for this could be that for ear traits the PC1 explained lower proportion of total variation than for stem traits. A661 showed the best general combining ability (Table 2) and the most resistant hybrids were A661 x PB60 in 1995 and A661 x F7 in 1996.

Table 2 -General combining ability for the first principal component (PC1) for stem and ear damage traits and for yield losses for inbreds in 1995 and 1996.

Genotypes	1995			1996		
	PC1 Stem	PC1 Ear	Yield loss	PC1 Stem	PC1 Ear	Yield loss
A509	-1.26	0.73	-1.54	-1.00	-0.69	-0.35
A637	0.40	0.19	-0.23	0.79	0.35	1.66
A661	0.39	-1.45	0.39	0.23	-0.66	2.24
CM105	0.08	1.03	3.71	0.53	-0.98	1.24
EP28	-0.62	0.54	-5.09	-1.06	-0.46	-2.25
EP31	0.21	0.75	2.56	1.17	-0.29	-2.16
EP42	1.88	-0.28	3.33	1.76	1.17	-1.24
F7	-1.38	-0.16	-8.58	-0.97	0.19	-4.11
PB60	0.83	0.60	1.89	-0.06	0.51	6.31
Z77016	-0.52	1.56	3.84	-0.33	0.86	-1.34
LSD (5%)	**0.49**	**0.49**	**2.04**	**0.46**	**0.36**	**1.48**

Resistance is not the only defense mechanism present in plants. Non preference and tolerance can also be considered in a breeding scheme. The study of yield loss allows to know the relative contribution of tolerance and resistance in the defense against the pest.

The relative contribution of resistance can be estimated as the part of yield loss which can be predicted from damage traits (regression of the yield loss due to corn borer attack). The deviation from the regression line reflects the tolerance component (Ortega *et al.* 1980).

The regression coefficient between yield loss and PC1 for stem traits was b =0.97 (r^2 =0.03) for hybrids and b= 2.70 (r^2 =0.18) for inbreds. The regression coefficients between yield loss and PC1 were b =1.57 (r^2 =0.09) for hybrids and b =2.11 (r^2 =0.19) for inbreds.

These regression lines mean that besides resistance components tolerance to *Sesamia nonagrioides* was also presented in plants. Kumar and Mihm (1995) also found both defence mechanisms (resistance and tolerance) operating in maize hybrids against fall armyworm, southwestern corn borer and sugarcane borer attack.

The inbreds PB60, EP31, and A661 showed stem tolerance in both years (Figure 1) while PB60 had also ear tolerance (Figure 2). The resistance of hybrids could be predicted from the performance of the inbred parents. However tolerance would not depend only on the tolerance of inbreds parents.

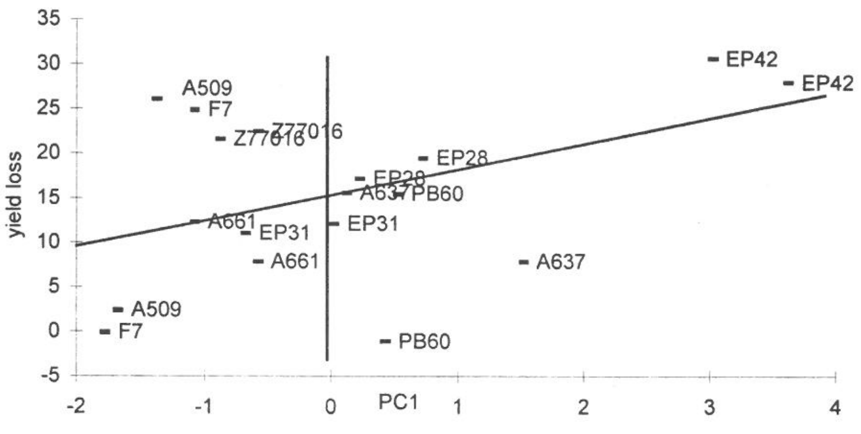

Figure 1 - Relationship between the first principal component (PC1) of stem damage traits for inbreds and percentage of yield loss.

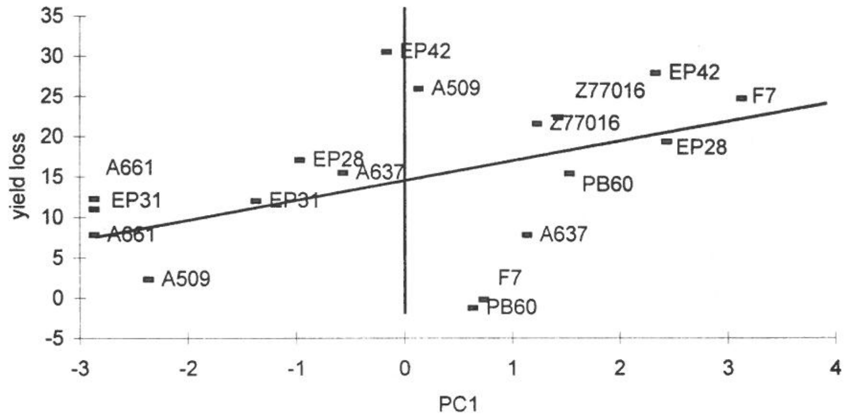

Figure 2 - Relationship between the first principal component (PC1) of ear damage traits for inbreds and percentage of yield loss.

Some genotypes had antibiosis whereas others had tolerance. Antibiosis and tolerance did not always occur in the same genotype and seem to be unrelated characteristics. Jarvis *et al.* (1991) found the same result for the attack of the second generation of the European corn borer.

Yield loss depends on tolerance and resistance of genotypes. Only a small part of the variation of yield loss can be predicted from stem or ear resistance. This indicates the need of selecting genotypes by a comprehensive measure such as yield loss (Ortega *et al.* 1980). Thome *et al.* (1994) studied the yield reduction in maize under infestation with southwestern corn borer and they also found that selecting directly for combining ability for yield across environments may be more useful than selecting directly for insect resistance without selecting for yield. Similar conclusions were given by Klenke *et al.* (1986), Jarvis *et al.* (1991) and Anglade *et al.* (1996). F7 showed the best general combining ability for yield loss and the best specific combinations were F7 x PB60 in 1995 and EP42 x F7 in 1996. The inbred line F7 could be used in breeding programs to improve the resistance to *Sesamia* and thus, to reduce yield losses.

In conclusion, resistance and tolerance are operating in maize against *Sesamia nonagrioides* Lef. A multitrait selection scheme using resistant traits and yield could improve the mechanisms of defense against pink stem borer.

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OBSERVATIONS ON OVERWINTERING LARVAL POPULATION OF THE PINK MAIZE STALK BORER *SESAMIA NONAGRIOIDES* Lef., IN SOUTHWESTERN FRANCE: SAMPLING FOR POPULATION DENSITY, INFLUENCE OF TEMPERATURE ON LARVAL MORTALITY

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ABSTRACT

A postharvest survey of the overwintering larvae of the pink maize stalk borer PMSB, *Sesamia nonagrioides* Lef., was developed during three winters (1990-1992), in a field of the Experimental Corn Unit, INRA, St Martin de Hinx, France. The aims of the study were 1) the estimation of the influence of temperatures recorded in the hibernation sites in the stalk on the mortality rates 2) the evaluation of sampling methods for low densities of poplations.

Data about borer density and locations in the stalk were collected monthly. Temperatures were continuously recorded at different levels of the stalk.

Temperatures in the upper part of the stalk were frequently observed below 0°C which appeared to be the only favourable hibernation site while negative temperatures were never recorded in the crown. Influences of duration of frosts on monthly mortality rates were detected. For the three years, larval populations were reduced to 7-15 larvae per 10 plants (94-87% mortality rate).

From the regression of mean crowding on general mean number of borers, sample number curves for fixed levels of precision were calculated. In the conditions of our experiments, the evaluation of the low levels of spring populations required a tedious and costly job.

Key words: Maize pests, *Sesamia nonagrioides,* overwintering larvae.

INTRODUCTION

The pink maize stalk borer (PMSB), *Sesamia nonagrioides* Lef., is one of the limiting factors for maize production in Southern Europe and in other Mediterranean countries. The major part of the last generation diapausing larvae overwinters in maize stubble, generally in the crown. High mortality rates are very often recorded during winter.

This aspect of the biology of *Sesamia* has been recently studied in France. Researches were initiated to assess the worth of winter cold susceptibility in explaining the apparent drastic decreases of population during severe winters. Laboratory studies (Peypelut *et al.*, 1992 ; Gillyboeuf *et al.*, 1994) have established that the supercooling points (SCP) ranged between -5° and -8° C. They also have shown that, for negative temperatures above SCP, mortality was clearly related to the intensity and the duration of the exposure to cold: for instance, 20, 45 and 100% mortality after 16 hours cold exposures at 0°, - 2° and - 4°C, respectively.

In the mean time, field experiment was conducted in view to survey the post-harvest diapausing larval population during all winter in southwestern french conditions. The work reported here was focused on three items : 1) distribution of the larvae in the stalk and crown, 2) relationships between the temperatures recorded in the stalk and the evolution of mortality rate from November to March, 3) evaluation of sampling technique for estimating population densities.

MATERIAL AND METHODS

Experimental field: Field studies on PMSB overwintering larvae were conducted at the INRA

Experimental Corn Unit, Saint Martin de Hinx, France, during three winters (1991-1993). Selected fields were cultivated with late-sown hybrids which have received high level of natural second generation. They were hand-harvested and stubble remained undisturbed until April.

Population evaluation: The evaluation of the larval population was made every 20th of each month from November to March, by dissection of samples of 10 plants and notation of the larval distribution changes in the stalk. The experimental field was divided into 6 sampling quadrats. A stratified random sampling pattern was used to locate the sample sites of 10 consecutive plants. The number of strata was empirically increased from 2-5 in November (expected high population) to 10-30 in March (expected low population). The general means for each monthly evaluation and their confidence limits at P = 0.05 were calculated.

Moreover, the degrees of crowding which represent the mean number per individual of other individuals in the same quadrat were estimated by the Lloyd's mean crowding (Kuno, 1991) as $x^* = x\ (s^2/\,x - 1)$. The linear regression of mean crowding x^* on mean x was calculated with the data collected in all of the sampling quadrats during the two first years y_1 = 1990-91 and y_2 = 1991-92 as $x^* = \alpha + \beta\, x$. Then, using the parameters α and β of the the regression, sample number (q) curves for various population densities were calculated for two levels of precision (D = 0.25 and 0.10) with the following formula (Iwao and Kuno, 1968) :

$$q = t^2 / D^2 (\alpha + 1/\ \overline{X} + 1) + \beta - 1)\ (1).$$

Finally, two precision response curves by setting either q = 20 for x = 1...10 or q = 50 for x = 0.25 ....10 (Poston *et al*, 1983) were calculated :

$$D^2 = t^2 /q\ (\alpha + 1/\ \overline{X} \pm 1)\ (2).$$

Temperature measurements : Temperature data were collected by the 21 x Micrologger (Campbell Scientific, Inc.). Sensors were arranged as follows : inside the maize stalk at 3 positions : in the crown and at 10 and 50 cm above the soil, in open air at soil surface and 1m above the soil. Each position was repeated four times in different stalks. Measurements were made every 5 minutes and hourly means were automatically calculated. In addition, temperature data under shelter and in soil were available.

RESULTS

Temperature: The differences among the three years for the fall and winter climatic conditions were characterized (Table 1) by the monthly mean soil temperatures and by the absolute daily minima recorded under shelter. Both two first years were characterized by an important decrease of soil temperature in December, moderate cold conditions in January-February and warmer temperatures in March. On the contrary, the last year was permanently milder than the others except severe late frost in March.

Table 1 - General climatic conditions at St Martin for the 3 years of observations

Periods	Absolute daily min./shelter			Mean soil temperatures		
	Y1	Y2	Y3	Y1	Y2	Y3
NOV	-	-	-	13.9	12.2	13.7
DEC	-4.2	-3.3	+0.9	7.3	7.2	10.7
JAN	-4.7	-2.9	-0.3	6.9	5.8	7.6
FEB	-5.2	-5.1	-0.5	5.2	5.9	7.6
MAR	+1.8	-0.8	-7.6	10.7	8.9	6.3

The analyses of the temperature data recorded for the different parts of the stalk including the crown have shown that the monthly mean temperatures in the crown were generally higher than in the upper parts of the stalk, mainly during frost period (Table 2). This small difference about -1°C would be explained by the different ranges in the daily maxima and minima between stalk and crown (Figure 1), the temperature remaining relatively more stable in crown.

Table 2 - Monthly mean temperatures recorded in upper stalk 50cm above the soil (ST50) in the first internode (IN1) and in the crown for two years of observations.

Periods	Year 2 (1991-92)		Year 3 (1992-93)		
	ST50	CROWN	ST50	IN1	CROWN
NOV	10.6	11.1	-	-	-
DEC	4.4	6.1	10.2	8.9	10.0
JAN	4.3	5.0	7.9	7.7	7.4
FEB	5.5	5.8	7.9	7.5	7.5
MAR	10.0	10.1	4.4	3.1	5.3
APR	10.1	10.7	-	-	-

In relation to larval mortality, it was appeared useful to consider monthly the number of frosty days, the total amount of hours below 0°C and the absolute minimum recorded respectively in stalk and crown. In fact, laboratory studies have shown the influences not only of the minimum temperature but also of the duration of cold exposure. Consequently, the data in Table 3 would be related to larval mortality in the field (see later) since negative temperatures were never recorded in the crown except for March 1993.

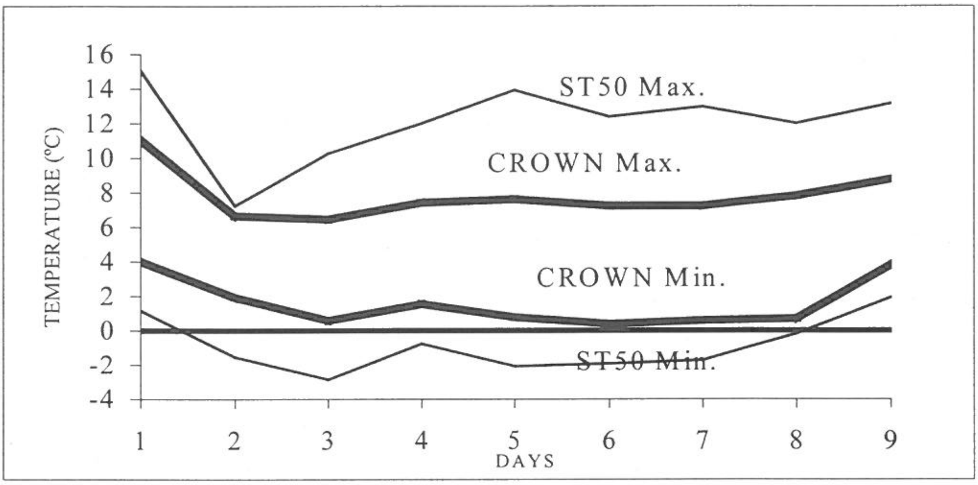

Figure 1 - Ranges of temperatures recorded in the stalk (ST 50) and in the crown during the first frosts in January 1991.

Population :

General observations: The initial overwintering larval populations were evaluated at the end of November by their means and their confidence limits at P = 0.05 as follows: 1990 = 125 (88-162) ; 1991 = 54 (41-67); 1992 = 119 (71-161) larvae per 100 plants which correspond to 71%, 44% and 72% infested plants, respectively.

The regression lines for the successive monthly evaluations expressed in decimal logs to numbers of days from November 20 (Figure 2) have shown similar slopes for the three years ($y_1 = 2.12 - 0.0093x$; $y_2 = 1.69 - 0.0075x$; $y_3 = 2.08 - 0.0072x$).

Depending to the initial levels and to differences in climate, the levels of residual populations might be evaluated at the end of March around 8-7 *l* /100 plants for the two coldest years and around 15 *l*/100 pl. for the mild year 1993, which correspond to 94%, 87% and 88% mortality rates respectively. Nevertheless highest monthly mortality rates were generally observed during the coldest periods (Table 3): in January Y_1 = 59%, Y_2 =

55% and in March for the late frosts $Y_3 = 53\%$ (Figure 2).

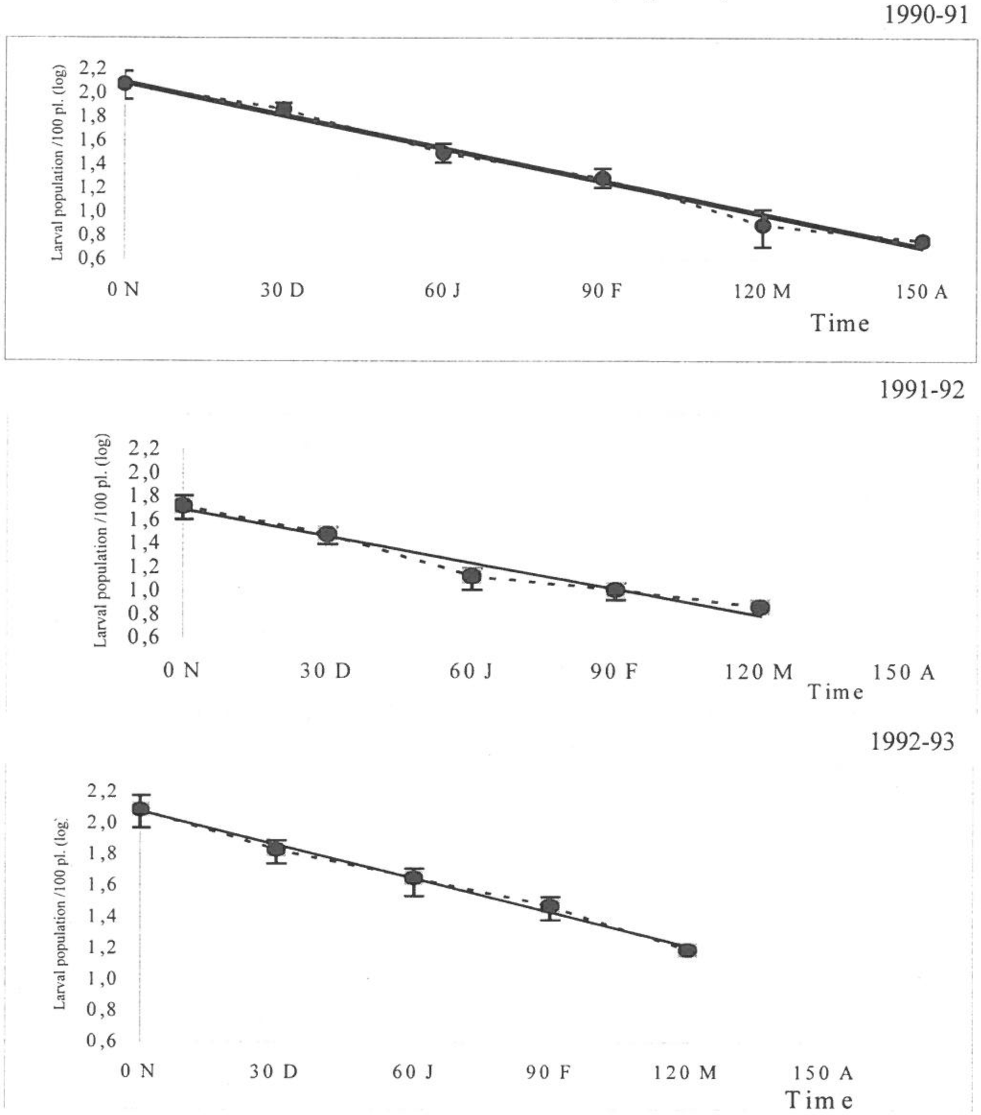

Figure 2 - Regression lines for monthly evaluations of population expessed in decimal logs with their confidence limits at P = 0.05 to number of days from 20 March. The highest monthly mortality rates are indicated with arrows.

Table 3 -Temperatures below 0°C: number of days, number of hours and absolute minimum (A.min.) for the different places : shelter and soil, localisations in the stalk : 50cm (ST50), first internode (IN1) and crown y_1 and y_2).

Year	Periods	SHELTER		SOIL	
		Days	A. min	Days	A.min
Y1	DEC	10	-4.2	0	+3.2
	JAN	7	-4.7	0	+1.6
	FEB	13	-5.2	0	+1.5

		ST50			CROWN	
		Days	Hours	A.min	Days	A.min
Y_2	DEC	6	60	-2.9	0	-0.5
	JAN	16	138	-2.9	0	-0.3
	FEB	16	116	-4.5	0	+0.2
	MAR	1	3	--1.1	0	+0.7

		ST50			IN 1			CROWN		
		Days	Hours	A.min	Days	Hours	A.min	Days	Hours	A.min
Y_3	JAN	6	46	-33	4	28	-2.5	0	-	+0.3
	FEB	5	17	-1.0	3	5	-0.3	0	-	+1.5
	MAR	9	77	-6.6	9	74	-6.3	3	17	-1.4

Location in stalk: In December and January (Y_2) the total number of larvae decreased according to their situation in the stalk (Table 4). For the upper parts of the stalk, many dead larvae were collected and the population decreased to 51 % in December and to 0 % in January Probably, these larvae died under the influence of daily variations of temperatures between 15° C to -3° C (Figure 1). For the lower part (first internode and crown) the number of larvae remained constant with a 7 % mortality rate. Similar observations were made during Y_3.

Table 4 - Total numbers of larvae recorded in the upper stalk (ST 50) and in the crown for different periods of observations (1991-1992) : actual numbers, percentages and mortality rates.

Situation	NOV No	%	DEC No	%	JAN No	%	Mortality %
ST 50	39	72	16	51	0	0	100
CROWN	15	28	15	49	14	100	7

Sampling : According to Iwao and Kuno (1968) the estimate of mean crowding, $x^* = \overline{X} + (s^2/\ \overline{X} - 1)$, was calculated for each universe for the two first years. Then, mean crowding was regressed on x (Figure 3) using the 66 universes for y_1 and y_2 : $x^* = 1.1303\ \overline{X} - 0.1487$, $r^2 = 0.938$. Sample numbers (q) curves for various population densities (from 0.5 to 10 larvae/10 plants) were calculated for two levels of precision (D = 0.25 and D = 0.10) of the true mean.

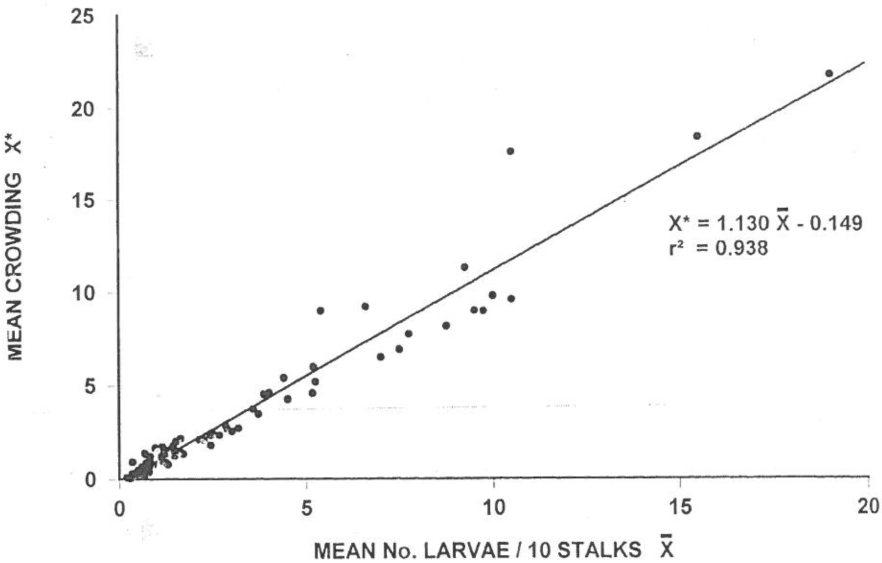

Figure 3 - Regression of PMSB mean crowding on mean density.

These curves are asymptotic to the ordinate and abscissa (Figure 4). Reciprocally, precision response curves for two numbers of samples (20 and 50) were calculated (Figure 5 et 6).

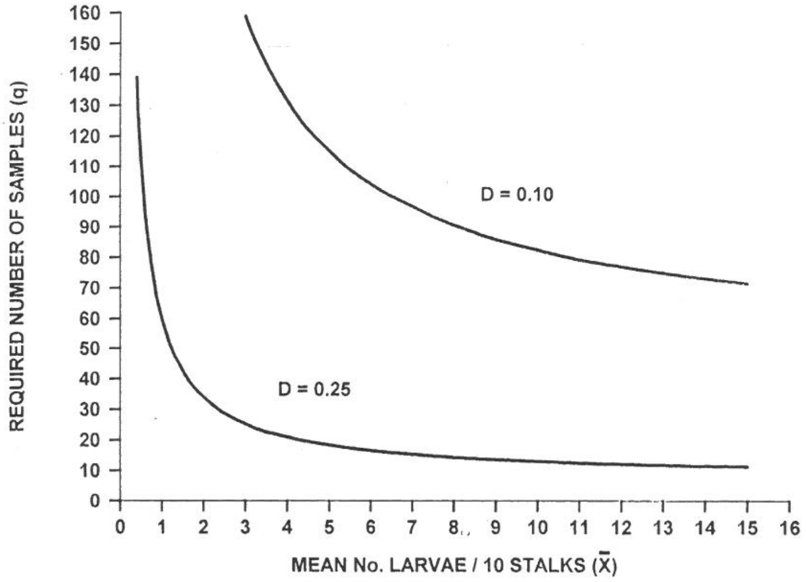

Figure 4 -Required number of 10-plant samples (q) for various PMSB densities at two precision levels

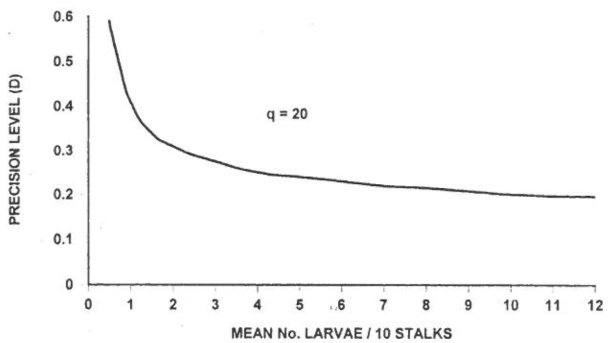

Figure 5 - Precision response curve calculated from equation (2) for q = 20

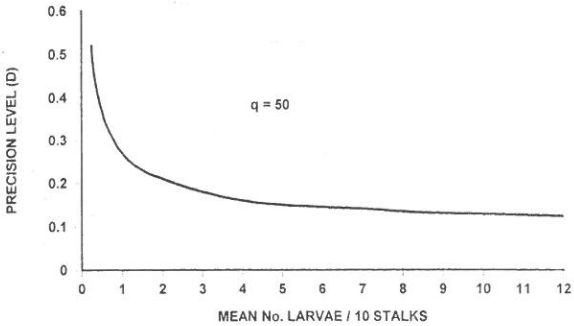

Figure 6 - Precision response curve calculated from equation (2) for q = 50

On sampling it can be seen that a sampling technique using q = 20 samples was adequate at population means of 4 or more larvae per 10 plants (D = 0.25). Using q = 50 samples, a same level of precision was obtained for population means for 1 or more larvae/10 plants.

DISCUSSION AND CONCLUSIONS

During the three winters of observations in Southwestern France, the *Sesamia diapausing* larvae experienced mild conditions alternated with short freezing periods generally in January but also at the beginning of March as in 1993. Temperatures recorded in the crown of the stalk never reached the supercooling point (SCP) measured in laboratory ranged between -5 and -8°C. Negative temperatures above the SCP were observed from time to time all along late fall and winter in the stalk from the first internode to 50 cm above soil level and only once in the crown, in March 93.

The mortality rates were fitted significantly with a linear regression on time. Nevertheless, they exhibited maxima values for months corresponding to the major durations of freezing periods. Moreover, the larvae observed in the upper stalk in October-November, which probably corresponded to the latest part of the population, disappeared, mainly in December, depending either to the action of the first frosts or to the unavailability of the hibernation sites in the crown. After January, all surviving larvae were exclusively located in this part of plant with only one larva per plant. The presence of a granulovirus in the southwestern populations recently confirmed by J.C. Veyrunes, (personal communication), might play with freezing periods a synergistic role in the mortality rate.

At the end of March, when larvae begin to pupate, the population densities were very low, around 1 insect per 10 plants. Our sampling study has shown the difficulties and the costs in estimating such a low level of population with an convenient level of precision. This low level of population may be reduced by the use of an uprooting equipment which exposes the stubble on the soil surface (Peypelut *et al*, 1996). Such levels are convenient for the application of mating disruption method by pheromone.

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THE CORN STALK BORER, *SESAMIA NONAGRIOIDES*, MODERN CONTROL CONSIDERATIONS

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ABSTRACT

The corn stalk borer, *Sesamia nonagrioides* (Lefebvre) (*Lepidoptera*, *Noctuidae*) is one of the most important pests in the Circummediterranean countries. The larvae cause extensive damage to the stalk as well as to the ears of maize. Late maize suffers higher losses than the early crop due to the high insect populations occurring later in the season.

The purpose of the present paper was to review the present knowledge and to consider information necessary for the design of a modern approach of control. This information derives from laboratory and field work that has been done on the biology, population monitoring, larval infestation-plant damage relations, chemical control, biological control agents, pheromones.

In Greece the insect has three and a partial fourth generations per year and it undergoes winter diapause in the last larval instar. In laboratory studies it was found that photophases between 6 and 14 hours, at 25^0C, induced 100% diapause. Temperatures higher than 25^0C decrease the percentage of larvae entering diapause. In the field eggs hatching in the middle of August enter diapause which terminates the following January. The first adults appear from early March to end April depending on the temperatures of the regions occurring from January to April. In warmer areas post diapause development as well as adult emergence is completed faster than that of colder areas. The population of the insect is low during the first two generations but in the third and the fourth there occurs a population explosion inflicting considerable damage to the crop. The population raise occurs usually in August. It is during this period that measures ought to be taken. Very seldom is there a high population in the first or second generation. Young plants seem to have considerable tolerance to the pest infestation which, however, is reduced later. Infestation at the 7-10 leaf stage results in considerable stalk damage and production of smaller ears weight, while infestation later, when the plants are 2.5 meters high and until flowering, damage of ears is higher.

The thermal constant has been estimated for the total development of the insect as well as the particular stages and instars of the larval stage. This data is useful for the design of a management system. Control of the insect is being done until now by chemical means usually empirically without determining exactly the right time of intervention. The implementation of the chemical control method is difficult when the plants are grown and special equipment must be used. Efficiency of control is improved by timely applications of insecticides when population monitoring of the insect is followed by pheromone traps. It is recommended that chemicals are applied about ten days after 3rd generation of the population starts rising. Experimental data has shown that two to three insecticidal applications by pyrethroids (deltamethrine) can offer adequately protection.

Activity of the parasitoid *Telenomus busseolae* Gahan occurs in Greece and it is possible that it can be proved effective against the egg stage of the borer as it has been found to play an important role in the regulation of the borer's population in the island of Evoia in Greece.

Some activity has been also recorded with the other egg parasitoids *Trichogramma evanescens* Westwood and *Telenomus n.s. near cirphivorus* Liu as well as the larval parasitoid *Lydella thompsoni* Herting. Pheromones have been successfully used for population monitoring. Their use for control by the mass trapping or the mating disruption technique have not been very effective and more work is necessary. The integration of the above techniques leads to the proposal of a control system. In early areas, sawing can be timed to avoid infestation since the emergence of adults can be estimated by the thermal summation method and taking into consideration the postdiapause development. In areas where the parasitoid *T. busseolae* could be established it might contribute to the control of the pest either by itself or in combination with the mating disruption technique. If these methods can not be applied chemicals could be used timely applied population monitoring with pheromone traps.

Key words: Maize pests, *Sesamia nonagrioides.*

INTRODUCTION

The corn stalk borer *Sesamia nonagrioides* Lef. (*Lepidoptera-Noctuidae*) has been recently reported in Greece since 1967 (Stavrakis 1967). During the last two decades it is considered to be one of the most important pests of maize in Greece as in other Mediterranean countries (Hilal 1977; Melamed-Madjar and Tam 1980; Tsitsipis 1988; Lopez *et al.* 1995). The insect damages the stalk as well as the ears. Infestation leads to lower yields quantitatively and qualitatively. The level of plant infestation in early crops varies from 2 to 97%, while the late crop is severely attacked and crop losses reach up to 80 - 100% (Gliatis 1983, Tsitsipis 1990). The economic importance of this insect has led many researchers to investigate the ecology, behaviour, and the life cycle of this insect as well as its control by chemical, biological and biotechnical methods (Tsitsipis 1988, Sans *et al.* 1993).

The present work aims at reviewing lab and field work done over a number of years (1983-1990) on aspects of the biology of the borer, such as seasonal appearance and aspects of its diapause, insect-host relationships, assessment of damage and crop loss from the insect, the effect of natural enemies and the efficiency of various control methods.

MATERIALS AND METHODS

1. Bioecology of S. *nonagrioides*

The study of the bioecology of the borer included the seasonal appearance of the insect, the developmental period in relation to temperature and aspects of the larval diapause.

The seasonal appearance of the corn stalk borer has been extensively studied for several years. Insect monitoring was made by modified International

Pheromones type plastic traps (Phytophil, Co. Greece). The exact methodology followed has been described previously (Tsitsipis 1988, 1990). The traps were baited with a pheromone blend composed of Z-11-16:Ac, Z-11-16:OH, Z-11-16:Al and 12:Ac and found at a ratio of 10:1:1:2 (Mazomenos, 1989). A dichlorvos plaquette (Vapona ®) was used as killing agent. Traps were placed in almost all maize producing areas Greece. During the first years light traps (Pennsylvania type) were used. The relationship between temperature and insect development was studied in the lab on artificial diet (Tsitsipis 1984) exposing the various stages of insect to different temperatures as well as on plants in the field (Thanopoulos and Tsitsipis 1989a). The effect of temperature also in the range of 15-35^{0}C in 2.5 ^{0}C increments was studied on egg production and survival of the borer reared on artificial diet (Thanopoulos *et al.* 1994).

To determine the type of diapause induction curve (Beck 1980) in *S. nonagrioides*, larvae (< 24 h old) were exposed to different diel photoperiods until pupation. Diapause incidence was expressed as the percentage of mature larvae failing to pupate by a certain time (age). Larvae that did not pupate by day 60 from egg hatch were considered to be in diapause. The effect of temperature on diapause induction was also studied by exposing groups of neonate larvae to various photoperiodic regimes at different temperatures.

Diapause development was investigated under field conditions. Corn plants were sowed at regular periods from May to August 1989, to have available suitable plants over an extended period. Each plant was artificially infested with 30 newly hatched larvae obtained from a lab colony. Infestations were made at 6 different times from July to September 1989. Within each treatment, the plants were cut seven days after the formation of the first pupa, as evidenced by the exit holes on the stem of the corn plants. In the last three infestation treatments, where larvae had entered diapause and were in the stalks, the harvest day was postponed until the plants became senescent. Cut plants were dissected and part of the larvae were placed in small plastic boxes with artificial diet. Part of the field-collected diapausing larvae were transferred at four different dates between November 1989 and February 1990 from a screen box, where the larvae were kept in the field, to the lab and held under either a development inducing photoperiod (16:8 h) or a diapause inducing (10:14 [L:D] h) at 25°C until pupation.

2. Insect-host plant relations

The main goal of this study was to assess of the crop losses caused by this insect. For this reason the study focused our study on the damage done by the insect to the plant in relation to infestation.

Natural crop losses caused by the stalk borer were estimated by random sampling of 100 plants per field at harvest time. Plants were dissected and stalk damage, caused by the larvae, was expressed as a percentage of the total volume of the stalk tissue. Damage evaluation in the spadices was similarly expressed as a percentage of the total seed area. Records were made of the infestation level of each plant associated with its spadix. The effect of insect infestation on the crop was studied by infesting artificially with two ready to hatch egg masses plants, at 10 different stages of development initiated when plants had 4-5 leaves and repeated at weekly intervals. At harvest time all plants were evaluated for stalk and ear infestation expressed as percentage of the total infested by the larvae. The seed weight of each ear was also considered.

3. Control methods

The effectiveness of a spraying program as well as natural parasitism of *S. nonagrioides* was studied.

An experiment was done to test a spray program with a synthetic pyrethrine against the corn stalk borer in an experimental field in Central Greece. The program included a combination of two to four insecticidal applications at three week intervals. The first spray was done 10 days after the initiation of the abrupt population increase in the first part of August and followed at 3week intervals. The following treatments were applied: two early, two intermediate, two late, three early, three late and four sprays. At harvest ten plants from each treatment were examined and the percent stalk and ear infestation as well as the dry ear seed weight were recorded.

Surveys made on the corn producing areas in Greece revealed a complex of parasitoids attacking the corn stalk borer. Three egg parasitoids *Telenomus busseolae*, *Telenomus n.s. near cirphivorus* (*Scelionidae)* and *Trichogramma evanescens* (*Trichogrammatidae*), were recorded in many areas. Also a larval parasitoid, *Lydella thompsoni* (*Tachinidae*) was found in maize agroecosystems. To study the effect of the three egg parasitoids extensive sampling was made in the area of Istiaia in Central Greece. Egg masses were collected and transferred in the lab. The pattern of parasitism was studied and % parasitism of egg masses were counted. Parasitoids were reared in the lab and their biology was studied. Duration of life cycle, egg potential and adult survival were investigated.

RESULTS AND DISCUSSION

1. Bioecology of *S. nonagrioides*

By adults monitoring of the corn stalk borer for many years in the major corn producing areas in Greece it was found that adults start appearing from the end of February to beginning of May. Early appearance occurs in warm areas and late in colder ones. Three generations are completed and there is a partial fourth. It was found that the seasonal appearance and the population density follow a more or less similar pattern, that is during the first two generations population remains low but it reaches very high numbers after mid August (Gliatis 1983, Tsitsipis *et al.* 1984, Tsitsipis et al. 1985, Tsitsipis and Alexandri 1989). The above is given in Figure 1.

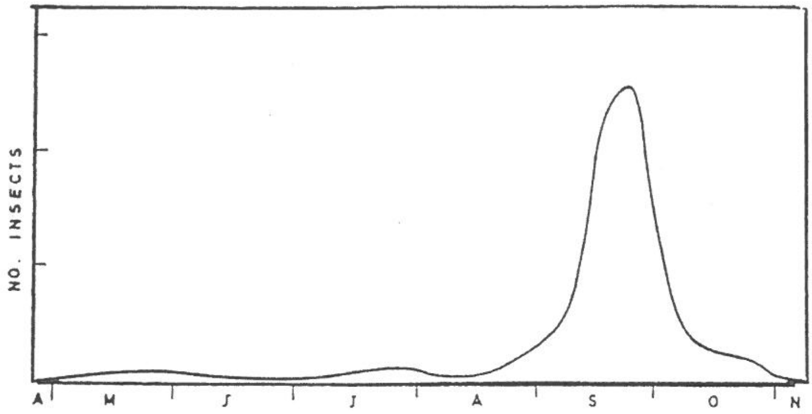

Figure 1 -Generalised curve of seasonal appearance of *S nonagrioides* adults caught in light traps in South, Central and North Greece during the years 1982-1985.

The developmental period expressed in day-degrees DD for eggs was found 102 DD while for larvae, reared on artificial diet, and pupae was 502 and 168 DD respectively.

Developmental zero for the three consecutive stages was 10.4 , 10.2 and 10.6 °C.

Larval and pupal development of the insects reared on plants was similar or longer than the development of insects reared on artificial diet, plants before flowering providing longer development than those after flowering (c.f. 735 with 667 DD) (Thanopoulos and Tsitsipis 1989a, 1989b).

Egg production, 130 eggs/female, was not differed from 15 to 17.5 ^{0}C while it increased to 27.5 ^{0}C (176-200 eggs/female) and after that it decreased to 32.5 ^{0}C (86 eggs/female). Survival decrease gradually from 15 (15^{0}C) to 3 days (35 ^{0}C).

Pupal weight was correlated almost linearly to egg production (Thanopoulos *et al.* 1994). Survival decrease gradually from 15 (15^{0}C) to 3 days (35^{0}C). Pupal weight was correlated almost linearly to egg production (Thanopoulos *et al.* 1994).

Larvae of *S. nonagrioides* exhibit a diapause of Type III (Beck 1980) which is under photoperiodic control. A diapause incidence of >90% was observed among larvae reared under a diel photoperiod of 8–10 h photophase (Fantinou *et al.* 1995) (Figure 2).

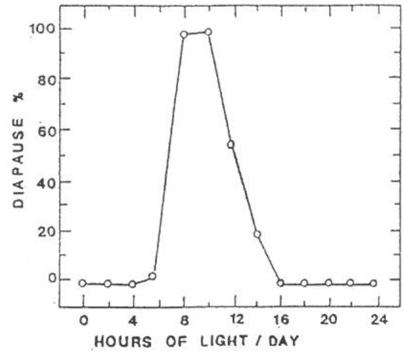

Figure 2 -Incidence of diapause induction among larvae of *S. nonagrioides* exposed to different diel photoperiods at 25° C (Fantinou *et al.* 1995).

There is also an interaction between photoperiod and temperature on diapause induction (Table 1). Diapause in *S. nonagrioides* was prevented by high temperatures at all photoperiods tested, whereas, low temperatures enhanced the inductive effects of daylengths at 6 and 14 h/d (Fantinou *et al.* 1995).

Table 1 -Diapause incidence (%) in S. *nonagrioides* larvae reared under various combinations of photoperiod (Light: Dark) and temperature

L:D	**17.5o**	**20o**	**25o**	**27.5o**	**30o**
6:18	97.3	92.8	3.0	0.0	0.0
10:14	96.7	96.8	100.0	26.9	4.5
14:10	96.5	58.3	49.0	0.0	0.0
16:8	4.3	1.2	0.0	0.0	0.0

Fantinou *et al.* (1995)

Results regarding the response of field-collected diapausing larvae to short- or long-day photoperiods are shown in Table 2.

Table 2 - Days needed for the pupation of field diapausing larvae of S. nonagrioides, collected from plants infested by newly hatched laboratory larvae at different dates, after their transfer to laboratory conditions (at 250 C and 16:8 or 10:14 [L:D] h).

Photoperiod (L:D) h	**Date of infestation**	**Date of larval transfer**							
		4 Nov. 1989		**15 Dec. 1989**		**25 Jan. 1990**		**25 Feb. 1990**	
		50%	100%	50%	100%	50%	100%	50%	100%
16:8	17 Aug.	10.0	15.5 (37)	-	-	6.0	8.5 (40)	4.0	5.5 (30)
	31 Aug.	-	-	-	-	7.0	9.5 (20)	4.0	5.5 (15)
	14 Sept.	-	-	-	-	7.0	10.0 (35)	4.0	5.0 (25)
	28 Sept.	-	-	11.0	15.5 (40)	6.0	10.0 (15)	4.0	5.0 (15)
10:14	17 Aug.	18.0	44.5 (37)	-	-	7.0	9.0 (40)	4.0	6.5 (39)
	31 Aug.	-	-	-	-	7.0	10.0 (20)	5.0	6.5 (20)
	14 Sept.	-	-	-	-	7.0	14.5 (35)	4.0	6.5 (35)
	28 Sept.	-	-	24.0	34.5 (40)	6.0	15.0 (15)	5.0	7.0 (20)

Numbers in parentheses indicate the number of transferred larvae. (Fantinou *et al.* 1977).

After transfer to 25°C, larvae proceeded immediately to pupation under both photoperiodic regimes but needed longer to complete pupation under 10:14 (L:D) h for samples collected until the middle of December. Therefore it seems that diapause did not end by the middle of December in the field population of *S. nonagrioides* as indicated by the differential responses of larvae to short- and long–day photoperiod (Fantinou *et al.* 1997).

After the 25th of January, diapause development ended as indicated by the same mean time of pupation after transfer of larvae to short- or long–day photoperiods. Despite the fact that diapause development ended early in the winter the 1st pupation record in nature occurred in mid-March (Fantinou *et al.* 1997) (Figure 3). This means that this species probably exhibited a postdiapause condition that protects the borers from unexpected temperatures of late winter and appears to synchronise the spring emergence of adults as suggested by Tauber *et al.* (1986) for other species.

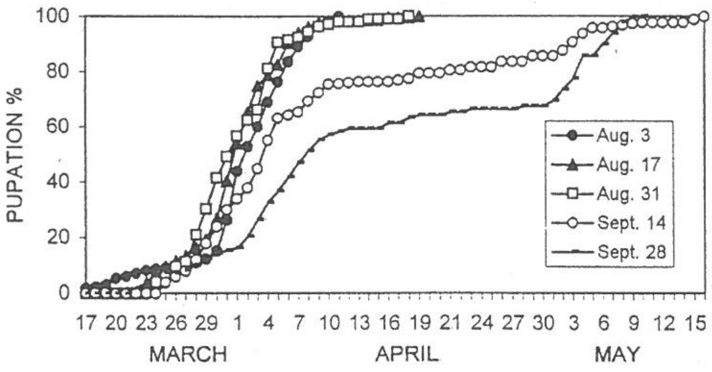

Figure 3 - Pupation curves of field diapause of *S. nonagrioides* collected from corn plants infested by newly hatched laboratory larvae at different days (Fantinou *et al.* 1997).

Temperatures for January, February, and March were probably quite low, around the larval developmental threshold (lower larval developmental threshold 10.85°C, Thanopoulos and Tsitsipis 1989b) and therefore the necessary summation of day-degrees (thermal units) needed for initiation of pupation has yet to be reached. This hypothesis is supported by the fact that adult emergence in Greece starts from February to March in warm areas (e.g., the area of Varda in Peloponnese) to April to May in more northern and colder areas (Kopais, Larissa in central Greece) (Tsitsipis, unpublished data). Hilal (1978) stated that the termination of diapause of this species is placed in SW France at mid-December. Results from other countries also confirm that the termination of diapause in this species is placed in mid January (Galichet 1982, Lopez *et al.* 1995). Galichet (1982) also stated that the same species spent the unfavourable period in a state of oligopause, according to definition by Mueller (1970), rather than in a true diapause, whereas the refractory phase was of short duration.

2. Insect-host plant relations

Evaluation of the impact of the corn stalk borer on the crop corn is shown in Table 3.

Table 3 -Infestation of stalks and ears of corn by *S. nonagrioides* in 1984. Sample size: 100 plants

District	Infestation %		Infestation intensity (%, LSD)					
	Stalks	Ears	Stalks			Ears		
Ilia	97	29	27.3	±	21.0	15.0	±	24.0
Larissa	43	9	12.6	±	14.2	9.0	±	16.0
Voiotia	37	5	9.5	±	14.0	3.7	±	4.7
Evros	2	0	1.5					
Phthiotis	65	25	15.5	±	16.7	7.2	±	8.3
Phthiotis+	49	50	9.1	±	12.2	14.1	±	18.3

\+ Late crop (Tsitsipis *et al.* 1985)

The infestation levels on the plant stalks varied considerably. Levels ranged from 2% in the Evros area, where there is practically no corn borer activity, to 97% in the Ilia area in the Peloponnese. The respective values for the spadices were 0% in Evros and 50% in the late crop in Phthiotis. The infestation intensity on the infested stalks ranged from 1.5 to 27.3% for the Evros and Ilia area respectively while the values for the spadices were 3.7% for Voiotia, 15% for Ilia and 14.1% for the late crop in Phthiotis. It is evident from these data that damage can be serious in the late crop. In the late sown field in Voiotia, where no measures against the corn borer were taken, a complete loss of the crop was recorded. Heavily infested plants had collapsed to the soil surface and all seeds were devoured by the larvae(Tsitsipis unpublished data).

The effect of infestation intensity of the stalks on the yield obtained is shown in Table 4. These data give an indication of the relationship between the two parameters measured. Stalk infestation at levels above 25% gave yields significantly lower than at levels ranging from 0-10%. It was of interest to see whether there is any adverse effect of the infestation level of corn, stalks to the quality of seeds produced by unifested ears.

Table 4 - Relationship between stalk infestation intensity of maize by *S. nonagrioides* and seed production. Sampling in Larissa 1984

Stalk infestation intensity %	Ears (No)	Mean ear seed weight (g)	Comparison	Student´s t values
0-10	78	195.6±71.5	1.2	2.03*
11-25	13	163.4±84.5	1.3	3.52*
>25	7	112.2±54.7	2.3	1.45

* p< 0,05 (Tsitsipis *et al.* 1985)

While there were no differences found in the lipid and ash content in seeds deriving from plants with very high or low infestation levels, there were statistical differences in the protein and the free nitrogen extractable matter between low level (28-40%) and high level (68-72%) stalk infestation in late sown corn in Voiotia. The respective values were for protein 10.68±0.4 and 12.49±0.7 and for free-nitrogen extractable matter 82.4±0.3 and 80.9±0.6 (per cent values of dry weight). It seems that high infestation levels affect protein metabolism of the plant (Tsitsipis *et al.* 1985). Seeds coming from heavily infested ears by the corn stalk borer (>70%) were tested for aflatoxin B_1. The test proved positive and the toxin was found at a concentration of 25±2 ppb, well below the tolerance level for animal feeds (50ppb). It showed nevertheless that the respective fungi producing the toxin were present and could produce it at certain levels (Tsitsipis *et al.* 1985). The host plant relationships showed that the plant growth stage shortly before and at flowering are more sensitive for yield reduction due to insect infestation than before or after those stages (Table 5).

Table 5 -Relationship between growth stage and S. nonagrioides field infestation. A completely randomised block design in 27 blocks. Treatments concerned artificially infested plants by ready to hatch eggs at weekly intervals. First infestation was made on June 4, 1987

Plant growth stage	% Infestation Stalks Ears	Plants with ear	Dry seed weight per ear (gr)	
4-5 leaves	42.6	0	8	82.6
5-7 leaves	25.9	0	17	68.6
7-9 leaves	37.2	4.5	12	66.8
9-10 leaves	55.0	0	2	50.0
1.7 m high	17.5	3.6	14	79.3
2.0 m high	33.5	13.0	11	82.5
2.5 m high	17.5	40.0	19	87.5
Flowering	28.8	32.8	16	65.4
Milk stage	18.2	16.0	19	80.8
End of milk	22.5	36.0	16	95.8
Control	4.5	0	19	95.0

Very young plants suffer high stalk infestation and a small part of them bear ears. The ear infestation was nil or very low until the 1.70 m high growth stage. Their yield was however adversely affected by early infestation indicating an intervention in the plant physiology. The infestation of ears was very high when it occurred one week before flowering and during flowering. During the latter stage, the yield is considerably lowered. Infestation at milk stage and later does not influence yields. For a control program the vulnerability to heavy infestation of the late crop should be considered (Tsitsipis 1990).

3. Control methods

The results of the protection of corn by the spray program is shown in Table 6. Two late applications resulted in high ear infestation and low yield. Then, it followed the treatment with two early sprays. Two intermediate ones provided good protection and so did three or four sprays. The data show that two to three applications can secure good degree of crop protection. The two sprays should be done however early to protect from heavy insect population loads (Tsitsipis 1990).

Table 6 - A spray program on late sown corn, variety "ARIS", with deltamethrine against *S. nonagrioides*. The program included 2-4 applications beginning August 20 and repeated every 21 days until October 22. Experiment was done in a completely randomised block design in 4 blocks.

No. of applications		Infestation % Stalks	Dry seed weight Ears	per ear (g)
1+2*	(2)	23.7	16.4	176.6
2+3	(2)	8.2	10.9	183.2
3+4	(2)	29.4	56.3	122.9
1+2+3	(3)	6.1	14.1	188.5
2+3+4	(3)	7.0	16.8	176.8
1+2+3+4	(4)	1.8	15.3	185.4
CONTROL	(0)	79.4	68.7	103.2

*Numbers denote spray sequence chronologically: 1=spray done on 20-8-87, 2=on 0-9-87, 3=on 6-10-87. Numbers in parentheses show the number of applications (Tsitsipis 1990)

Sampling of egg masses of the corn stalk borer at Istiaea showed that the most abundant egg parasitoid was *T. busseolae* attacking from 27.6 to 76.2% of the egg masses in three consecutive years (Table 7). The other two parasitoids were of minor importance. The two *Scelionids* have a longer developmental period in the host eggs which is 18.6 and 17 days for *T. busseolae* and *T. n.s near cirphivorus* respectively (Table 8). Egg production of the two Scelionids equally reached the 100 eggs. Adult females of the two species lived for 20-25 days. Among these parasitoids *T.*

busseolae was considered the most promising for a biocontrol strategy for its high specialised host preference (Alexandri and Tsitsipis 1990). The effectiveness of this parasitoid has been also established in Italy on *S. nonagrioides* (Giacometi and Bin 1994) and in Benin (Setamou and Schulthess 1995) and in Nigeria (Bosque-Perez *et al.* 1994) on *S. calamistis*.

Table 7 - Parasitism of eggs of *S. nonagrioides* by egg parasitoids in the area of Istiaea

Year	No. of egg masses	Parasitism %					
		T. *busseolae*	T. n.s. near *cirphivorus*	T. *evanencens*			
		masses	eggs	masses	eggs	masses	eggs
1986	936	76.2	42.8	-	-	2.0	0.4
1987	2446	27.6	12.9	2.0	0.8	2.4	0.4
1988		69.2	29.6	-	-	5.2	0.6

Table 8 - Development of *T. busseolae*, T. n.s. near *cirphivorus* and *Trichogramma evanencens*.

Parasitoid Species	Immature stage development (d)	Egg production	Adult survival (d)	
			Males	Females
T. *busseolae*	18.6	100	18	25
T. n.s. *near cirphivorus*	17.0	105	20	20
T. *evanencens* *	10.0	31-68	17-25	

* Data from Russo & Voegele 1982, Bourarach & Hawlitzky 1989

CONCLUSION AND FUTURE RESEARCH

Studies of the insect population management require knowledge on the basic life-history parameters such as developmental rates, survival, longevity, fecundity and diapause. Knowledge and understanding of the relationship between such parameters and temperature can be useful in the construction of life-tables and developmental models, and assist in a successful design of pest management strategies and provide a basis for further studies.

According to the data described in the present study a schematic representation of the population fluctuation of *S. nonagrioides* in relation to the existence of maize or alternative hosts is given in Figure 4.

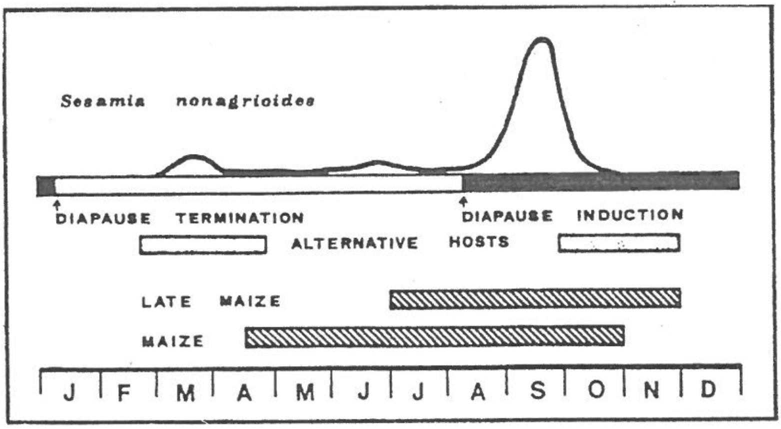

Figure 4 -Schematic representation of the population fluctuation of *S. nonagrioides*, the period marked between diapause induction and termination and host availability (Tsitsipis 1986).

These data could be used to synthesise a successful control program for the management of this serious pest. Therefore, the following general points should be considered:

Reduction of the spring populations.

For this purpose cultural methods, such as shredding and ploughing under of the crop remnants after the harvest to kill off part of the overwintering population that would provide the spring generation, could be used. Burning could be used but it is less efficient, as part of population survives it.

Avoidance of the early borer attack (warm areas)

The first adult occurrence can be predicted by the use of thermal summation model and verified by trapping. Depending on the severity of the winter, the first adult emergence occurs, in mild areas, in the end of February and in colder ones up to early May. In mild winters early appearance of adults comes before corn is present and the fate of the short living adults is doomed. Following the winter temperatures it would be possible on many occasions, to predict adult emergence (thermal constant, lower temperature threshold) and time sowing accordingly to avoid coincidence of adults with presence of maize. In very cold winters (temperatures below 6-8 °C), a large part of the population could be killed and therefore the early crop does not encounter any serious pest problems.

Introduction and /or stimulation of the activity of natural enemies

Two important biological control agents could prove to be of importance. The egg parasitoid *T. busseolae* can provide a good density dependant factor for population regulation of *S. nonagrioides*. High parasitism rates have been recorded (Alexandri and Tsitsipis 1990). The larval parasitoid *L. thompsoni* has been also found in many areas of Greece. A survey recorded *lepidopterous* species of the family Noctuidae, *Archanara geminipuncta, A. dissolute, A. spargani and Nonagria typhae*, obligatory intermediate hosts of the parasite, attacking hydrobiotope plants such as , *Phragmites communis, Typha* sp., *Sparganium* sp., found in many maize agroecosystems (Tsitsipis *et al.* 1991). It is possible to increase parasitism by managing the alternative host plants (usually weeds) properly.

Timely application of pesticides

If other methods fail and *S. nonagrioides* population is high insecticides could be used at the end of the crop period after mid-August. New insecticides such as growth regulators, chitin synthesis inhibitors, often compatible with parasitoids, could be tested for sufficient borer control.

Investigation of the use of pheromones for control

The role of pheromone could also be extended beyond the population monitoring, possible for direct control purposes by the mass trapping technique (Mazomenos and Vardas 1988) or probably even better by the sexual confusion technique. The use of these technics needs further study to improve efficiency (Sans *et al.* 1993).

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FIELD EVALUATION OF POPULATION DEVELOPMENT OF *SESAMIA NONAGRIOIDES* Lef. AND *OSTRINIA NUBILALIS* Hüb., AT ALENTEJO (PORTUGAL)

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ABSTRACT

Field studies were conducted in 1992 and 1993, at Alentejo, in order to study the biology of *Sesamia nonagrioides* (Lef.) and *Ostrinia nubilalis* Hüb., and its behaviour in maize. This study included the installation of pheromone traps and a weekly scheme of plant sampling. It was confirmed the existence of a complete generation and another incomplete for *O. nubilalis*, and two complete generations and another incomplete for *S. nonagrioides*. Based on plant dissections, it was verified that both species remained as larva during the winter, inside the crop stalks, and returned to normal development in the following spring. It was also verified the predominance, in population terms, of *S. nonagrioides* during most of the crop cycle.

Key words: Maize pests, *Ostrinia nubilalis, Sesamia nonagrioides,* generations pattern.

OBJECTIVES

The objectives of this work were, at first, the study of epidemiology of the corn borers *O. nubilalis* and *S. nonagrioides*, at Alentejo, and its behaviour in relation with the crop and the region. It was also intended the identification of which phenological stage (s) of maize was (were) more attractive to the laying.

MATERIAL AND METHODS

To obtain those objectives, four pheromone traps were installed during 1992 and 1993, from sowing until harvest. In 1993, a dissection scheme was decided upon where 20 maize plants, registered with predominant phenological stage, were dissected on a weekly basis, began during the phenological state two-three sheaths, and ended at harvest, totalling 23 weeks. In each plant was registered the number of egg plaques, larvae and pupae of each species, and also the number of exit holes.

The methodology used was described by Pereira (1994).

RESULTS

Males of *O. nubilalis* captured in traps indicated the existence of a complete generation and another incomplete; for *S. nonagrioides* it was verified the existence of two complete generations and another incomplete, each year. These results are in accordance with other studies carried out previously (Arias & Alvez, 1975; Gonçalves, 1980; Figueiredo & Araújo, 1990). Graphics in Figures 1 and 2 represent, respectively for *O. nubilalis and S. nonagrioides*, the results obtained with pheromone sexual traps.

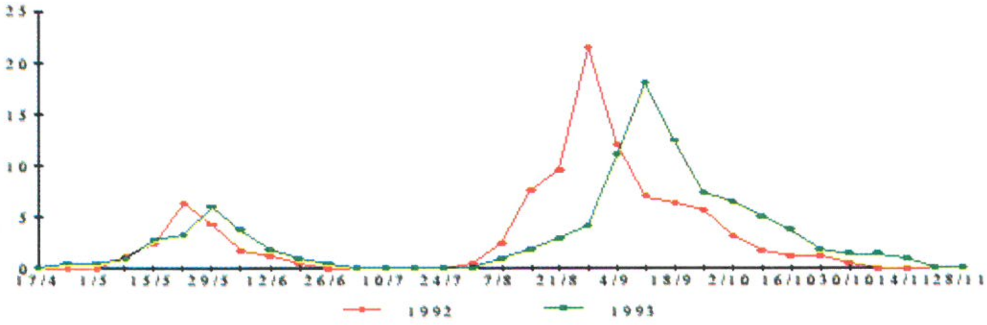

Figure 1 -Percentage of total males of *Ostrinia nubilalis* captured in pheromone sexual traps.

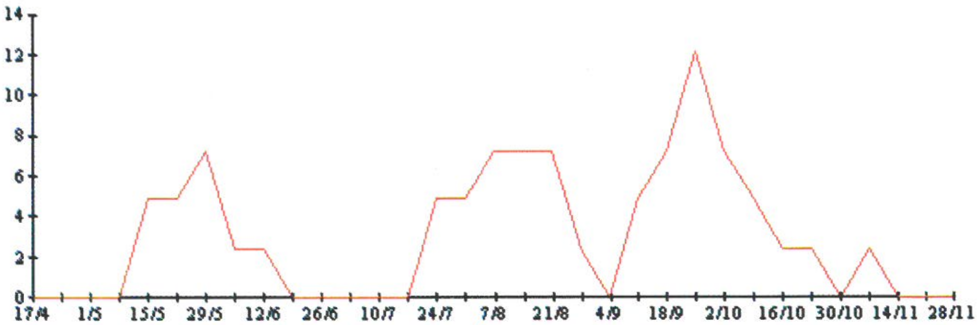

Figure 2 - Percentage of total males of *Sesamia nonagrioides* captured in pheromone sexual traps, in 1993.

For both species, the biggest part of captures occurred on the last flight of the year. Comparing the second flights of *O. nubilalis*, in 1992 and 1993, there is a difference about one week between them, although, in 1993, the activity of this species started two weeks before. The results for *S. nonagrioides* aren't enough strong, because the number of males captured was very small, and so, they must be analysed carefully.

Based on plant dissections, done through the crop cycle, Table 1 was built; only alive larvae and pupae were considered.

Table 1 - Results of plant dissections

Sample N°°	Phenologic Satge	N°of Larva		N° of Pupa		Egg Plates	
	(*)	*S .nonagrioides*	*O. nubilalis*	*S. nonagrioides*	*O. nubilalis*	*S. nonagrioides*	*O. nubilalis*
1	B-C	0	0	0	0	0	0
2	C	0	0	0	0	1	1
3	C-D	1	0	0	0	2	1
4	D	3	4	0	0	0	0
5	D	9	1	0	0	0	0
6	E	26	3	1	0	0	0
7	F	23	1	5	1	0	0
8	G	13	0	5	2	0	0
9	H	7	0	8	1	1	0
10	H-I	5	0	6	0	3	0
11	I	8	31	5	2	3	1
12	I	6	0	0	0	0	1
13	I-J	12	2	0	0	1	0
14	J	31	2	0	0	0	2
15	J	25	11	1	0	0	2
16	K	12	26	9	0	0	1
17	K	10	28	8	0	4	1
18	L	14	24	3	0	1	1
19	L	25	16	1	0	0	0
20	M	29	18	0	0	0	1
21	M	21	12	0	0	1	0
22	N	18	10	3	1	0	0
23	N	23	15	5	1	0	0
Sub-total		**321**	**204**	**60**	**8**	**16**	**12**
Total		**525**		**68**		**28**	

(*) Gadavour-Cargill Scale (Pereira, 1994).

There is no doubt about the predominance of *S. nonagrioides*, more obvious until flowering (stage J); since then, the number of larvae from both species are similar. From a total of 593 specimens founded inside the crop stalks, 88% were larvae, and nearly 61% of them belong to *S. nonagrioides*.

The number of *S. nonagrioides* larvae was very small until the 4th sampling date (26 June), which did agree with the final of the first flight. Also for *O. nubilalis*, the number of larvae and pupae remained low until the 11th sampling date (14 August), coincident with the second flight. These situations may be related to a delayed sowing of maize, and, by consequence, to the fact that the plants were not in an attractive stage for egg laying, by females from the winter generation, and also to the survival of new born larvae.

So, and for both species, the last generations are the most abundant, either in larvae and pupae number or in males captured. The phenological stage with more larvae and/or pupae risen after stage H (beginning of flowering) (Table1).

With the purpose of getting a view from the evolution of each species development along the crop cycle, Figures 3 and 4 were elaborated, respectively for *O. nubilalis* and *S. nonagrioides,* with the results obtained from plant dissections and pheromone sexual traps.

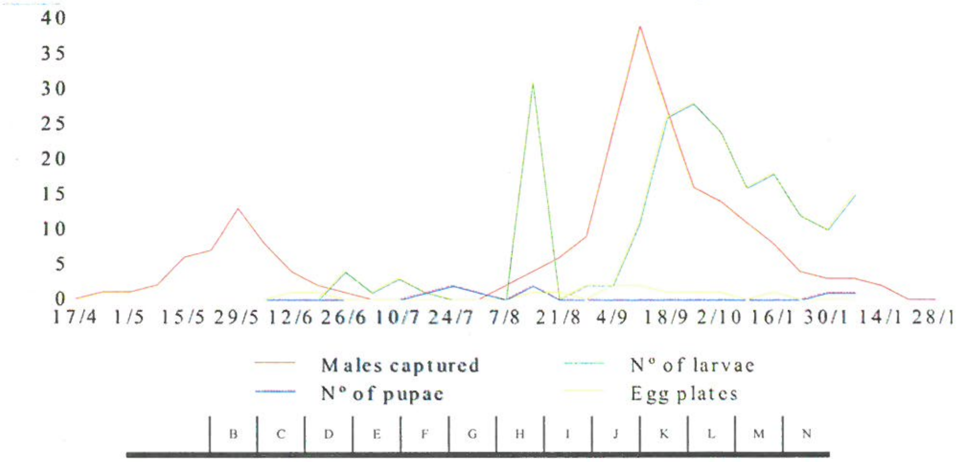

Figure 3 - Annual development of *Ostrinia nubilalis* Hub.

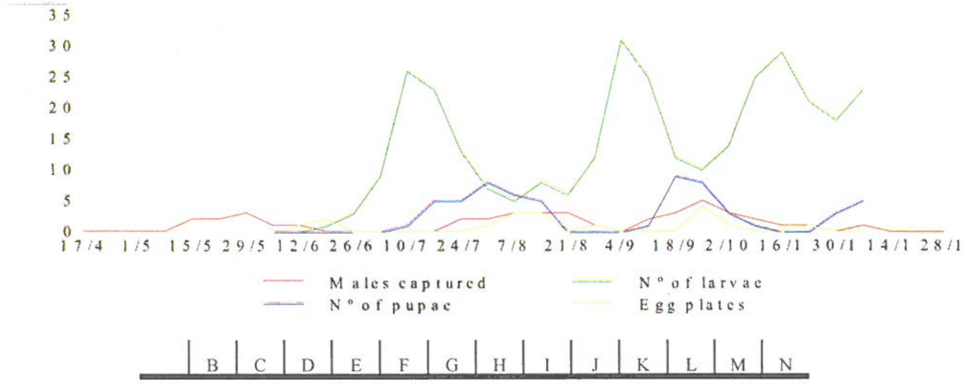

Figure 4 - Annual development of *Sesamia nonagrioides* (Lef.).

From Figures 3 and 4 it is obvious the difficulty of setting up the frontiers between development stages of both species, mainly on the final of the crop cycle.

DISCUSSION

The results of male captures and plant dissections are in accordance with the expected values, namely to the number of generations and the local predominance of *S. nonagrioides*.

The displacement between the peak of *S. nonagrioides* first flight (29 May) and the peak of larvae founded inside the crop stalks (17 July) seems excessive (about 1,5 month), but confirm the difficulty and weakness of the work done with so few captures.

The experience demonstrated that in plants where the two species overlap in time and space, the *S. nonagrioides* larvae were founded, almost always, in the stalk lower half, while *O. nubilalis* larvae preferred the top.

The durability of each development stage shows some variations between generations, and decrease along the year. The establishment of frontiers between those stages is a hard problem, due, in part, to a long period of flight, which give rise to a spread egg laying period.

These results don't give any information about the attack intensity of the maize field, because the plants dissected were selected only if attacked.

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THE STUDIES ON *OSTRINIA NUBILALIS* Hübner (LEP.:*PYRALIDAE*)AND *SESAMIA NONAGRIOIDES* (Gahan) (LEP.:*NOCTUIDAE*) IN TURKEY

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ABSTRACT

In Turkey, the maize is cultivated as main crop and second crop. Since 1982, the second crop maize has been grown in Mediterranean and Aegean region. But, the second maize crop production is limited by *Ostrinia nubilalis* Hübner (Lep.: *Pyralidae*) and *Sesamia* spp (Lep.: *Noctuidae*). In the Black Sea region where maize is grown as a only main crop, *O. nubilalis* is the key pest on corn fields. In the Black Sea region, *O. nubilalis* has two generations and in the Mediterranean region four generations per year. There are two species of *Sesamia* spp. in Turkey. But, *S. nonagrioides* Lefèbvre is dominant and it has five generations per year in the Mediterranean region. *S. cretica* Lederer was recorded in Southeast Anatolia and in Marmara region.

Many natural enemies of both pests were found. Among these natural enemies, egg parasitoid, *Trichogramma evanescens* Westwood (Hym.:*Trichogrammatidae*) for *O. nubilalis* and *Platytelenomus busseolae* (Gahan) (Hym.: *Scelinioidae*) for *S. nonagrioides* were the most effective. In the Mediterranean region, the population development of *T. evanescens* and *P. busseolae* was correlated with their host egg populations. The natural parasitism rate on the eggs laid by *O. nubilalis,* during the main and second maize growing seasons was 48.96%, 75.26% and 73.02% in 1988, 1989 and 1990, respectively. After these studies, *T. evanescens* were released against to *O. nubilalis* in IPM programme, and the obtained results were enough to control *O. nubilalis*. In the same place, the natural parasitism rate of *S. nonagrioides* eggs by *P. busseolae* was 56.80%, 49.37%, 38.63% and 48.08% in 1990, 1991, 1992 and 1993, respectively.

Key words: Turkey, Maize pests, *Ostrinia nubilalis, Sesamia nonagrioides.*

INTRODUCTION

Maize is cultivated as main crop and second crop in aproximately 600.000 ha in Turkey. There is a great potential for second maize crop production in Mediterranean, Aegean and Southeast Anatolia region of Turkey. But, the second maize crop production is limited by corn borers, *Ostrinia nubilalis* Hübner (Lep., *Pyralidae*) and *Sesamia* spp. (Lep.

Noctuidae), except Southeast Anatolia region, which is newly irrigated and up to date the population of corn borers is much lower than in the other regions. In the Black Sea region, where maize is produced as a main crop, only *O. nubilalis* is a pest on the corn fields.

Almost all plants are infested, and crop losses of the order of up to 100 % are not usual. The problem of crop protection from both pests is complicated by many parameters. Insecticidal applications should be timely made, since insects are susceptible to the chemicals only for a short period of time, usually 2-4 days egg hatch for both, while young larvae have not entered inside the stem or other parts of the plant. Another problem, after the plants are grown is that the applications are difficult, since special equipment ought to be used, and even on such occasions with questionable results. The different studies by researchers have conducted to control both pests and to improve a new control strategy for Integrated Pest Management programs since 1970. On the other hand, when farmers apply chemical insecticides, the studies of biological control have been carried out against both pests. Many natural enemies of these pests were found, and the efficiency of the native egg parasitoids, *Trichogramma evanescens* and *Platytelenomus busseolae* were tested on the eggs of *O. nubilalis* and *S. nonagrioides,* respectively. Similar studies about the use of these two parasitoids as biological control agents have been continued by researchers. In this paper, results from some important research aiming to build up control measures for both pests, are reported.

THE POPULATION DEVELOPMENT OF *OSTRINIA NUBILALIS*

O. nubilalis overwinter as diapausing mature larvae in Turkey. Özdemir (1981) determined that *O. nubilalis* has two generations in the Black Sea region. The adult flight of the first generation started after the second half of May and reached the highest peak in mid-June then, decreased by early July. The adults of the second generation appeared in mid-July, reached the highest peak in early August and then, decreased in early September. Then, the larvae entered diapause in the corn stalk and others parts of the plant.

Kayapınar (1988) has found four generations per year in the Mediterranean region of Turkey. In Table 1 and Figure 1, the first occurrence and the population development of the different stages of *O. nubilalis,* are shown.

Table 1 - First occurrence dates of the different stages of European Corn Borer in the Mediterranean region (Çukurova Plain) in 1987(From Kayapınar, 1988)

Stages	Over wintered generation	First generation	Second generation	Third generation	Fourth generation
Adults	11 April	23 June	24 July	25 August	-
Eggs	-	13 May	26 June	24 July	28 August
Larvae	-	18 May	5 July	28 July	4 September
Pupae	23 March	16 June	21 July	18 August	-

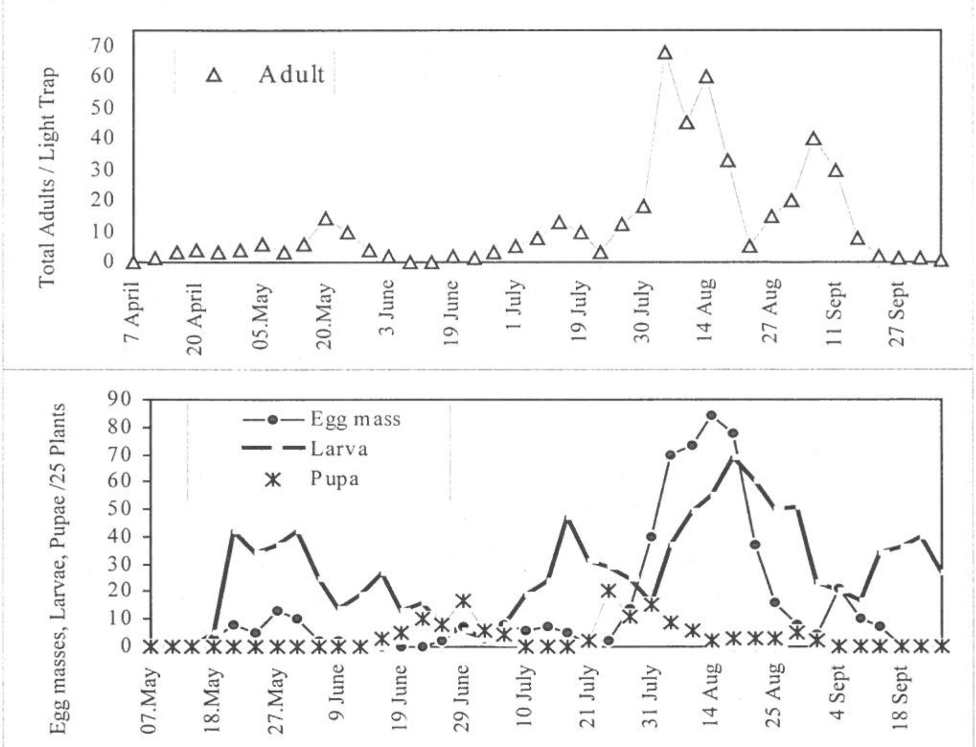

Figure 1 -The population development of adults and other stages of *Ostrinia nubilalis* in the Mediterranean region of Turkey (in Balcalı-Adana) in 1987 (From Kayapınar, 1988).

The diapausing was started as a mature larva into stalk in late August. Diapaused larvae pupated in late March and the pupa period of the overwintered generation continued by early April, and the activity of the adults started in mid-April. There are four peaks during the growing season: one in second half of May, one in mid-July, one in the first week of August, and one in early September.

The eggs of the overwintered generation were seen in the second half of May, then fluctuated till mid-September. Here again, four peaks were found, occurring in late May, early July, mid-August and early September. Changes in larval population were similar to the egg population and once again four peaks appeared. After the second half of August the larvae started to enter into diapause. The highest peak was observed in the third generation in August.

Also, similar results were obtained by other researchers in the same area showing that *O. nubilalis* doesn't damage the main crop, but it is a serious pest on the second maize crop.

THE POPULATION DEVELOPMENT OF *SESAMIA NONAGRIOIDES*

S. nonagrioides overwinter as larvae in corn stalks. The larval diapausing was not clear, because there are different larval instars during winter in the Mediterranean region of Turkey. Kavut and Derin (1988) determined that *S. nonagrioides* overwintered as fully mature larvae in the corn stalk and stuble in the Aegean region. The emergence of moths started in Spring, increasing to the end of April/beginning of May and decreasing to the end of May. Kayapınar and Kornoşor (1988) reported that the overwintered larvae started pupating by early February, and continued until March. The adults began to emerge in mid-March and the adult activity continued untill the end of Nov. There are 4-5 generations per year.

In the Figure 2 it is shown that the adult population was low in the early growing season, but rapidly increased in early Sept. and reached the highest peak at the end of Sept. or early Oct. in the Mediterranean region (Adana Province).

S. nonagrioides mainly lays its eggs between the plant stem and leaf sheats or sometimes into ear husks. The newly hatched larvae spend 2-4 days in the area of the egg deposition and then enter directly into the interior of the plant. Therefore, it is only during this short period that the larvae can be reached by insecticides. The plants attacked by *Sesamia* larvae do not grow, the larvae tunnelled within the stalk and can cause extensive damage to the stalks as well as to the ears of maize, especially depending on the planting time.

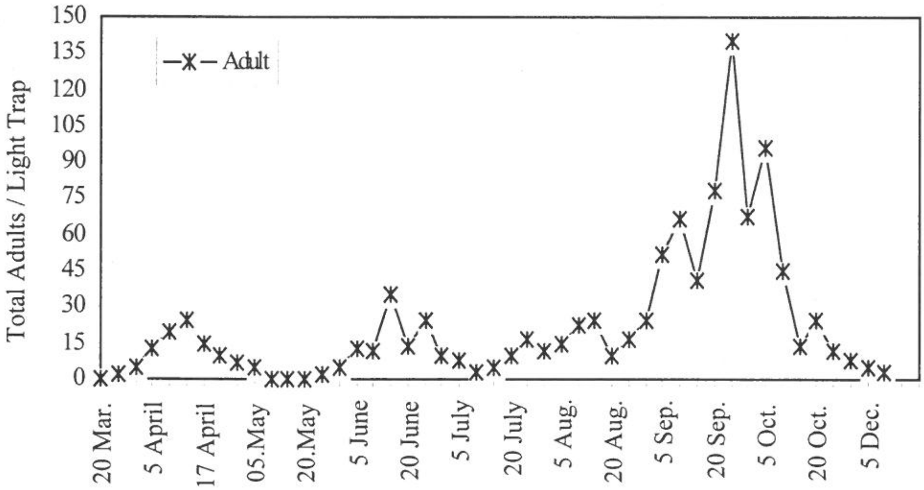

Figure 2 - The population development of *Sesamia nonagrioides* in the Mediterranean region of Turkey, in 1987 (From Kayapınar and Kornoşor, 1988)

Finally, *O. nubilalis* and *S. nonagriodes* cause significant losses during the growing season especially on the second crop of corn. In the first maize crop no control measures for both pests are needed, but for the second crop in the Mediterranean and Aegean regions, such controlling measures are required. The usual ways of controlling the insect has been the use of insecticides. The farmers usually apply periodically the contact insecticides to control these pests, but can not take required results due to the feeding behaviour of the larvae and plant phenology.

Insecticides can not reach larvae feeding inside the stalk and other parts of the plant. If the larvae are not affected by insecticides, the farmer increases the use of these chemicals leading to the killing of the natural enemies and so giving rise to new alternative pests which are not considered here.

This stresses the need for developing an effective pest control programme against these two pests in corn production.

THE BIOLOGICAL CONTROL OF *OSTRINA NUBILALIS*

O. nubilalis has been reported to have an extended list of biological control agents, mainly larvae and egg parasitoids in Turkey. Among the natural enemies, the native egg parasitoid, *T. evanescens* was found very effective on the egg population of *O. nubilalis* in the field conditions (Özdemir, 1981; Kayapınar and Kornoşor, 1992).

In the Black Sea region, on the first and second generation eggs of *O. nubilalis* were parasitized up to 96 and 42 % by *T. evanescens* (Özdemir, 1981).

In the Aegean region, on the second generation eggs of *O. nubilalis* were parasitized 6.5-100 % by *T. brassicae* (Uzun, 1994).

According to Özpınar and Kornoşor (1995) the total number of sampled *O. nubilalis* eggs were parasitized by *T. evanescens* at the rate of 53.34, 48.96, 75.26 and 73.02 % in natural condations in 1987, 1988, 1989 and 1990, respectively. This indicated that, the parasitoid could be a promising biocontrol agent.

As shown in Figure 3, the population development of *T. evanescens* was correlated to the number of eggs laid by *O. nubilalis*. Particulary, *T. evanescens* populations were more increased, when eggs of the population of *O. nubilalis* reached their highest density by August.

As shown in Figure 3, the population developments of *O. nubilalis* and *T. evanescens* were similar during the four years, but the population density was different in the last two years.

In 1991, *T. evanescens* was released (200 000 /ha) once at the beginning of the oviposition period of the third generation of *O. nubilalis* in the second maize crop and the parasitism was 78 % (Özpınar, 1996) and in 1993, as a result of two releases (150 000 /ha) with one week interval, parasitism rate was 88.48 - 95.96 % (Coşkuntuncel and Kornoşor, 1996). It was enough to control *O. nubilalis* within a short time.

At present, releases have been continued in experimental fields.

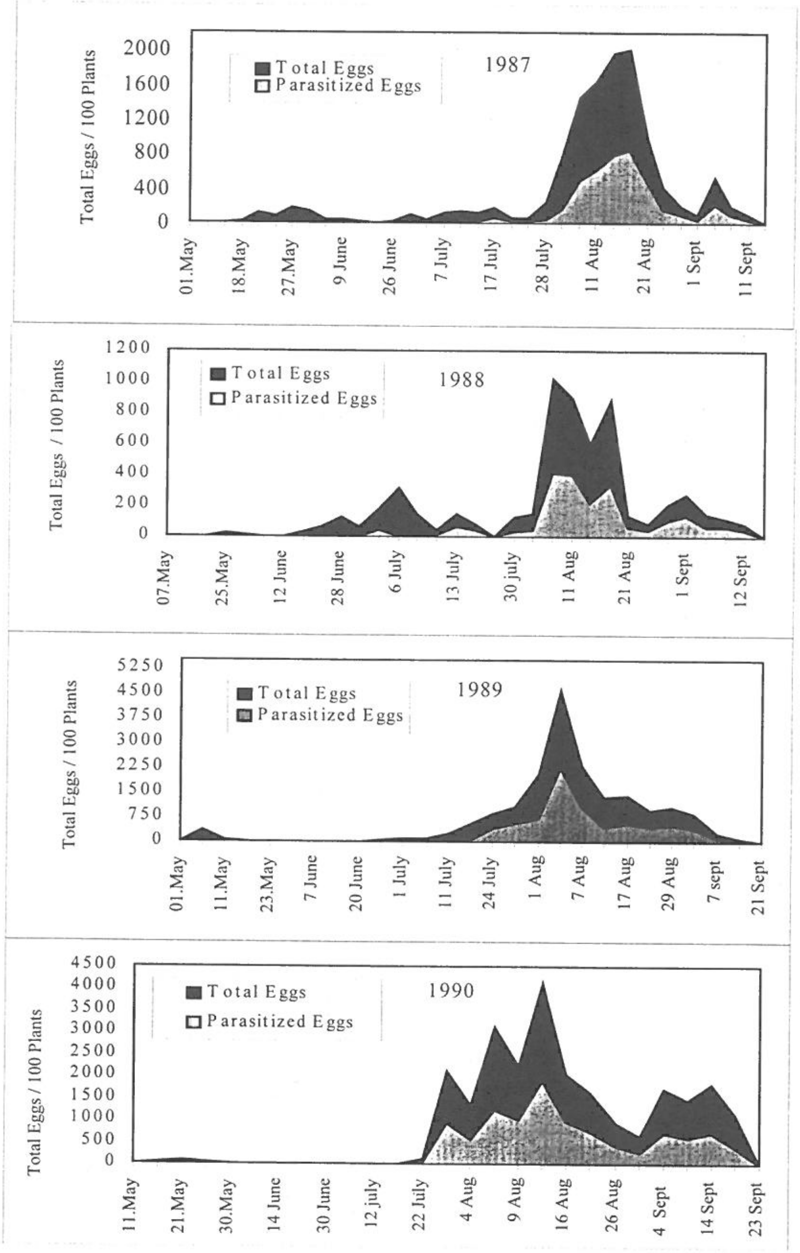

Figure 3 - The parasitization of *Ostrinia nubilalis* eggs by *Trichogramma evanescens* in the (From Özpınar and Kornoşor, 1995).

THE BIOLOGICAL CONTROL OF *SESAMIA NONAGRIOIDES*

Also, *S. nonagrioides* has many natural enemies in Turkey. Egg parasitoid, *Platytelenomus busseolae* (Gahan) was first reported in 1988 in the Çukurova plain of the Mediterranean region (Kayapınar and Kornoşor, 1990). It was found that *P. busseolae* was the most effective natural enemy of *S. nonagrioides* and the parasitization rate was 49.37, 38.63 and 48.08 % in 1991, 1992 and 1993, respectively (Kornoşor *et al.*, 1994). As shown in Figure 4, the parasitoid appeared first in May, and continued till November. But, in the beginning of the season, the parasite population was very low, so the relationship with its host eggs is not visible in Figure 4. However, by the end of the season, the relation between host and parasitoid is clear, reaching its peak in August (Kornoşor *et al.*, 1992).

Similar results about both pests and their egg parasitoids were obtained in other studies, and at present, these studies have been continued in different regions of Turkey. The egg parasitoids, *T. evanescens* and *P. busseolae* have proved to be a useful tool for the regulation of both pest populations either alone or together with others in the IPM program.

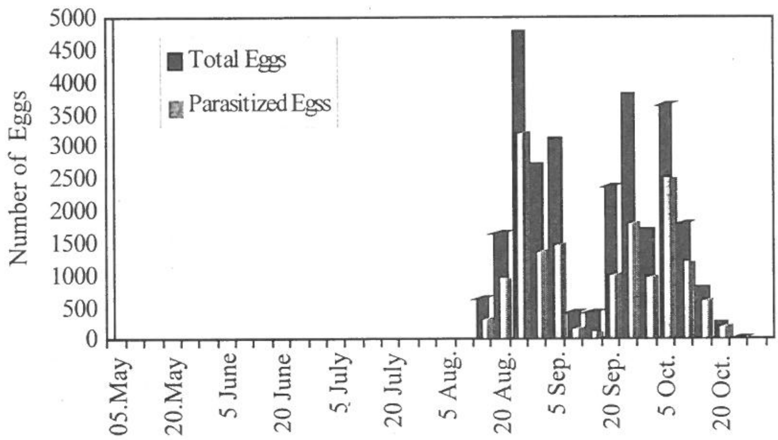

Figure 4 -The parasitization of *Sesamia nonagrioides* eggs by *Platytelenomus busseolae* in the field, in the Mediterranean region of Turkey, (in Balcalı-Adana) in 1988-1990 (From Kornoşor *et al.*, 1992).

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MAIZE PESTS IN PORTUGAL AND THEIR CONTROL

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ABSTRACT

In Portugal the most important maize pests are the species *Sesamia nonagrioides* and *Ostrinia nubilalis*. Their attacks may occur in all national regions. However, other species may also present some importance in some years and places.

In the present work some comments are made on the importance of the main pests of the maize in the Ribatejo region (Ferreira, 1991).

Based on experiments carried out in some regions of the Portuguese territory, a set of graphs, showing flight periods and highest catches of *Sesamia nonagrioides* Lef. and *Ostrinia nubilalis* Hüb., as well as the amount of losses, are presented.

Finally some points are emphasized on the control of the main pests of this crop. A table referring the authorized active ingredients is also presented.

Keywords: *S. nonagrioides*, *O. nubilalis*, *Agriotis* sp., *Agriotis* sp., *Aphididae*, *Tetranychus*.

INTRODUCTION

Maize is cultivated in almost every region. However, the greatest incidence in national yield is due to the regions of Entre Douro e Minho, Beira Litoral and Ribatejo e Oeste, which contribute with 85% of the national yield (Rocha, 1995).

Control, in many crops, constitutes the most significant factor on the costs. In this way, it is generally assumed that the knowledge of the main crop pests, the extent of the losses they cause and the period of risk are the factors to consider in minimizing costs.

As to the enemies of maize in Portugal the existent studies until 1980 are scarce but the situation improved in last years.

In an inquiry made to farmers in Ribatejo region, these considered by decreasing order of importance the following pests: mites, *Agrotis* spp., corn borers, aphids, *Agriotis* spp. and tomato crawlers (Ferreira, 1992).

Some considerations are presented on pests and control strategy, and in relation to corn borers a great development is made on flight curves, flight and maximum capture periods, damages verified and their importance.

MATERIALS, METHODS AND SOME RESULTS

Corn borers (*S. nonagrioides* and *O. nubilalis*)

These *Lepidoptera* are considered the main maize pests.

The first is a *Noctuidae* of the Mediterranean Region and its development is favourable in zones with a gentle winter and hot summers, while the second one is a *Pyralidae* and it develops preferably in hot and humid forests and dry weather seems to be unfavourable.

In the same national zones, light traps were installed and captures made from the species *S. nonagrioides* (Figures 1,2,3 and 4) were considerable at Braga and Idanha-a-Nova areas in 1981, 1982 and 1983 and Alvalade do Sado in 1983 and 1984. However, in the central zone of the country, Vila Nova de Ourém and Golegã in 1980 and 1991 (Evaristo 1984; 1985; Baeta, 1991) the captures were in less number.

In relation to *O. nubilalis* (Figures 5,6,7,8 and 9) captures were made in greater number in the areas of Braga and Idanha-a-Nova, in less number in the interior area of Vila Nova de Ourém and almost non-existent in the interior of Alentejo, more specifically at Alvalade do Sado.

<u>Flight curves and maximum capture periods</u>

S. nonagrioides

From the capture of adults, both with light and sexual traps, flight curves, flight periods and maximum capture periods were determined.

In Braga (Figure 1) two flight periods were determined:

- The first, begins in the end of April and has the maximum capture period (MCP) from the end of May to mid June.

- The second, may occur from July to October with a MCP during September.

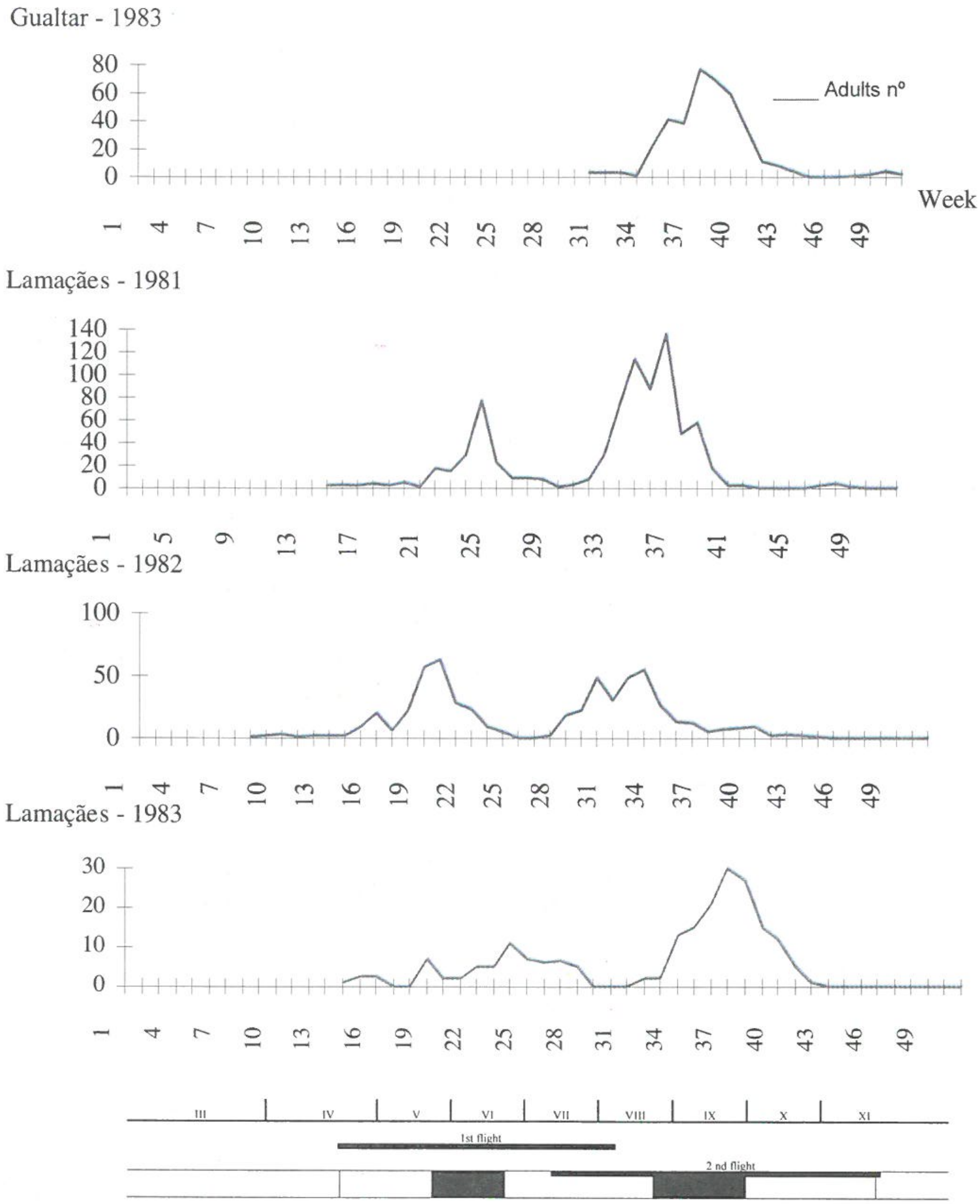

Figure 1 -Captures of *S. nonagrioides* adults with light traps at Braga (Gualtar) in 1983 and Braga (Lamações) in 1981-1983 Graphs based on data published by Evaristo (1984-1985).
Representation of flight periods [ ] and maximum captures [■]

In Idanha-a-Nova (Figure 2) three flight periods were observed. The first, goes from April to mid June with the MCP during June. The second period goes from the end of June to the end of August with a MCP in the end of July. The third period goes from August to mid October with a MCP occurring in mid September.

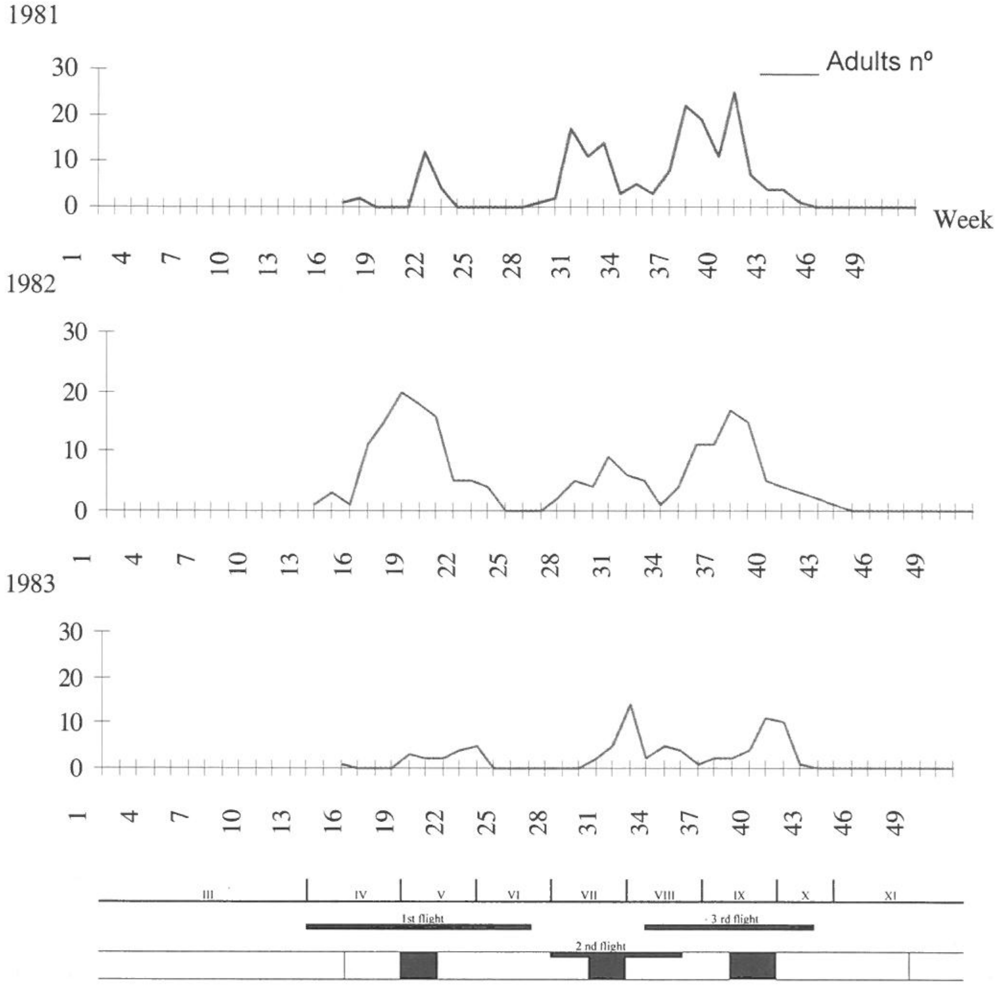

Figure 2 -Captures of *S. nonagrioides* adults in light traps at Idanha-a-Nova in 1981, 1982 and 1983 (Graphs based on data published by Evaristo (1984-1985).
Representation of flight periods ☐ and maximum captures ■

In the regions of Santarém/Golegã (Figure 3) the captures carried out did not allow clear separation of the flights, mainly at the region of Santarém. However, they seem to indicate the existence of three flights. The first, in spite of insufficiently characterized, may occur from April/May to mid June. The second, from mid June to mid August, with a MCP occurring at the end of June/beginning of July. The third occurs from mid August to October with a MCP at mid September.

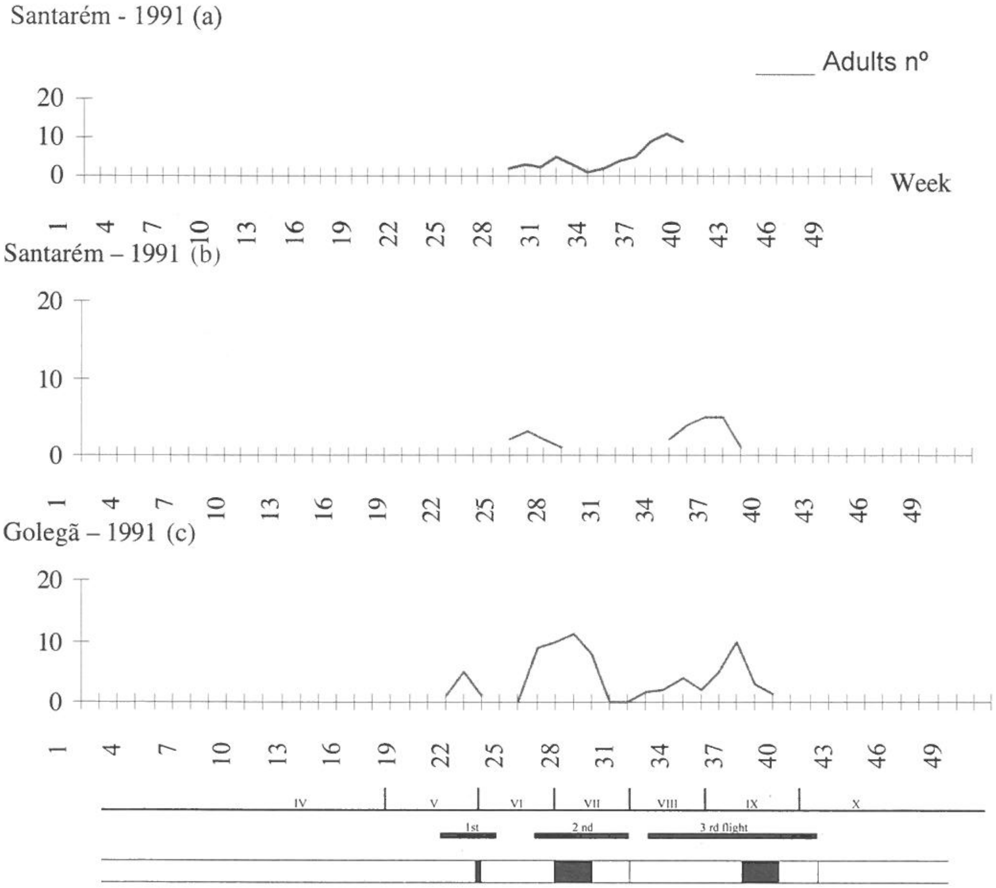

Figure 3 - Captures of *S. nonagrioides* adults with light traps (a) and sexual traps (b e c) located at Santarém and Golegã in 1991 (Baeta, 1991).
Representation of flight periods ☐ and maximum captures ■
Flight period insufficiently defined ....

Considering the observations made on the crops attacked at the region of Alvalade do Sado/Odemira, (Figure 4) although the captures do not allow a very precise definition of the first flight, a possibility of developing three flights is suggested. The first, from May to mid June with a MCP during June. The second from mid July to the end of August with a MCP in mid August. The third occur would from September to November with a MCP in the end of September beginning of October.

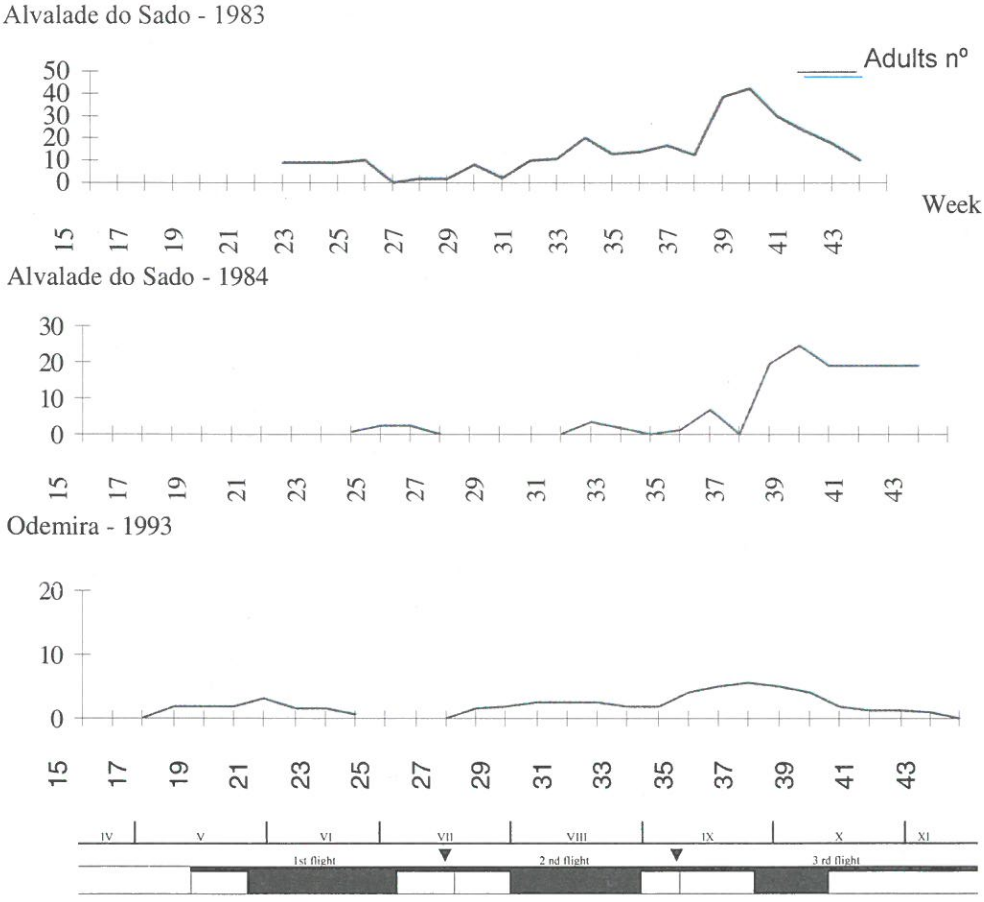

Figure 4 - Captures of *S. nonagrioides* adults with light traps located at Alvalade do Sado (1983 and 1984), and on sexual traps in Odemira (1993). Graphs based on data published by Evaristo (1984-1985) and Pereira (1993).

Representation of flight periods ☐ and maximum captures ■

O. nubilalis

At the region of Braga the occurrence of two flights was observed. The first, during May-June with a MCP in May. The second, goes from mid July to mid November with a MCP in August (Figure 5).

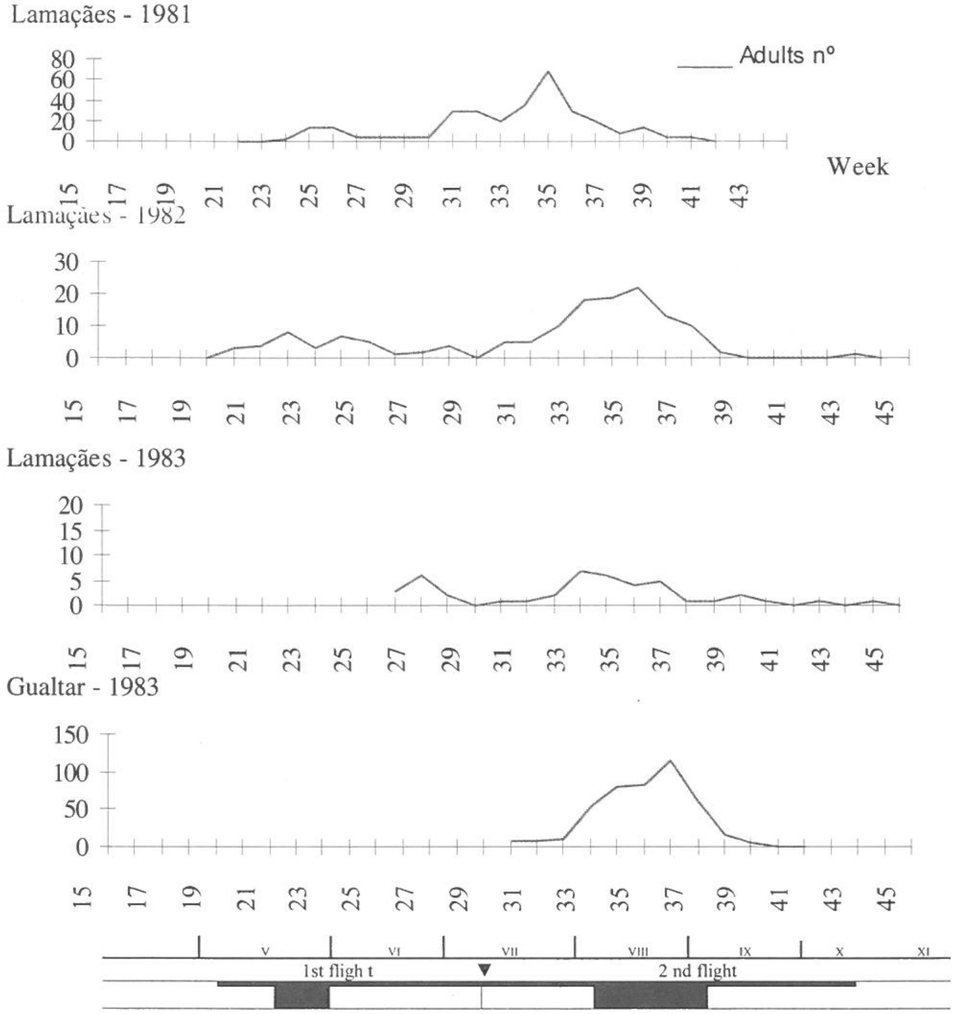

Figure 5 - Captures of *O. nubilalis* adults with light traps located at Braga (Lamaçães) (1981, 1982 and 1983) and on sexual traps at Braga (Gualtar) (1983). Graphs based on data published by Evaristo (1984-1985). Representation of flight periods [ ] and maximum captures [■]

At the region of Idanha-a-Nova the occurrence of two flights can be considered. The first, goes from April to mid June, with a MCP in May. The second, seems to occur between July and mid September with a MCP in mid August (Figure 6).

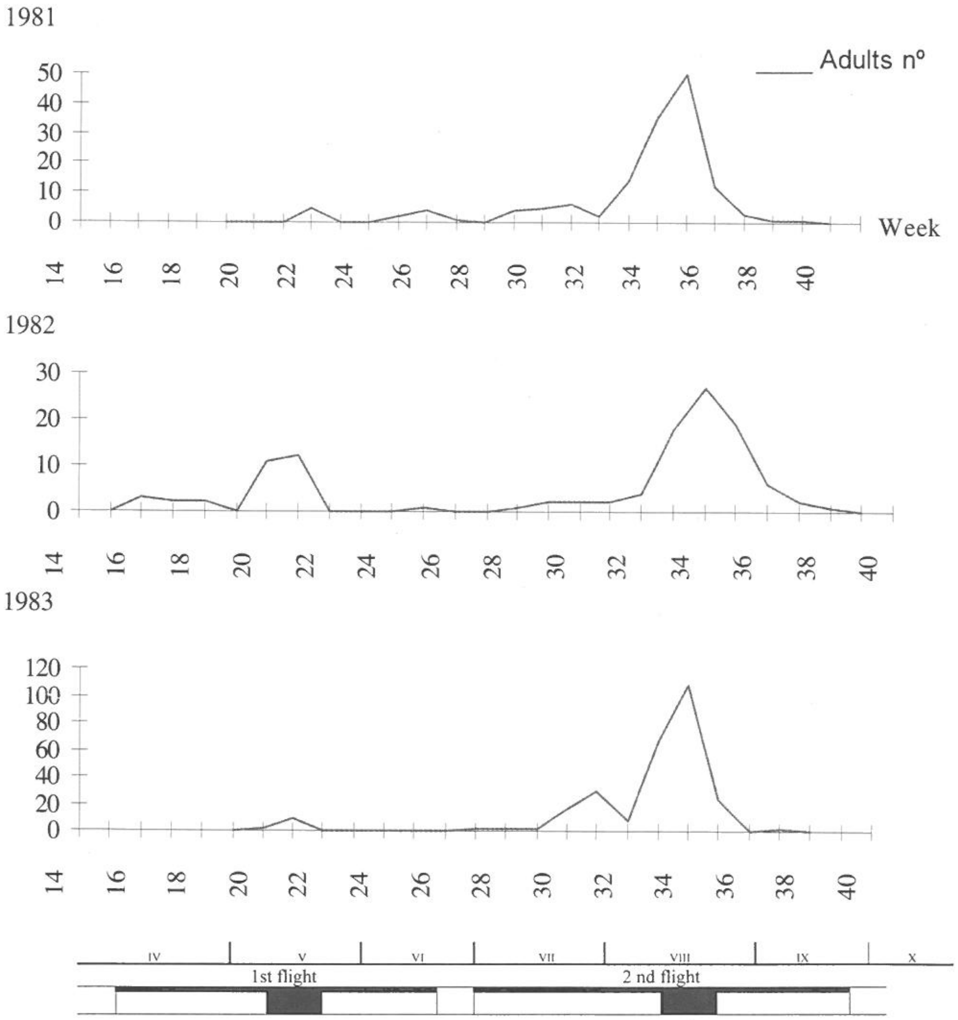

Figure 6 - Captures of *O. nubilalis* adults with light traps located at Idanha-a-Nova (1981, 1982 and 1983) Graphs based on data published by Evaristo (1984-1985).
Representation of flight periods ☐ and maximum captures ■

At the region of Vila Nova de Ourém/Golegã although the first could not be defined, the occurrence of two flights can be considered. The second flight occurs between August and September with a MCP in mid August (Figure 7).

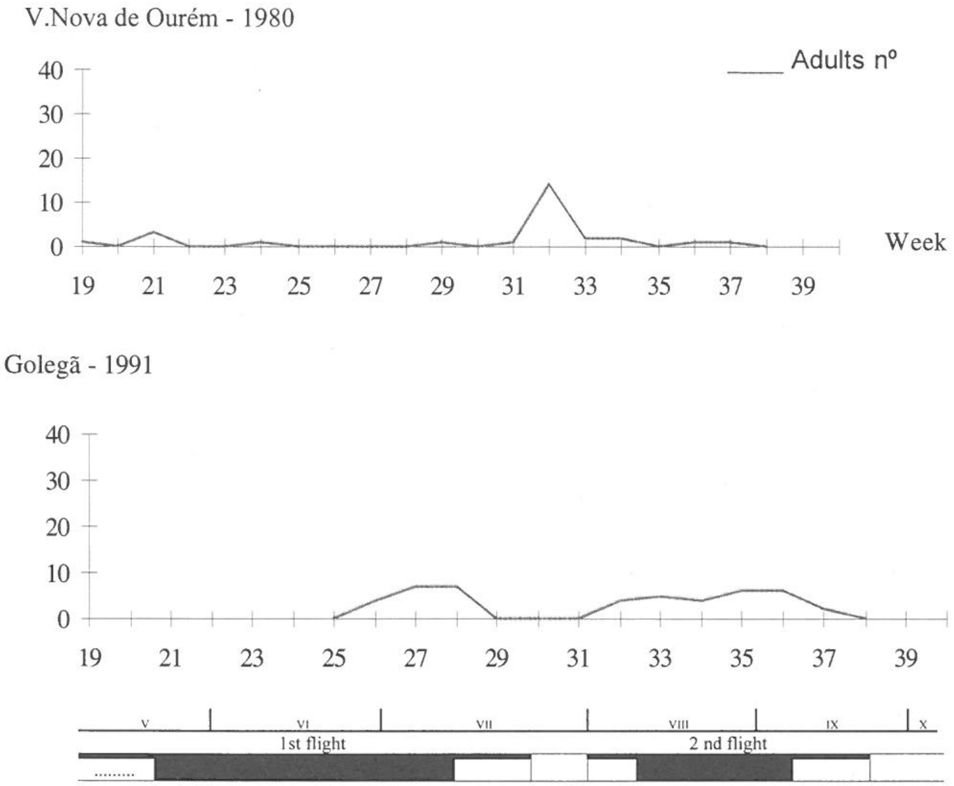

Figure 7 - Captures of *O. nubilalis* adults with light traps located at V. Nova de Ourém (1980) and on sexual traps at Golegã (1991). Graphs based on data published by Branco (1980) and Baeta (1991).
Representation of flight periods [ ] and maximum captures [■]
Flight period insufficiently defined ....

At Alvalade do Sado, captures of *O. nubilalis* were not carried out.

At the region of Odemira two flights were observed. The first, from the end of April to the end of June with a MCP at the end of May. The second, goes from the beginning of August to mid November with a MCP between the end of August and mid September (Figure 8).

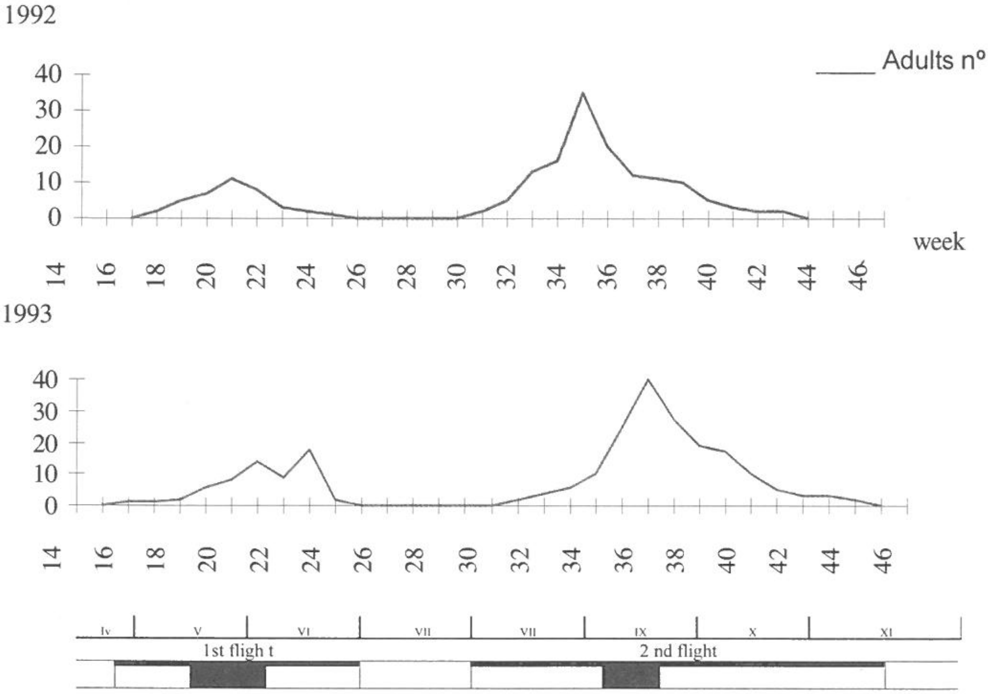

Figure 8 - Captures of *O. nubilalis* adults on sexual traps located at Odemira. Graphs based on data published by Pereira (1993).
Representation of flight periods [ ] and maximum captures [■]

In summary, it can be concluded that *S. nonagrioides* seems to present two flights in the North of the country and three flights in the South. On the other hand, *O. nubilalis* seems to present only two flights in all regions.

Periods of attack and number of plants and ears affected

Field trials were carried out at the regions of Alvalade do Sado (979-1988) and Santarém/Golegã (1989 and 1990) with hybrid maize. These trials were performed in randomized block design with four or six replications and a variable number of entries.

Data were collected from 100 plants per plot for observing the incidence of plant attack. At the harvest,100 random plants were observed and the following data were registered: number of attacked plants, larvae per plant, number and weight of the ears and grain weight (Figure 9).

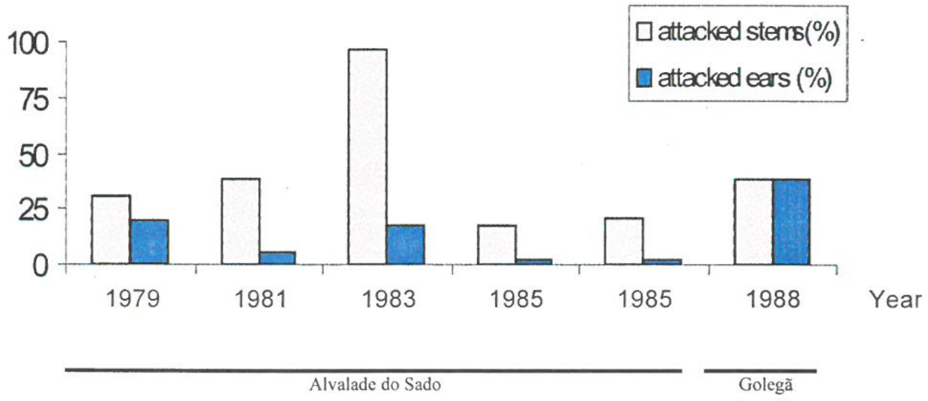

Figure 9 -Attacked stems and ears by *S. nonagrioides* (Alvalade do Sado) and by *S. nonagrioides* + *O. nubilalis* (Golegã).

From the overall data of these trials it can be concluded that:

- In the first generation, weak attacks were registered (attacks may occur from May/June to July). However, attacks to young plants generally cause total destruction.
- In the second and third generations, in hybrid maize with developed plants and with an average larvae number always bellow one larva/ plant, the pest attacks never showed significant effects on the yield. However, Pereira (1994) seems to have found significant differences among open pollinated maize regional varieties for infestation levels of two larvae/plant.
- The intensity of the attacks decreased from year to year and may be considered low when compared with those referred in other countries (Bennani, 1974; Larue, 1984).
- In these trials significant differences between control and treated entries were not found.

Agrotis sp.

Some species of the *Noctuidae* family, also called corn borers, are polyphagous and may cause damages in vegetable crops and cereals. In maize and in our conditions, *Agrotis segetum* Denis & Shiffermülller and *Agrotis ipsilum* Hufenagel are the most important species.

Observations carried out at Ribatejo, Braga, Idanha-a-Nova, Elvas and Alvalade do Sado allowed to conclude that *A. segetum* is the species with the highest significance (Evaristo, 1985; Ferreira, 1992).

At the region of Idanha-a-Nova *A. puta* seems to have a great representation.

Captures made by light traps allowed to determine three flight periods at Ribatejo (Figure 10). Damages are caused to the young plants by larvae in Spring (May-June). A second attack may occur later on, between the end of June and July.

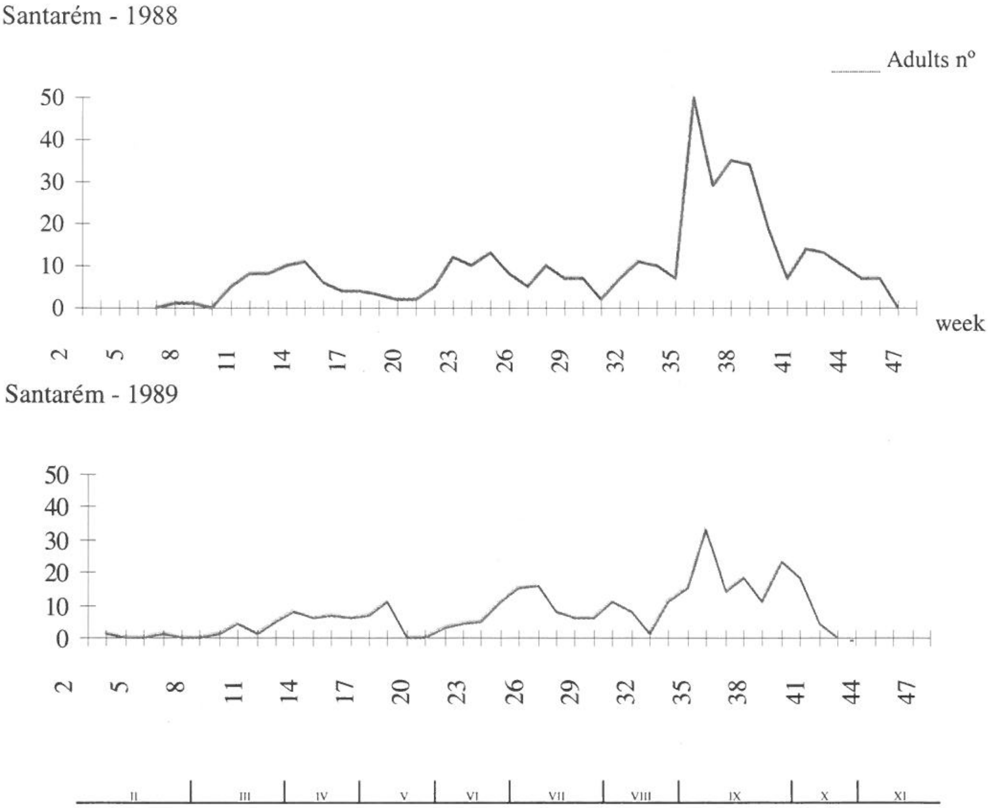

Figure 10 - Captures of *A. segetum* adults on sexual traps located at Santarém (1988 and 1989). Graphs based on data published by Pereira (1993).

Agriotis sp.

Larvae of *Agriotis* genera present night activity and cause high damages. Observations carried out in May of 1988 at the region of Chamusca, allow to conclude that high damages in maize can be caused in Spring, shortly after the emergence. In certain years the untreated fields may have significant attacks. At the region of Chamusca severe attacks were observed with 40% dead plants.

Scutigerela immaculata

Maize plants, once attacked by this myriapod, turn red. This pest generally occurs in permanent and limited spots and does not seem to be much sensible to crop rotation.

From a survey carried out among farmers at the Ribatejo region about soil pests and corn borers, it was shown that most of the farmers (95%) make at least two insecticide applications during the crop cycle (Ferreira, 1992). The first application is made in the row during the sowing in order to control *Agrotis* sp. and *Agriotis* sp..The second application is made over the crop, in the Spring, to control *Agrotis* sp., *S. nonagrioides* and *O. nubilalis.*

Aphids

In Portugal, the following species have been observed in maize: *Rhopalosiphum maidis* Fich; *R. padi* L); *Tetraneura ulmi* L.; *Macrosiphum euphorbiae* Thomas and *Sitobion avenae* Fabricius (Ilharco, 1973-1979). By contrast, in France, *R. padi* and *S. avenae* seem to play an important role in the maize crop (Naïbo, 1982).

From our field trials it could be noticed that *R. padi* behaved as a pre-maturation pest, the first adults arising in June and installing themselves in the foliage without showing, however, high multiplication levels. In July they reach the panicles and, in favourable conditions resulting from unbalanced high nitrogen fertilizations and the use of chemicals with high toxicity on predators and parasitoids, they may reach high multiplication levels. This is a very favourable situation for the development of a melada and the formation of fumagine which may inhibit the normal process of photosynthesis.

However, the presence of this pest near or at the maturation period does not seem to cause serious damages (Naïbo, 1982).

S. avenae seems to present no great danger to maize. In trials carried out at Alvalade do Sado it was observed that this pest shows its highest multiplication at the final stage of the crop cycle. Although they feed on the plant, the damage does not seem to be relevant.

Tetranychus sp.

In the maize crop, in Portugal, the following species were detected: *Tetranychus atlanticus* Mc Gr. (*T. turkestani* Ugarov & Nicholskii) and *Tetranychus telarius* L. (*T. urticae* Koch.) (Carmona, 1980).

In 1991-1992 important attacks were observed in fields with overflow irrigation at Ribatejo. In the region it was observed that the weeds were hosting the mites wich gave rise to the attacks on maize.

From our trials carried out at Chamusca and designed to analyse the population evolution of *T. turkestani*, data collected between June and August showed a magnitude of attack reaching 80 mites/leaf. As a consequence, large spots of totally dried plants were observed. To control such attacks, the following active chemical ingredients (a.i.) were tested, all of them with good performance: dicofol+tetradifon, cyhexatin and hexatiazox.

SOME CONCLUSIONS AND CONSIDERATIONS

O. nubilalis and *S. nonagrioides*

Although in most years the attacks of the first generation reveal themselves harmless, they may exceptionally present some significance. For this reason, the installation of sexual traps is advisable for the mentioned species.

Starting from the flight peak, periodic laying observations should be started, in 100 plants chosen at random, on the third or fourth leaf, in relation to *S. nonagrioides* and in the interior leaf relatively to *O. nubilalis*.

At the first perforations in the stem, a treatment can be carried out on the spots, previously detected by general observations in the field. In relation to the second generation of *O. nubilalis*, the application of granulated insecticides at the peak of the second flight seems to present a satisfactory efficiency.

However, generalised treatments at the second and third generations with pesticides under granulated or emulsion concentrate formulations (Table 1), demand special application equipment not always available to farmers.

The total coverage with the application of insecticides during the damage period, would require a considerable number of applications, which may implement the development of other pests, like mites and aphids, and so alternative means of control may be considered: cultural control, biological control, genetic control.

Soil insects (*Agrotis* sp., *Agriotis sp.* and *Scutigerella immaculata*)

The larvae of the genera *Agrotis* reveal a high polyphagy due to which their control is difficult by crop rotation. Although there are numerous antagonists, namely *hymenoptera*, *tachina* and viruses, sometimes it is necessary to use chemical control (Table 1) (Bonnemaison, 1962).

To control *Agriotis* sp. larvae, crop rotation may be used. Peas and beans are crops to be considered in the rotation, since the respective cropping practices are not favourable to the development of this pest.

The crops which demand a higher soil moisture, such as pastures, are favourable to their laying and the development of the larvae, since these stadia are very sensitive to soil drying. Winter or Summer ploughings may also contribute to the reduction of their populations.

The beneficial biological controllers of this pest seem to perform a little poorly.

The soil insecticides, carbamates, organophosphorates or pyrethroids, in granules are effective against the most soil pests as well as against those which attack the aerial part of the plants (Table 1). The application should then be considered whenever these pests constitute a menace to the development of maize (Naïbo, 1985).

Table 1 - Insecticide and fungicide active ingredients registered for maize crop.

Disease / pest	Active ingredient	Form	Authorized dosages
Seeds treatment	mancozeb	ds	162g a.i./100kg of seed
Agriotes spp.	carbofuran	gr	625g a.i./ha
	diazinon	gr	10 kg a.i./ha
	fonofos	gr	4 kg /ha
	furathiocarb	gr	600g a.i./ha
	tefluthrin	gr	50-75g a.i./ha
Sesamia spp.	carbaryl	wp	100
	endosulfan	ec	123,5-247
	endosulfan	wp	123,5-247
	lindan	wp	20
	trichlorfon	sp	160
	trichlorfon	gr	0,375-0,625kg a.i./ha
Melolontha spp.	diazinon	gr	10kg a.i./ha
	fonofos	gr	4 kg /ha
Agrotis spp.	diazinon	gr	
	Lambda-cyhalothrin	ec	7,5g a.i./ha
	tefluthrin	gr	50-75g a.i./ha
O. nubilalis	cypermethrine	gr	0,05g a.i./ha
	Lambda-cyhalothrin	ec	20g a.i./ha
Gryllotalpa gryllotalpa	diazinon	gr	1,2 -1,8 kg a.i./ha
	lindan	wp	180g a.i./100kg
Scutigerella immaculata	carbofuran	gr	625g a.i./ha
	chorpyrifos	gr	3kg a.i./ha
	fonofos	gr	4 kg /ha
	tefluthrin	gr	50-75 g a.i./ha

Aphids

On maize, frequently small aphid populations are observed in the Spring and near harvesting.

However, in this crop, there are also predators of these aphids, namely *Coccinelidae* and *Chrysopidae*, and a high parasitism rate has been already observed, mainly in the aphid populations which appear near the harvest. Because on maize, most of the times, no treatments are carried out along the growing cycle, favourable conditions for the development of the beneficial predators contribute to the establishment of a desirable balance between aphids and their natural enemies.

In France, the application of products which reveal a higher toxicity to beneficials, such as pyrethroids in the control of *O. nubilalis*, has lead to situations of large aphid population development. Also unbalanced fertilizations rich in nitrogen may induce favourable conditions to the development of this pest (Costes, 1985).

Mites

The maize crop due to its big plant size and high plant densities, presents some limitations for the spraying of chemical treatments, especially from June on. For this reason, the pest control shall be carried out, whenever possible, in pre-emergence or at the first stage of development.

In what mites control is concerned, host weeds shall be detected and destroyed during the Winter and Spring. Crop rotations using non-hosting crops of this pest shall also be considered and their irrigation shall be well scheduled and, whenever possible, by sprinkling, in order to provoque a higher mortality of the mobile forms. A special care shall be paid to the fertilization process, which requires a good balance of the main nutrients, namely nitrogen, which favours the mites proliferation.

Finally, maize beneficial predators may play an important role as large amounts of *Coccinelis* (S. *punctilum*), *Phytoseids* and *Chrysopids* are generally present.

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COMPARISON OF TRAPPING METHODS FOR MONITORING OF *DIABROTICA VIRGIFERA VIRGIFERA* Le Conte ADULTS

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ABSTRACT

After it's first appearance in Yugoslavia (1992, near Belgrade airport), in the following four years *Diabrotica virgifera virgifera* Le Conte has quickly spread through the Northern plain part of the country (the main corn growing region). However, this appearance even could not be properly registered due to the lack of any kind of traps. Only in 1996, by 160 sex pheromone traps in the framework of the plant the protection forecast service, it was established that Western Corn Rootworm (WCR) has already occupied almost 60 % of Serbia territory (2).

In 1995, by using cucurbitacin traps and also visually, a few adults of the new pest were first found in Bačka (in some locations 60-80 km a North-western of Belgrade). In June 1996, in the above mentioned region, on some fields with continuous corn, damage caused by larvae had already been registered, as well as high adult populations later on during the Summer. This gave us the opportunity to monitor the population density and the dynamic of WCR beetles in continuous corn field at Temerin (20 km northern of Novi Sad) where visual countings showed density of 5-15 adults/plant. Methods including yellow sticky (Multigard "Ecogen" inc.) traps, cucurbitacin traps (3) and sex pheromone ("Csalomon" type) panel trap were used for catching the adults. Sampling was conducted at two week intervals from 11 July (yellow and cucurbitacin traps) and from 23 July (sex pheromone trap) to 04 September, and once a month during September - October 1996. Captures per one trap/day were considered.

The activity of WCR adults was continuous from July to October, with a peak in early August on all three types of traps. Comparing catches among the traps it was found that the highest number of beetles had been observed on yellow traps, less on pheromone traps and the smallest number of WCR was captured on cucurbitacin traps.

The number of captured beetles on yellow traps was much higher than Hein & Tollefson's (1) economic threshold (6 beetles/trap/day) which has been used in USA for Pherocon AM traps. Also, the value of visual countings was significantly higher than nominal economic threshold of approximately one beetle per plant which has been suggested in USA. For that reason great risk of damage by WCR larvae in Spring 1997 had been predicted if corn were continuously grown without chemical control.

These results should be considered as preliminary because we did not have enough traps for valid statistical analysis, except between yellow and cucurbitacin traps. This year studies will be continued at the same field and probably completed with records of larvae damage.

Key words : Maize pests, *Diabrotica virgifera virgifera* trapping methods.

INTRODUCTION

After it's first appearance in Yugoslavia (1992, near Belgrade airport), in the following four years *Diabrotica virgifera virgifera* Le Conte has quickly spread through the Northern plain part of the country (the main corn growing region). However, this appearance even could not be properly registered due to the lack of any kind of traps. By using 160 sex pheromone traps in the framework of the plant protection forecast service, during last year it was established that Western Corn Rootworm (WCR) has already occupied almost 60% of Serbia territory (Sivčev, Draganić, 1996). The area with registered damage increased from 0,5 ha in 1992 to about 11000 ha in 1996.

This means that WCR should be considered a serious pest for continuous corn in Yugoslavia.

In 1995, both by cucurbitacin traps and also visually, a few adults of the new pest were first found in Bačka (in some locations 60-80 km North-western of Belgrade). In June 1996, in the above mentioned region, on some fields with continuous corn, damage caused by larvae had already been registered, as well as high adult populations later during the summer. This gave us the opportunity to monitor the population density and the dynamic of WCR beetles in continuous (2nd year) corn field (0,28 ha) at Temerin (20 km Northern of Novi Sad) where visual count on entire corn plants showed density of 5-15 adults/plant.

In USA, where this pest is one of the largest on continuous corn, different sampling techniques and programs have been developed for all stages of WCR in order to prevent economic losses. Sampling of adults to predict subsequent larval damage seems to be the most promising due to its simplicity and low cost. The plant counting has become the standard rootworm scouting tool throughout the Midwest. The use of trapping methods is advantageous, because population estimates are determined over an extended period, whereas countings measure the population only at a specific time. The use of traps would also eliminate the need for well-trained scouts to carry out a whole plant sampling. Hein & Tollefson (1984) recommended Pherocon AM sticky trap as the practical choice for sampling work due to compactness, easy installation and use, commercial availability, and low cost. Shaw *et al.* (1984) found a good correlation between cucurbitacin trap catch and peak visual countings and suggest that this type of trap has great potential use in IPM for monitoring the population density of WCR in cornfields. During 1995 in Hungary it was concluded that pheromones traps were more suitable for detection of WCR beetles when compared with cucurbitacin traps (Prinzinger, 1996).

The objective of this study was to compare the efficacy of three trapping techniques for monitoring WCR beetles and larval damage prediction of this new corn pest in Yugoslavia.

MATERIALS AND METHODS

Methods including yellow sticky (Multigard "Ecogen" inc.) traps, cucurbitacin traps and sex pheromone ("Csalomon" type) panel trap were used for catching adults.

Yellow sticky Multigard traps ("Ecogen" inc., 610 Central Avenue, Billings, MT, 59105) were made commercial, but unfortunately we have had only 14 of them. For that reason we did not have the possibility to use more than three of them in a field, positioned about 10 m from each other and approximately as far as from the cucurbitacin traps. The color of the Multigard trap is visually attractive to a number of pests, therefore, no additional bait is needed. Traps were wrapped around the corn stalk at ear height. They were left in the field during the whole investigation period, but in each check (except at 6 August by mistake) the new traps were added.

The cucurbitacin traps were almost the same as Cucs traps used in Illinois, USA (Shaw *et al.* 1984). We made them from amber plastic medicine vials measuring 3 cm in diameter and 9 cm in length, with 5 mm holes drilled in equal intervals around the vial. The bottom was removed and covered with screen wire, and the vial was capped with a piece of net cloth and rubber ring. A clear plastic insert measuring 2.5 cm by 7 cm, cut from a sheet of transparency film and trimmed to fit inside the vial, was coated with a mixture of powdered dried squash root (*Cucurbita fetidissima*), corn grouts as carrier and carbaryl (Sevin) insecticide in proportion 65 : 22 : 13 %, respectively.

These inserts were replaced with new ones each time the traps were examined. The traps were attached to the ear zone of a corn plant with a piece of gardener's wire. Three of traps were placed in field, positioned ca. 10 m apart.

The pheromone trap was "Csalomone" type, made in Budapest Plant Protection Institute (Hungary), with pheromone on rubber septa. Previous research (Toth *et al.* 1996) showed that panel trap baited with the pheromone is more suitable for detection of corn rootworms occurrence than triangle designed trap. Since we have had only one panel trap we could not change pheromone lure after 4-6 weeks as recommended, but it did regularly catch during experiment. The trap was tied directly to corn plant at ear height.

Sampling was conducted at two week intervals from 11 July (yellow and cucurbitacin traps) and from 23 July (sex pheromone trap) till 04 September, and once a month during September - October 1996. Captures per one trap per day (or 14 days) were considered. In lack of more pheromone traps, the analysis of variance was possible only to test differences between number of WCR beetles captured on yellow and cucurbitacin traps. On yellow traps was also determined their durability, and on cucurbitacin traps sexual index of WCR beetles.

RESULTS AND DISCUSSION

The mean number of WCR beetles collected by different traps per day during the investigation period is presented in Figure 1. The activity of WCR adults was continuous from July to October, with a peak of their appearance in early August on all three types of traps.

The number of collected beetles per yellow trap per day varied from 38.8 (23 July), through 56.9 (6 August) to 1.2 at the end of growing season (21 October). The maximum number of captured beetles per one yellow trap per two weeks was 823 (at the peak of appearance), while the minimum number was 25 per 21 day (at the end).

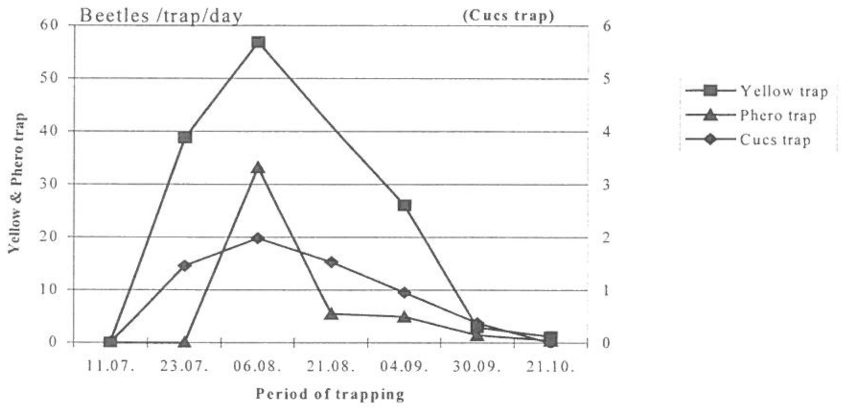

Figure 1 -Efficacy of three trapping techniques for monitoring population density of *Diabrotica virgifera virgifera* Le Conte adults in Temerin (Yu), 1996.

On pheromone trap the catch was the highest at 6 August (33.21 per day or 465 per 14 days), and after that time it suddenly declined during each successive period to the end of October. This is similar to the results reported by Meinke *et al.* (1989). Comparing the efficacy of WCR pheromone encapsulated in a starch borate granule with rubber septa pheromone delivery system the mentioned authors found both systems equally effective. The mean number of collected beetles were the highest between 8-12 August (22.0 per trap per day), and after that time declined until early September 1986 in Nebraska.

On cucurbitacin traps catches varied from 1.46 (23 July), through 1.98 (6 August) to 0.38 (30 September), while the two weeks maximum and minimum values per trap were 33 and 4 beetles. It is quite similar to data from Illinois (Shaw *et al.* 1984), where Cucs traps captured 2.5 and 1.6 WCR beetles per day in 1981 and 1982, respectively, and could be left in the field for a period of 8-12 days.

The analysis of variance and LSD for P 0,05 conducted on data from yellow and cucurbitacin traps (Table 1) indicates that significant differences (*) occurred among average catches on two trap types during each collecting period. Especially high differences (**) were at the peak of appearance ($F = 2473.3$; $df = 4$; $LSD = 71,2$; $P < 0,01$).

Table 1 - The number of WCR beetles captured by Multigard yellow and cucurbitacin traps, in 2nd-yr cornfield in Temerin (Yu) during July-September 1996.

Trap	**Average capture per trap per 14 days in different trapping periods**		
	11 - 23 July	24 July - 6 August	22 August - 4 September
Yellow	504,7 *	796,5 **	369,0 *
Cucurbitacin	19,0	27,7	13,3
$LSD_{0,05}$ =	386,4	42,9	231,4

The durability of yellow Multigard traps in our trials is shown on Figure 2. In all sets traps installed for 14 days trapped beetles quite well, but in the next 14 days their efficiency significantly dropped. Karr & Tollefson (1987) demonstrated that yellow Pherocone AM traps installed for 6-21 days trapped beetles equally well, but were not as effective as 3-d-old traps. They also found that traps were 100% efficient through 6 days in lst-yr corn fields, whereas in higher-population fields (continuous corn), the trap performed at 100% efficiency through 10 days. Their results indicate that Pherocon AM trap is a rather flexible sampling tool that can be installed for a duration that is convenient for the sampler.

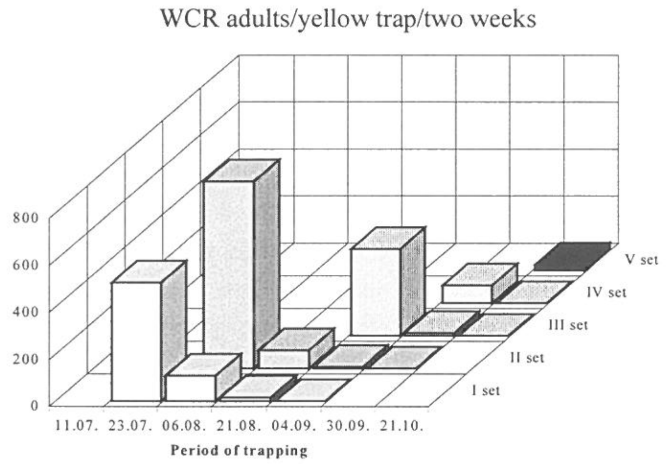

Figure 2 - Durability of Multigard yellow traps for *Diabrotica virgifera virgifera* Le Conte adults in Temerin (Yu), 1996.

We conclude that similar as Pherocon AM traps, Multigard yellow traps are very efficient during 14 (and maybe more) days in 2nd-yr corn fields.

The Cucs traps readily captured male and female WCR beetles, attracted by cucurbitacin as feeding stimulant and killed by carbaryl. As they were not sticky they were more convenient to determine sexual index on them (Table 2). In the middle of July males were predominant (89% of population), while at the end of August and at the beginning of September females became predominant (80%). This is similar to what was showed by Shaw *et al.* 1984 and Meinke *et al.* 1989.

Table 2 - Sexual index of *Diabrotica virgifera virgifera* in cucurbitacin traps (Temerin, 1996).

Period of collection in 1996		**Number of collected adults**	**Sexual index (in %)**	
dates	days		males	females
11 - 23 July	12	102	89,2	10,8
24 July - 6 August	14	138	77,5	22,5
7 - 21 August	15	127	48,8	51,2
22 August - 4 September	14	77	19,5	80,5

Comparing the average total catches among the traps during the whole season it was found that the highest number of WCR beetles per trap (1848) had been observed on yellow traps, much less (665) on pheromone traps and the smallest number (93) of beetles was captured on cucurbitacin traps. In all cases, yellow traps caught far more beetles than pheromone and especially cucurbitacin traps. This is oposite to the results from Hungary (Z. Ilovai., unpublished data 1996) where it was found that only few catches were observed on the yellow traps.

These results should be considered as preliminary because they were obtained after a year of study and with small number of traps to make statistical analysis valid. However, they show that yellow Multigard sticky and "Csalomon" pheromone traps can consistently sample WCR populations for extended periods of time. We agree with Hein & Tollefson's (1985) opinion that in fields near or below the economic treshold, an extended sampling technique may be more economically feasible than weekly plant-counting sampling. In these situations trapping procedures would be more usefull than plant-counting procedures in predicting the potential for larval damage.

The number of captured beetles on yellow traps in our experiment was much higher than Hein & Tollefson's (1985) economic threshold (6 beetles/trap/day) which has been used in USA for Pherocon AM traps. Also, the value of visual counting was significantly higher than nominal economic threshold which is suggested in USA of approximately one beetle per plant. Because of such high adult population great danger of damage by WCR larvae in the vicinity of Temerin during spring 1997 had been predicted, on corn grown continuously without chemical control.

In 1997 the investigations were continued at the same field and implemented with records about larvae damage.

ACKNOWLEDGMENT

Research reported here was partially supported by contract funds approved by the Ministry of Sciences and Technologies of Republic of Serbia(Project 12M17). Special thanks are due Dr. Franja Bača and Dr. Harald K. Berger (Institute for Phytomedicine, Wiena, Austria), for supplying us with powdered squash root, Cucs traps and Multigard traps and made our experiments possible.

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HARMFULNESS AND CONTROL OF THE WESTERN CORN ROOTWORM IN YUGOSLAVIA

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ABSTRACT

In Yugoslav conditions, the harmfulness of *D. virgifera virgifera* refers to damages caused by larvae in the corn root in repeated sowing or in monoculture. Damages from larvae in corn in plant rotation have not been registered. Imagoes are harmful in corn where the root is damaged by larvae. Damages from imagoes in corn in plant rotation have not been registered.

The main protection measure in corn from *D. virgifera virgifera* is plant rotation. The replacement of culture is the most efficient protection measure in corn. The application of soil insecticides with sowing is the optimal solution for control of larvae in infested corn. In the trial during 1996, soil insecticides based on terbufos and tebupirimphos showed the best efficiency against larvae.

Key words: Maize pests, *Diabrotica virgifera virgifera.*

INTRODUCTION

Maize has been grown in Yugoslavia for over 350 years (Camprag, 1994). Among cultivated plants, corn is grown in the largest areas. This mass growing is in considerable percentage achieved by corn growing in repeated sowing for one or several years.

Ever since it was brought to these areas, the main problems in corn growing were caused by local flora and fauna. The harmfulness, however, never had the level which would seriously endanger the production. Spread corn growing in repeated sowing led to the population growth of some harmful insects, such as *Tanymecus dilaticollis* Gyll (Camprag, 1994).

The appearance of a new insect species in Europe, *Diabrotica virgifera virgifera* LeConte, known in the North American Continent as one of the most significant corn pests, has worried all producers. Although there is a lot of research work about this pest in American literature and there are numerous publications, many questions have been raised as for its status of pest in new conditions.

The purpose of this work is to show the data gathered during the 1992-1997 period referring to the harmfulness of a new insect species in our conditions and ways of control being developed by us.

MATERIALS AND METHODS

Harmfulness

Monitoring the new territories population and areas of harmfulness was done in cooperation with 40 regional stations for plant protection. During 1993 - 1995 the visual method was used to find the presence or absence of imagoes at randomly selected fields. When the presence was found, imagoes were counted in 100 plants. During 1996 and 1997 pheromone traps were used. The number of damaged corn fields by *D. virgifera virgifera* was established by field check-up at regional level. The characterization of the new pest harmfulness was made on the basis of monitoring the attacked plants and corn fields.

Efficiency of soil insecticides

The experiments were set up on infested corn field (ZP 677) in monoculture in Glogonj with damages occurring in the last 2 years. Infestation was determined visually in 1996, during maximum adult density at the beginning of August. Population was heavy with over 20 imagoes per plant. On the yellow sticky trap "Multigard" - Scentry during one week (July 23 - 29), 632 imagoes were caught. All treatments as well as the untreated check were conducted in four repetitions. Plots were 6 rows wide and 11.9 m in length in a randomized block design. The insecticides were manually applied with sowing on the band, 15 cm wide, over the seeds and covered with soil. In order to find out the efficiency during the whole sowing period (April 10 - 30), the application of soil insecticides was made on April 11 and April 25. The evaluation of soil insecticides was determined on roots by rootworm damage ratings. Ten plants were randomly selected per plot during July 14 -18. Roots were removed, washed and brought to the lab. Root damages were evaluated on an Iowa scale 1-6.

RESULTS

Population and harmfulness spreading area of *D. virgifera virgifera* 1992-1996

When the presence of Western Corn Rootworm (*Diabrotica virgifera virgifera* LeConte) was registered in 1992, the population was already numerous, causing damages in corn. This indicates that, besides the pest was introduced much earlier, there are all prerequisites for development of the pest and its harmfulness manifestation on new territories. Corn in monoculture provides food for larvae and, besides the climate, soil and other factors, it is the factor which mostly contribute to the pest multiplication, thus contributing to its wide spreading.

Figure 1 shows how *D. virgifera virgifera* was spread in Serbia up to 1996. Considering that significant part of corn is grown in monoculture, the population was reproduced every year more and more. The air currents probably had an important role in its spreading.

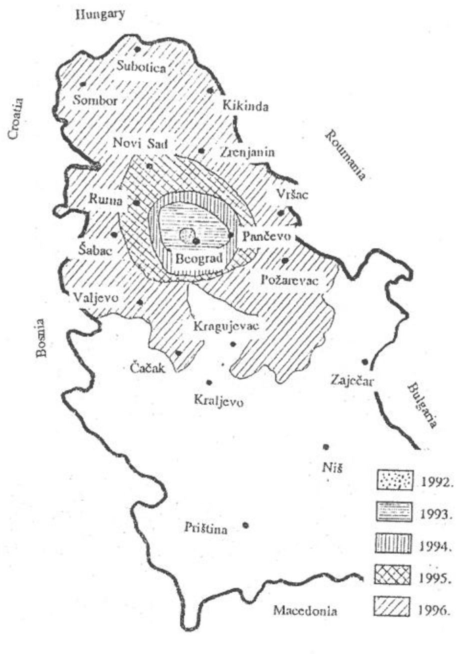

Figure 1 – Areas in Servia infested by *D. virgifera virgifera* in 1992 – 1996.

Figure 2 shows the areas with population reproduction to the extent when damages occur and how the harmfulness zone was spread during 5 years. It is interesting to note that within that period imagoes were populating the territories North, North-east and North-west of the place of introduction, while damages were spread in the direction East -West. Dominant winds during Summer are blowing from South-east and North-east direction.

Figure 2- Areas in Servia with damages from *D. virgifera virgifera* in 1992 – 1996.

The territory North of the introduction place (South Banat) is known by a great corn participation in monoculture and about 90% damaged fields were registered there in the period up to 1996. It is certain that in the following years the territory spreading where damages occur will be subject to corn presence in repeated sowing.

Harmfulness of *D. virgifera virgifera*

Monitoring the damages from 1993-1997 shows that the part of corn production in repeated sowing is significantly imperiled. The most significant are the damages which larvas made to the corn root sown in infested field. Registered corn fields damaged by larva *D. virgifera virgifera* within 1992-1996 are shown in Table 1. It was evaluated that the largest number of fields had yield decrease of 20%, but fields with 80% lower yield were also registered. Damages from larvas in corn root in plant rotation have not been yet registered. It was also found that the root of the first corn row in plant rotation could be attacked by larvas from the next infested field, but strongly damaged plants were never seen. In seed corn, typically grown in plant rotation, damages from larva were not registered. In other growing plants there were no damages from larva *D. virgifera virgifera* either.

Table 1 - Damaged corn fields registered in Yugoslavia 1992-1996

Year	Damaged corn fields
1992	0.5 ha
1993	6 ha
1994	60 ha
1995	275 ha
1996	10.787 ha

The difference in damage degree in corn root depending on the period of sowing is visible. The root of corn sown at the beginning of optimal sowing time (April 10) is less damaged than the root of plants sown at the end of optimal sowing time (April 30). The root of corn sown considerably out of optimal time, at the end of May, was not damaged.

Differences in damage manifestation in infested fields are noticeable. In years with enough rainfall, root regeneration and partial plant recovery take place already by the end of June and in July.

The heaviest damages occur in drought years. In 1996 the summer was drought in South Banat with only 21.8 l rain/m^2 in June. The damages were manifested in the strongest form also with a great number of lodged plants. Plants with less damaged roots remained upright and formed corncobs. A week before the harvest, however, there were abundant rainfalls which softened the soil by soaking, and a strong wind knocked these insufficiently rooted plants down and caused additional damage. Besides, weaker damage manifestation is visible in manured fields.

The significance of imago as a pest is considerably less. Mass imago occurrence is in corn damaged by larvas. It is frequent in such fields, especially in drought years, that plants with damaged root fall behind in the development, and their florescence period coincides with the maximum imagoes number. In those cases the silk is completely eaten up, and as a result the corncobs are with decreased number of kernels. In plant rotation corn being sown during the optimal period, there were no imago damages. In 1996 the harm by imago was registered in seed corn sown about two weeks after the optimal sowing period, as well as in sweet corn in lateral sowing which reached the florescence about midst of August. During July, in sunflower, soybean, alfalfa and other flowering plants, there were imagoes of *D. virgifera virgifera*, but no damages registered. In 1996, the imago attack was registered in the first half of July in *Cucurbitaceae* flowers.

Control of *D. virgifera virgifera*

The main measure of control of *D. virgifera virgifera* is plant rotation. It is well known to our farmers that plant rotation is effective preventive measure of control. It is enough to replace the type of plant for one year. Plant rotation introduction, however, is not a simple task for many small farms and requires economic steps. To induce the farmers to grow other plants, the Ministry of Agriculture develops the market and demand for those plants and their products. A considerable part of area with corn in monoculture is expected to be replaced with plants or their products in demand, such as wheat, sugar beet, lucerne and other forage plants.

Chemical protection of corn against *D. virgifera virgifera*

Although plant rotation is the most efficient and cheapest measure of control of *D. virgifera virgifera*, it seems that areas with corn in repeated sowing will not be completely substituted with other plants. The possibility of insecticides was, therefore, considered in corn protection as well.

Preventive measure eliminating danger of damages by imagoes is corn sowing within the optimal period. Control of *D. virgifera virgifera* imagoes with insecticides, as a preventive measure to decrease the number of eggs laid, is difficult to be done because of small size of fields and variety of plants growing there. Considering that control of imagoes should be done by the end of July or beginning of August, when the corn is high up, only application from air is possible. Besides, efficiency of insecticides applied and number of treatments required for the successful preventive measure are factors which will direct the farmers to prefer the plant rotation and soil insecticides for control of larvae. Problems occur as well when choosing the insecticides. In August 1996 a strong imagoes attack was registered in sweet corn. Due to law restrictions concerning the pre-harvest timing of insecticides application, it was possible to use only pyrethroid in sweet corn which did not give good results at the temperature of 35^0C. Because of these problems with imago in 1997, the producer gave up sweet corn sowing during July and decided for sowing in April to avoid imagoes attack.

For these reasons, the major application of insecticides is expected to be in control of larvae. Optimal application of soil insecticides is with sowing. Sowing is done during the second and third decade of April, and the preparation applied should provide the protection of corn root against larvae appearing at the end of May, and largely in June. Field trials testing the efficiency of soil insecticides have been carried out since 1993. No soil insecticide has so far got permanent license. It is expected that the first soil insecticide to get the registration will be the one on the basis of terbufos.

Results of biological efficiency of soil insecticides in 1997

The degree of harmed root plants from early treatment (April 11) and late treatment (April 25) is considerably different. The larvae attack was more strongly manifested in plants of later sowing and differences in treatments were easy to be seen there. All samples of roots from trials in both treatments were attacked and more or less damaged.

The results of biological efficiency of applied soil insecticides along with sowing at the beginning of optimal sowing period (April 11) are shown in Table 2. Average degree of corn root damage in treatments with terbufos and tebupirimphos in both doses is the lowest and within the tolerance limits. The best efficiency was shown with terbufos in the quantity of 1250 gr a. i/ha (Counter G-5 25 kg/ha).

The results of biological efficiency of applied soil insecticides along with sowing at the end of optimal sowing period (April 25) are shown in Table 3. Average degree of corn root damage in treatments with terbufos and tebupirimphos in both doses is also the lowest in this treatment and within the tolerance limits. The best efficiency was shown with terbufos again in the quantity of 1250 gr a. i/ha (Counter G-5 25 kg/ha).

Based on the results of the trial, it can be concluded that only two soil insecticides, terbufos and tebupirimphos + cyfluthrin have shown good efficiency in control of *D. virgifera virgifera* larvas.

Table 2 -Efficiency of soil insecticides against corn rootworm larvae applied with sowing on April 11, 1997.

Treatments	Rates kg/ha l/ha	Active ingredient	Root damage ratings (1 - 6) Mean/LSD
Counter G 5	25 kg	terbufos	2.350 a*
Arriba 2.1 GR	6 kg	tebupirimfos + cyfluthrin	2.475 ab
Counter G 5	20 kg	terbufos	2.575 ab
Arriba 2.1 GR	8 kg	tebupirimfos + cyfluthrin	2.650 ab
Regent 200 FS	1 l	fipronil	3.250 bc
Dursban G 7.5	25 kg	chlorpyrifos	3.575 cd
Dotan G 5	10 kg	chlormephos	3.600 cd
Dursban G 7.5	15 kg	chlorpyrifos	3.650 cd
Regent 5 GR	2.5 kg	fipronil	3.725 cd
Regent 5 GR	5 kg	fipronil	3.775 cd
Dursban G 7.5	20 kg	chlorpyrifos	3.850 cd
Nurelle D	3 l	Chlorpyrifos + cypermethrin	3.875 cd
Check	untreated		4.025 cd
Dotan G 5	12 kg	chlormephos	4.175 d

*Values followed by the same letter are not significantly different at the 5% (LSD test)

Table 3 -Efficiency of soil insecticides against corn rootworm larvae applied with sowing on April 25, 1997.

Treatments	Rates kg/ha l/ha	Active ingredient	Root damage ratings (1 - 6) Mean/LSD
Counter G 5	25 kg	Terbufos	2.100 a*
Arriba 2.1 GR	8 kg	tebupirimfos + cyfluthrin	2.425 a
Arriba 2.1 GR	6 kg	tebupirimfos + cyfluthrin	2.475 a
Counter G 5	20 kg	Terbufos	2.750 ab
Dursban G 7.5	25 kg	Chlorpyrifos	3.700 b
Nurelle D	3 l	Chlorpyrifos + cypermethrin	3.775 c
Regent 200 FS	1 l	Fipronil	3.825 c
Dursban G 7.5	15 kg	Chlorpyrifos	3.975 c
Check	untreated		4.400 c
Regent 5 GR	4 kg	Fipronil	4.450 c
Dotan 5 G	12 kg	Chlormephos	4.600 c
Regent 5 GR	2 kg	Fipronil	4.600 c
Dotan G 5	12 kg	Chlormephos	4.675 c
Dursban G 7.5	20 kg	Chlorpyrifos	4.700 c

* Values followed by the same letter are not significantly different at the 5% (LSD test).

Comparing early and late treatments it is obvious that there are no significant variations in the efficiency of both insecticides. In 1997, later appearance of larvas and imagoes has been registered for the first time which can be ascribed to somewhat lower temperatures and abundant rainfall. Good efficiency of only two insecticides in 1997 is probably being shown owing to their sufficiently long persistence and slow mobility in soil.

One possible way of more complete documentation of efficiency and differences between applied soil insecticides is the distribution of frequency of the evaluation of corn root damage degree (Table 4 and 5). Acceptable values of damage evaluation are 1, 2 and 3. It is shown in the Table that with the most efficient preparations there are stronger plant damages as well. In contrast to the plant rotation, the application of soil insecticides decreases the root damage to the tolerant degree.

Table 4 -Frequency distribution of root damage ratings (Iowa scale 1-6), when insecticides were applied with sowing on April 11

Treatment	Root damage ratings Number of plants						n=
	1	2	3	4	5	6	
Counter G-5 25 kg/ha	0	27	12	1	0	0	40
Counter G-5 20 kg/ha	0	22	15	3	0	0	40
Arriba 2.1 GR 8 kg/ha	0	15	24	1	0	0	40
Arriba 2.1 GR 6 kg/ha	0	24	13	3	0	0	40
Check	0	3	9	14	12	2	40

Table 5 -Frequency distribution of root damage ratings (Iowa scale 1-6), when insecticides were applied at sowing on April 25

Treatment	Root damage ratings Number of plants						n=
	1	2	3	4	5	6	
Counter G-5 25 kg/ha	0	36	4	0	0	0	40
Counter G-5 20 kg/ha	0	21	11	5	3	0	40
Arriba 2.1 GR 8 kg/ha	0	29	7	2	2	0	40
Arriba 2.1 GR 6 kg/ha	0	27	8	4	1	0	40
Check	0	2	6	10	18	4	40

CONCLUSIONS

Since 1992, when damages from *D. virgifera virgifera* were registered for the first time, until 1996, the pest has populated the main production areas of corn in Serbia and Yugoslavia. Dominant direction of the new pest expansion is towards the North.

During the 1992-1996 period, considerable damages in corn appeared in repeated sowing. The largest number of damaged cornfields was in South Banat, where monoculture was greatly used.

In infested cornfields great damages are caused by larvas of *D. virgifera virgifera.* The yield decrease goes up to 80%, but in 1996, for example, in most fields the yield decrease was 20%. Imagoes have sporadic significance and rarely cause damages.

The main way of control of *D. Virgifera virgifera* is the plant rotation. Its mass application is subject to the market demand for production of various plants.

The plant rotation alternative is the application of soil insecticides. In field trials, the best efficiency against larvas of *D. virgifera virgifera* have been shown by insecticides terbufos (Counter G-5) and tebupirimphos + cyfluthrin (Arriba 2.1 GR).

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ESTABLISHMENT POTENTIAL OF THE WESTERN CORN ROOTWORM (*DIABROTICA VIRGIFERA*) IN GERMANY

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ABSTRACT

D. virgifera would find maize in all regions of Germany but the most endangered areas, those with the highest maize concentration, are the Northwest and the South of Germany. The climatic conditions in Winter favour an establishment in Germany and mortality is expected to be lower than in the native areas. The temperature sum model calculated for an average of 9 years indicate that 50% of the first larvae would emerge in Germany between 10th June (Southwest) and 20th July (North). In most parts of Germany 50% of the first larvae would appear between 23rd June and 08th July. So the third instar as the most damaging stage would be expected between 30th June and 20th August. First adults would appear in the Rhein-Main area (Southwest) and in East Germany between 10th and 27th July and in other parts between 27th July and 13th August. Apart from extremely cold years in North of Germany (the North of Schleswig-Holstein) populations would be able to multiply.

Germany has suitable climatic and trophic conditions for the establishment of *D. virgifera*.

Key words: Maize pests, *Diabrotica virgifera virgifera*.

INTRODUCTION

The leaf beetle species *Diabrotica virgifera* is the most serious insect pest on maize in the USA and Canada and causes sometimes considerable crop losses (Krysan & Miller, 1986; Mcbride, 1972; Perez-Dominguez & Najera-Rincon, 1991; Spike & Tollefson, 1991; Csamprag *et al.*, 1994). Since the introduction in Europe (Yugoslavia) in 1992 the pest has spread to most of the neighbouring countries. Therefore, it is to be expected that the pest will reach Germany, too.

The establishment potential is a part of pest risk assessment. Host availability and climatic conditions are the main factors for the establishment. The analysis of these factors will allow to predict the development conditions and finally the possibility of the establishment of *D. virgifera* in Germany.

MATERIALS AND METHODS

At first we analysed host distribution in Germany. Maize concentration and especially continuous maize were defined for several regions.

Further, we performed climatic comparisons for the Winter time between the region of origin in the USA and local conditions. In regard of mortality of the overwintering eggs the January long-term mean temperatures over 25 years from 5 representative meteorological stations were calculated because this month is the coldest in Germany.

The influence of the climatic conditions (temperatures) on the development of the Western Corn Rootworm in the growing season was involved by means of temperature sum models. The emergence of the individual development stages was calculated for 38 localities throughout Germany for an average of 9 years (110,000 data) and in addition for a year (1992) warmer and a year (1987) colder than average (years with extreme weather conditions).

The temperature sums for larvae are based on the lower development threshold of 11 °C and a development time of 265 degree-days, which were determined for Ontario in Canada (Schaafsma, 1991). This basis development temperature was used because the thresholds and sums from the USA, South Dakota (Fischer, 1989; Jackson & Elliot, 1988) and Illinois (Levine *et al.* 1992) are not as suitable for German conditions as the Canadian ones. To predict the other stages, the only available lower development threshold of 9 °C and development times of degree-days of JACKSON and Elliot (1988) were involved in further simulation.

Host

Maize is the main host of *D. virgifera*. Adults feed on leaves, pollen and later on silks. Most damage is caused by the larvae, which feed on roots. In Germany 1.6 million ha are cultivated with maize. They occupy more than one third of the total maize-growing area of the EC (3.8 million ha). Therefore, Germany has beside France (1.7 million ha) the largest maize-growing area in the EC (Stratmann *et al.*, 1997).

Maize differs in concentration in Germany from region to region and varies between less than 5% and more than one fifth of arable land (Figure1).

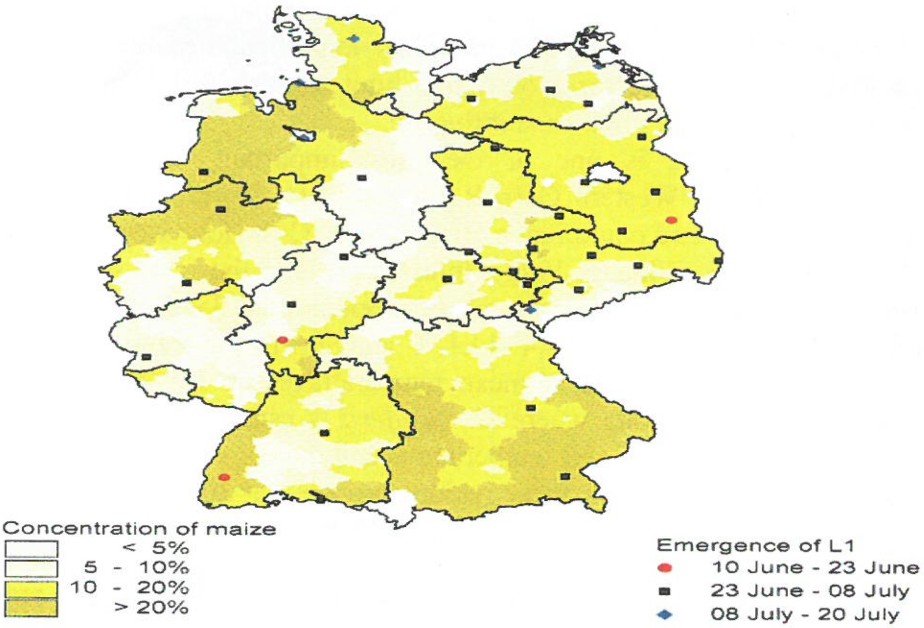

Figure 1- Simulation of time of emergence (model) of the first instar of *Diabrotica virgifera* for Germany

The highest concentration of maize is located in the South of Baden-Württemberg, in the South of Bayern and in the Northwest of Niedersachsen and Nordrhein-Westfalen. High potential risk arises from areas with maize after maize in crop rotation. They cover some regions in the Southwest of Bayern more than 80% of the arable land. More than one tenth of the maize-growing area (15,000 ha) in Baden-Württemberg and more than 70% in the Oberrhein region are cultivated with continuous maize. Some farms grow maize as monoculture thus providing the best conditions for the build-up of a high abundance of the Western Corn Rootworms. Especially these regions are endangered by Western Corn Rootworm.

<u>Climatic conditions in Winter time</u>

The establishment of a pest is most dependent on the climatic conditions in a region. Moisture and temperature have an influence on mortality, generation time and increase of population are finally responsible for the survival of the insect. *D. virgifera* has no particular requirements for moisture so that this factor would not limit an establishment area of Germany, which is under maritime influence.

So, temperature is one of the most important factors for the establishment of the Western Corn Rootworm. Egg mortality depends on Winter temperature and could reach 50% at - 10 °C for four weeks and at - 15 °C for one week (Chiang, 1973). Infested areas in the USA show great differences in January long-term mean temperatures. January long-term mean temperatures range from 4.4 -14.1 °C and reach an average of -4.8 °C. The coldest January long-term mean temperatures, up to -14.1 °C, are registered in North Dakota where *D. virgifera* is established. The above-mentioned temperatures are air temperatures and the eggs overwinter in soil at a depth of 15 to 30 cm, in these regions probably at 30 cm or more. An adaptation of the population to stronger Winter conditions cannot be excluded.

In Germany long-term Winter temperatures are not as cold as in USA because Central Europe is influenced by maritime climatic conditions. January long-term mean temperatures range from 2.8 to -3.8 °C and are on average -0.5 °C. Coldest temperatures in the Southeast of Germany, mainly in Bayern, amount up to -3.8 °C and are far above the critical point of -10 °C. For 25 years soil temperatures of -5 °C and less at 5 cm depth were reached only in 45 to 107 days. The coldest soil temperature was -13 °C in one day.

Eggs normally require a cold-induced diapause period before they will hatch. A mild chill temperature of 7.5 °C would be more suitable than lower temperatures of 0 or 5 °C over winter time (Chiang, 1974). This minimum chill temperature would be sufficient in Germany to induce diapause. Germany provides suitable Winter conditions for the overwintering of the Western Corn Rootworm. Winter mortality rate is expected to be low.

<u>Period of development</u>

Temperatures in the growing season are responsible for the successful finishing of a generation, increase of population and finally the establishment of the species.

The model calculated for an average of 9 years indicate that 50% of the first instar of *D. virgifera* would emerge between 10th and 23rd June in the Rhein valley (southwest) and in the Southeast of the Federal State Brandenburg (Fig.1). The Rhein valley has a special microclimate and Brandenburg is already under the influence of continental climate. In most parts of Germany 50% of the first larvae would appear between 23rd June and 08th July. Only in the North (Niedersachsen, Schleswig-Holstein and Mecklenburg-Vorpommern) half the population of the first larvae would hatch between 8th and 20th July (Figure 1).

Third instar as the most damaging stage are expected to hatch between 30th June and 17th July in Rhein valley and in large parts of Brandenburg, Sachsen and Sachsen-Anhalt (Figure 2). In most parts of South, West and North Germany the third instar emerges between 17th July and 3rd August. Only at the North See and in Schleswig-Holstein this larval stage appears between 3rd and 20th August.

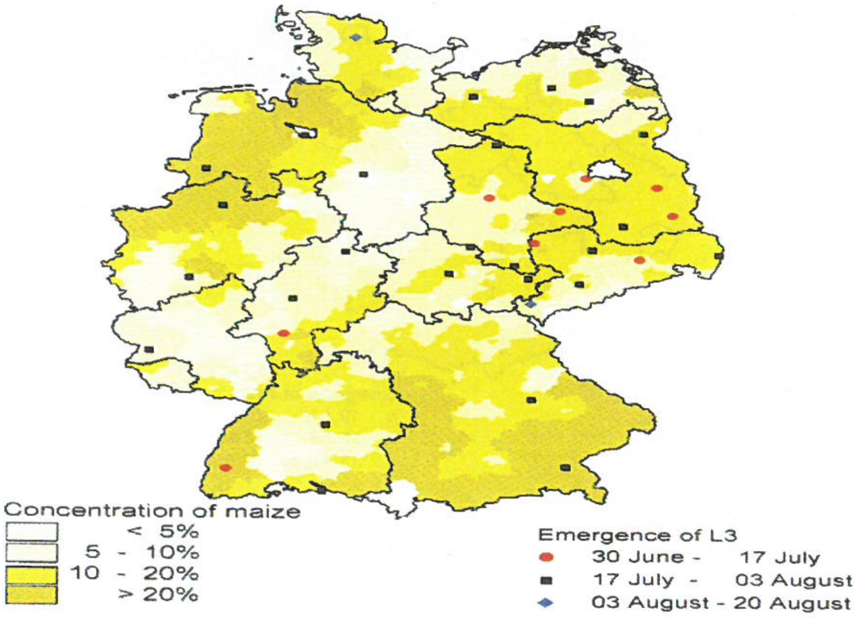

Figure 2 -Simulation of time of emergence (model) of the third instar of *Diabrotica virgifera* for Germany.

First adults would appear in the Rhein-Main region and in East Germany (parts of Brandenburg and Sachsen) between 10th and 27th July and in the main part between 27th July and 13th August (Figure 3). As to the third instar late emergence (13th to 30th August) would be expected in North Germany (at the North See and in Schleswig-Holstein). Time of emergence has greater differences for adults in the different regions of Germany than time for the first larvae. In spite of this the adults of the Western Corn Rootworm are able to finishing the life cycle in the average of the years in all Germany.

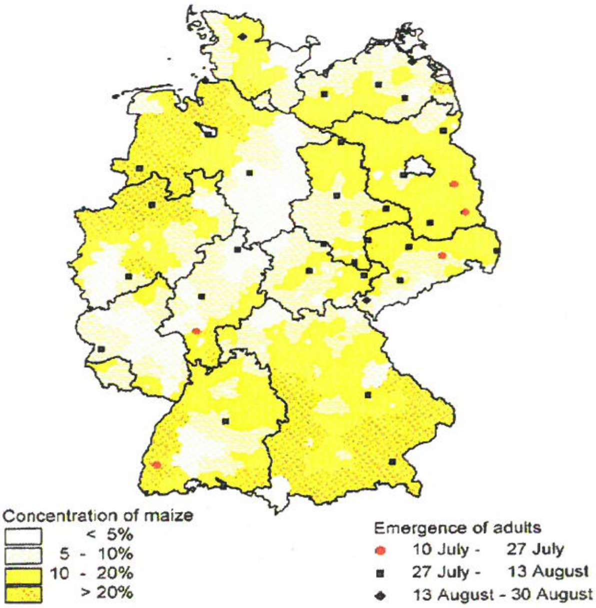

Figure 3 -Simulation of time of emergence (model) of the adults of *Diabrotica virgifera* for Germany.

In extremely warm (1992) and cold years (1987) the first larvae would have emerged in the Southwest on 5th June and 27th June respectively and in the North on 28th June and 21st August respectively (Figure 4). In cold years emergence of first instar would be much later in the North (Schleswig-

Holstein) than in the Southwest (Rhein valley). In addition the differences of appearance in the same regions between extremely warm and cold years would be greater in the North (54 days) than in the Southwest (22 days).

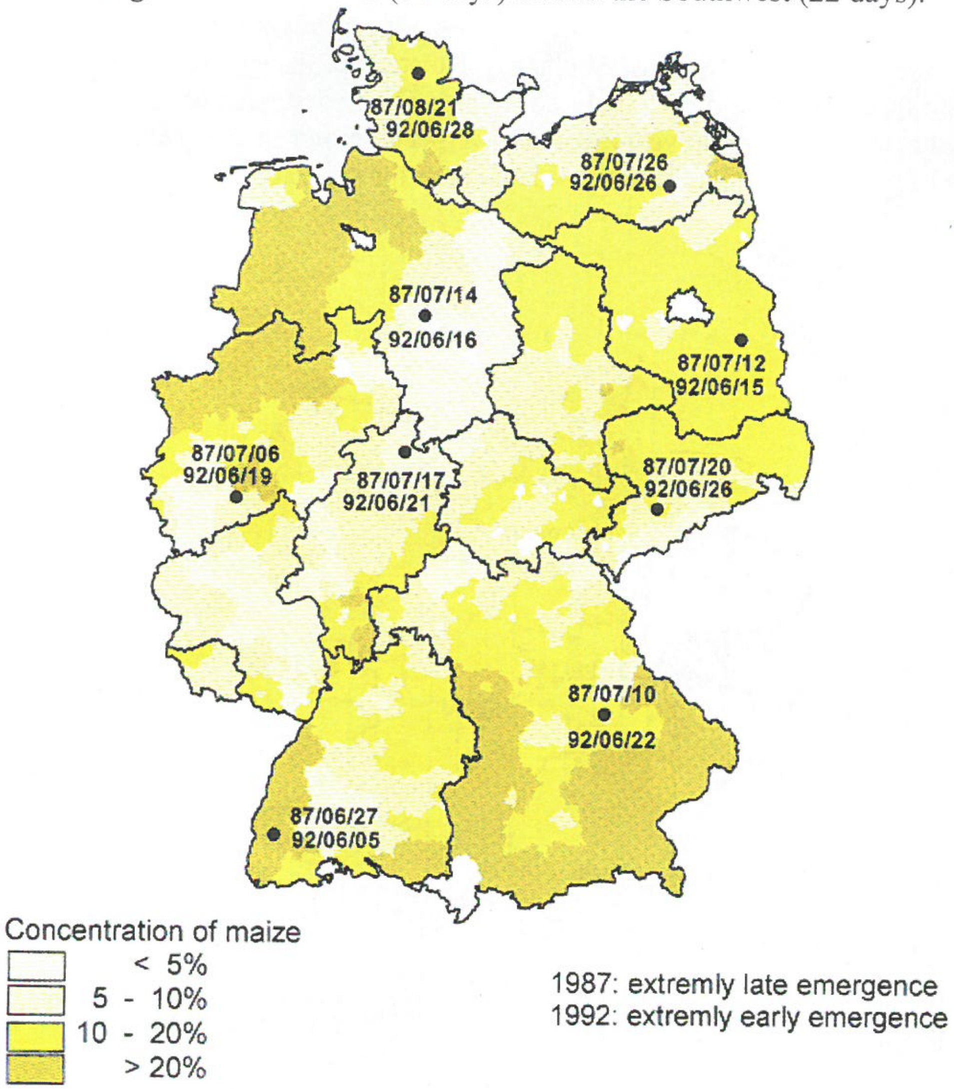

Figure 4 -Simulation of time for an extremely late (1987) and extremely early year (1992) of emergence (model) of the larvae of *Diabrotica virgifera* for Germany.

Under these conditions a similar phenomenon would have appeared for adults. The adults would have been expected between 14th (extremely warm) and 29th July (extremely cold) in the Southwest and between 30th July and 11th October in the North (Mecklenburg-Vorpommern) (Figure 5). In one case, in the North of Schleswig-Holstein adults would not have been able to appear in the extremely cold year. The differences of emergence of adults in such extreme years would have been greater in the North (73 days) and smaller in the Southwest (15 days) than for first larvae.

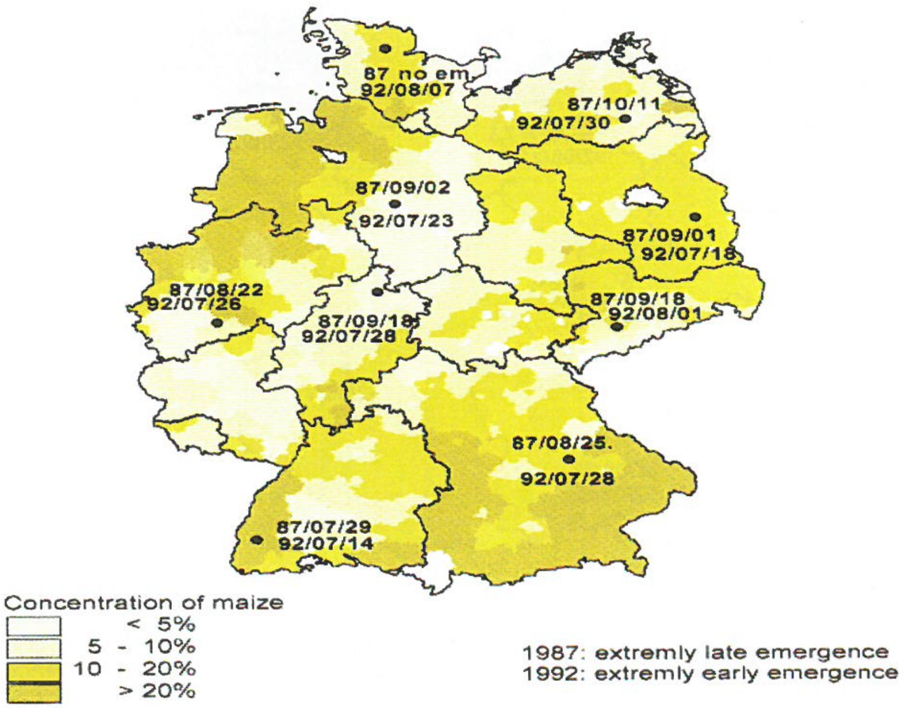

Figure 5 -Simulation of time for an extremely late (1987) and extremely early year (1992) of emergence (model) of the adults of *Diabrotica virgifera* for Germany.

CONCLUSIONS

D. virgifera would find its host maize throughout Germany and the climatic conditions are suitable for the development of this species. The most endangered area is the South of Germany. It has the highest maize-growing concentrations as well as continuous maize and the best climatic conditions for the development of the generations. Therefore, in this area the German Plant Protection Service started monitoring with cucurbin attractants in 1997. Beside this area the Southeast of Germany (Bayern) and the Northwest (Niedersachsen and Nordrhein-Westfalen) have also a high maize concentration (including continuous maize areas) and suitable climatic conditions for the multiplication of populations. In these areas the Western Corn Rootworm could become an important maize pest in Germany.

The Winter conditions are not a limiting factor for the establishment. In the contrary a lower Winter mortality than in the USA would be expected. Apart from extremely cold years in the North of Germany (Schleswig-Holstein) populations would be able to finish the generation and increase abundance. This guarantees the establishment of the non-native pest in Germany. Crop rotation could limit losses, but would not prevent an establishment (Baufeld *et al.*, 1996).

The assessment of the establishment potential in Germany reveals that the Western Corn Rootworm would find suitable climatic and trophic conditions. *D. virgifera* has not reached its ecological range in Europe up to now. Therefore, the maize pest is able to spread further on throughout Europe and the pest risk for Germany as well as for other maize-growing European countries is high.

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APPEARANCE AND SPREAD OF THE WESTERN CORN ROOTWORM *(DIABROTICA VIRGIFERA VIRGIFERA)* IN CENTRAL-EASTERN EUROPE

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ABSTRACT

The Western Corn Rootworm *Diabrotica virgifera virgifera* Le Conte, was imported to Europe probably througt the Belgrade airport in 1988-90. Its damage to plants has been registered since 1992. In 1996 Sincsev and Dragonics published a report on dispersal of WCR in Serbia, wich was prepared on the basis of yearly monitoring data. Since 1994 the IOBC IWGO working group has been co-ordinating a monitorig of the pest´s dispersal in Europe by the harmonization of recording methods. Besides traditionally used cucurbitacin and yellow sticky-traps a pheromone plain-capsules-traps developed by IPP HAS (Tòth *et al.*, 1996) seems to be the most effective for trapping.

In 1993 study was done in Serbia on the forms of *Diabrotica virgifera virgfera* injury and circle of host-plants. Experiments were carried out on sex-pheromone traps for comparison of effectiveness of plain and deltoid form of traps. In 1995 panel traps were successfully applied in the monitoring program of WCR. In 1996 a county-wide system was worked out for records of the presence of the pest. This system is based on Integrated Forecasting System. The density of traps placed was determined on the basis of phytogeographical an ecological corn-growing areas.

The new generation of WCR adults feeds on silks, leaves and in the end of flowering period on young cods. Larval infestation of roots causes "gooseneck". Due to the damage, plants fall down whit stronger winds. A macroscopic examination of roots shows "corridors" made by larvae in the root tissue. Besides corn, feeding on beans leaves and sunflower was observed.

The application of pheromone traps proved a head spreading of WCR. However, no damage was registered, even at places with high number of trapped beetles. According to Serbian observation, eggs are lied in concentrated areas, Beetles are able to migrate for a distance of 80-150 kilometres far from the place of hatching. The data on trapped adults collected in 1996 predestine a probablity of damage in 1997 year.

In 1997 the FAO Technical Co-operation Programme on development and implementation of containment and control of WCR in Europe has been started. It contains the monitoring of the newcomer by the apllication of sex-pheromone traps in the concerned countries. A trapping insecticide material is going to be tested in demonstrative experiments in Hungary and Serbia on ane area of 988 hectares. There is a need for research on materials for females mass-trapping. This could limit a massmultiplying of the pest in zones of monoculture and also restrict its further spreading in Europe.

The WCR ability to adapt itself to the new conditions and the absence of its natural enemies, makes the study on ethology and ecology of this potentially dangerous pest a especially important international task.

Key words: Maize pests, *Diabrotica virgifera virgifera*

INTRODUCTION

The appearance of the Western Corn Rootworm, WCR (*Diabrotica virgifera virgifera* Le Conte) in Yugoslavia opened a new period in the European maize production. After its first detection in Yugoslavia in 1992 (Bača, 1994), the pest has reached the neighbouring countries and its occurrence is already known in Hungary, Romania and Croatia. In the first years after the establishment, Yugoslavian authors reported about the pest in several publications (Bača, 1994; SivČev *et al.* 1994; SivČev *et al.* 1996; Sivcev and Draganic, 1996). The book published with the edition of Čamprag (1995) summarizes the most important knowledge on the pest, its biology and ecological conditions, the expected directions of the European spread, available methods for the detection and control of WCR.

After being aware of the occurrence of *D. virgifera virgifera* in Yugoslavia, the Hungarian plant protection organization made steps in order to obtain as many information as possible on the pest. The major steps were as follows:

- Publication of the first Hungarian language article on *D. virgifera virgifera* (Čamprag, D. - Sekulic, R. - Bača, F.:AGROFÓRUM, 1994. 5 (7) 41-42.)
- Invitation of Yugoslav entomologists to Hungary to give lectures about WCR (Hódmezõvásárhely, July 1994 and Budapest, February 1995)
- Lecture of Prof. Richard Edwards on WCR and a field visit at starting monitoring (July 1995)
- Visit of Hungarian entomologists to Yugoslavia to study WCR *in situ* (July and August 1995)
- Organization of the 2nd International Workshop on *Diabrotica* together with the Gödöllõ Agricultural University (November 1995), laying down "Recommendations" approved by the participants.

According to the statement of the 1st International *Diabrotica* Workshop (Berger, 1996), from July 1, 1995 a monitoring system has also been set up in Hungary. As a result of that the first WCR individuals were found for the first time in the Summer of 1995 in Southern counties, near to the Yugoslav and Rumanian border.

This new situation brought up the need for extending the former bilateral Hungarian-Yugoslavian cooperation to the adjacent countries within a FAO financial support.Therefore, from 1997, monitoring studies have been conducted with the joint participation of the related countries, Hungary, Romania, Yugoslavia, Croatia and Bosnia, in a agreed way within the FAO TCP framework. The present work gives account of the results of the three year monitoring of WCR in Hungary.

MATERIALS AND METHODS

In 1995, monitoring studies were organized according to the statement of the first *Diabrotica* workshop in Graz.

The Plant Protection Department of the Ministry of Agriculture organized the monitoring network on the territory of plant health and soil conservation stations of four counties adjacent to the border of Yugoslavia, Croatia and Romania. Systematic detection has begun in 20 districts of the 400 km long border zone. Monitoring sites are shown in Figure 2.

Traps baited with cucurbitacin were placed on 29 June 1995. The traps were fixed near the developing cobs of the marked maize plants, 50 m far from the edge of the indicated maize fields. Five traps were used at all the twenty sites from 15 August to 15 September. Besides, one pair of pheromone traps was set up at each of the further six places (especially on international roads, at Budapest airport and the duty-free port of the Danube).

From 1996 the monitoring system has been extended to the whole territory of the country. During the establishment of the monitoring system the organizational framework of the national Integrated Forecasting Network was taken into consideration. The special detection system was based on forecasting regions as well as phytogeographical properties, agri-ecological districts and maize growing areas. The territory of the country was divided into three zones illustrated by Figure 1. It was followed by the determination of monitoring sites in the particular zones by the entomologists and forecasting specialists of the county plant protection stations on the bases of acreage and intensity of maize production. Thus in zone I, II and III 70, 51 and 37 sites were determined, respectively. Later on these monitoring sites were completed by observations in other institutes and farms. Altogether, catches from 162 fields were analyzed in 1996. In 1997, considering the presumably higher number of beetles, number of monitoring sites was increased. Monitoring was based on the use of sex-pheromone traps type "CSALOMON".

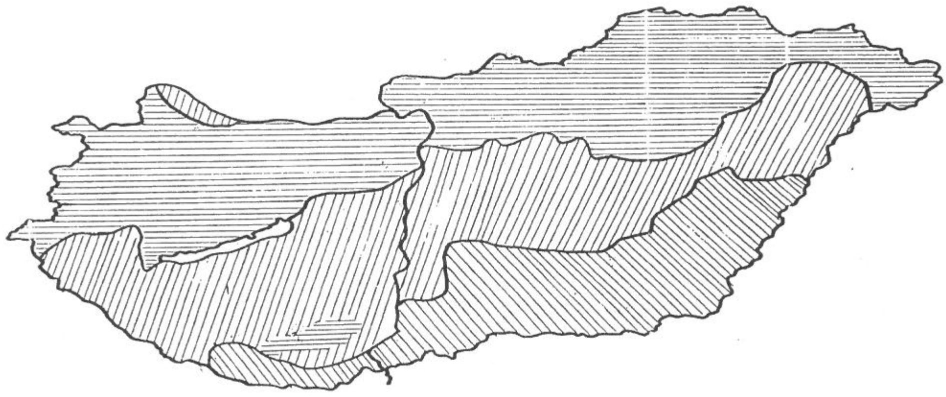

Figure 1 – Zones of the monitoring system for western corn worm in Hungary, 1996

Monitoring was made in July, August and September, using one trap in each field. Dispensers were renewed in every four weeks. Captures were assessed weekly and the captured insects were removed from the sticky surface. In 1996 at one site per county of zone I yellow sticky traps (Ferocon) and traps baited with cucurbitacin were also set up. Catches were summed up as well as the appearance and spread of the species were plotted on a Grid map. At the end of the trapping, plants were also analyzed at each catching site to assess eventual damage caused by the pest. In 1997 in the frame of the FAO TCP line and consecutive trapping were also introduced in zone I, with sex-pheromone and yellow sticky traps type "Multigard" being paralelly used at every 7-10 km. In zone II, the number of observation sites was doubled. Places with pest occurrence in the previous year were checked for the presence of larvae or root damage.

The monitoring system staff is based on entomologists, forecasting specialists and phytosanitary inspectors working at the plant health stations. They learnt the trapping techniques, the properties of the pest and types of damages. Two regional training courses were organized. A slide collection was made on developmental stages of WCR and on the types of damage.

RESULTS

The appearance of Western Corn Rootworm in Hungary and its spread in 1995, 1996 and 1997 are shown in Figures 2, 3 and 4.

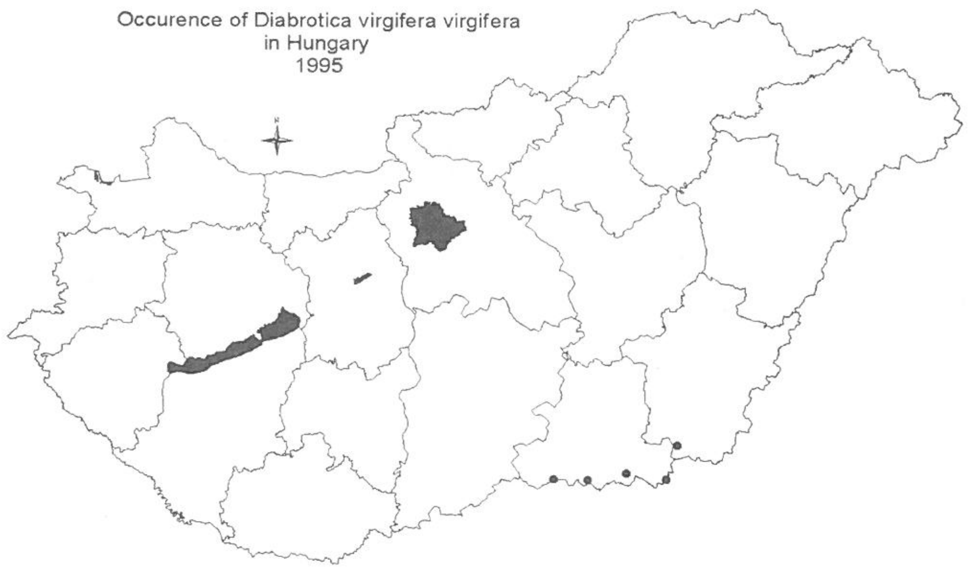

Figure 2 – Ocorrence of *Diabrotica virgifera virgifera* in Hungary 1995

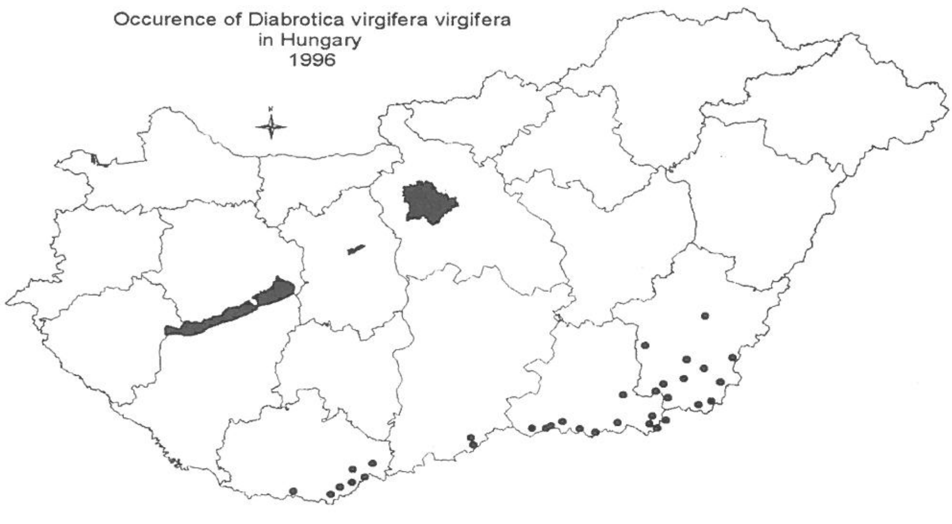

Figure 3 - Ocorrence of *Diabrotica virgifera virgifera* in Hungary 1996

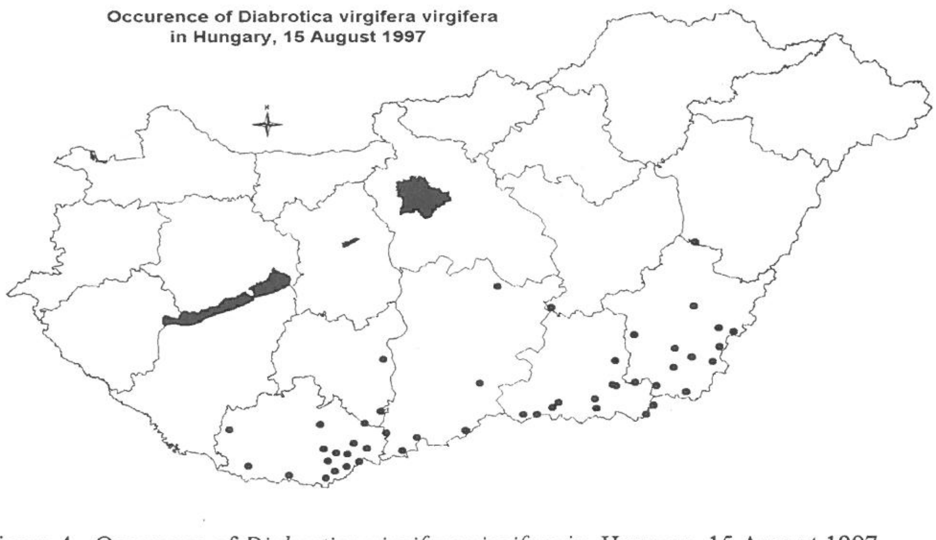

Figure 4 - Ocorrence of *Diabrotica virgifera virgifera* in Hungary, 15 August 1997

As a result of the monitoring, Western Corn Rootworm was found in maize fields in Hungary in 1995. The first adult of the pest was found in a cucurbitacin trap on 30 June 1995 near Mórahalom (Csipak). The female adult was identified with the participation of Prof. R. C. Edwards. Later on, this type of traps had no catches here or at any other monitoring sites.

From 15 August males of *D. virgifera virgifera* were found in sex-pheromone traps set up at other places (Table 1). Between 21 and 28 August the pest was identified at four monitoring sites along the Yugoslav and Rumanian border. In all cases the insect was observed only once in the traps.

During monitoring in 1995 only the adults of the pest were observed in maize, no form of damage could be observed. After the first occurrence of *D. virgifera virgifera* in Hungary, we informed the responsible organization of the countries most exposed to the risk of the pest, Rumania and Croatia.

In 1996 instead of the 5 observation sites of 1995, adults were caught at 36 sites.

Table 1 -Data on monitoring the western corn rootworm (*Diabrotica vir*gifera *virgifera)* in Hungary in 1995

No	Date	Locality, code	Trap type	Caught adult		Damage on		
				sex	piece	leaf	silk	root
1	30. jun	Csipak, 11	Cucurbitacin	Female	1	0	0	0
2	21. aug	Tiszasziget, 13	Sex pheromone	Male	5	0	0	0
3	22. aug	Nagylak, 15	Sex pheromone	Male	3	0	0	0
4	23. aug	Mezõhegyes, 16	Sex pheromone	Male	5	0	0	0
5	28. aug	Kiszombor, 14	Sex pheromone	Male	2	0	0	0

All catches were observed in zone I (Tables 2 and 3). The spread of the species is demonstrated by Figure 2. At present, the observation zone covers an area of 210 km wide and 40 km deep, including 30 % of zone I. The activity of the adults is continuous from late July to September, with most catches of adults in August. The highest activity was observed in late July - early August (Figure 5).

Table 2 - Distribution of *Diabrotica virgifera virgifera* in different zones of Hungary, 1996

Zones	Number of plots observed			Results of trapping	
	all	pest found in	%	adults number	%
I. zone	70	31	44	1191	100
II. zone	51	0	0	0	0
III. zone	37	0	0	0	0

A comparative study was conducted to evaluate traps at some locations and it was found that only a few catches were observed on the yellow traps (Table 3). The plant and root analysis made in 1996 in some areas showed no damages. However, the ratio of population increase was 1 to 19 as an average.

Table 3 - Catches of *Diabrotica virgifera virgifera* by different traps

County	Cucurbitacin				Yellow				Sex pheromone			
	July	Aug	Sept	All	July	Aug	Sept	All	July	Aug	Sept	All
Baranya	-	-	-	-	-	-	-	-	4	10	1	15
Csongrád	-	-	-	-	2	5	1	8	369	356	74	799
Békés	-	-	-	-	0	5	0	5	60	260	30	350
Bács	-	-	-	-	-	-	-	-	3	10	1	14

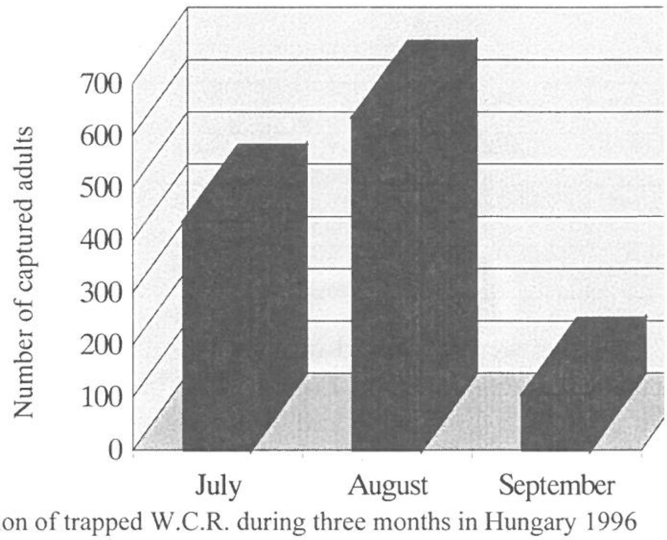

Figure 5 – Distribution of trapped W.C.R. during three months in Hungary 1996

In spite of the large number of catches in 1996, no damages and symptoms could be recorded.

Results obtained up to now in 1997 show further spread of the pest (Figure 4). Adults were observed in other counties or at new places by the specialists of the county station, at 58 locations till the middle of August. WCR is distributed in an area of about 300 x 100-120 km from the Southern and Eastern boundaries of Hungary. At some places along the Southern border the pest can be found in spots, where the adults were observed in greater quantities in 1996 with a population density extremely high (e.g. at Szeged, Csanádpalota). At such places even larvae were found in 1997, but lodged plants and the economic losses caused by this damage have not been observed.

Up to now adults of WCR have been found on maize, sunflower, beans, soybean, alfalfa, squash and among weeds as ragweed *(ambrosia)* and pigweed (*Amaranthus retroflexus*). On the firs three host plants adults were feeding on leaves and flowers, while larvae were feeding on maize roots.

DISCUSSIONS

The Western Corn Rootworm, spreading from Yugoslavia, has reached the Southern part of Hungary in the Summer of 1995 and some adults were recorded in the cucurbitacin or sex-pheromone traps set up at the observation sites.

In 1996 the pest spread further on in a width of 200 km and depth of 40 km, stepping over the Danube line. In 1997 the species continued its way to the North, and adults were found in the sex-pheromone traps in a depth of 100-120 km from the border. Population density is changing, but it is generally low, except in some locations along the Southern and South-Eastern border of Hungary, where adults were observed in greater quantities, in some localities. At these places even larvae were found for the first time in 1997. *D. virgifea virgifera* is considered definitely established in Hungary.

All these results coincide well with the observations made in Croatia and Romania, where reports were published on the spread of the insect to a similar extent (IGRC Barcic and Maceljski 1996; Vonica 1996). The sex pheromone traps developed in the Plant Protection Research Institute, Budapest have been successfully used in monitoring of WCR for three years. Their application was accepted by the Technical Cooperation Program followed by the mentioned countries and supported by FAO.

The fact that economic losses caused by the larvae have not been observed in Hungary indicates that the pest is still in the initial phase of its establishment. Analyzing Yugoslavian data (Sivcev and Draganic 1996), it can be concluded that spread of adults is not immediately followed by the occurrence of damage. It is presumed that damage caused by larvae will be found gradually, in spots and, due to the biology of the pest, especially in maize grown in monoculture.

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THE SUITABILITY OF SEX PHEROMONE TRAPS FOR IMPLEMENTING IPM STRATEGIES AGAINST *AGRIOTES* POPULATIONS (*COLEOPTERA*: *ELATERIDAE*)

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ABSTRACT

Preliminary studies on the suitability of newly developed sex pheromone traps to implement IPM strategies against the wireworm species (*Agriotes ustulatus* Schller, *Agriotes sordidus* Illiger, *Agriotes brevis* Cndeze, *Agriotes litigiosus* Rossi) which cause damage to maize crops in North-eastern Italy are discussed. This research took place over a 3 year period (1995-1997) in the Veneto region using synthetic pheromone baits prepared in Budapest, Hungary. The respective pheromone components (geranyl isovalerate for *A. litigiosus*; geranyl hexanoate for *A. sordidus*; geranyl butyrate and E,E-farnesyl butyrate for *A. brevis*; E,E- farnesyl acetate for *A. ustulatus*) were tested in several dispenser types, dosages, and trap designs at several localities. *Agriotes* larval populations were estimated before and after the monitoring period of adults (pheromone trapping) via the use of soil sampling and attractive traps. In evaluating practical implications of pheromone trapping data obtained by both funnel and sticky traps baited by optimal dosages (high dosages performed better than low ones) in polyethylene dispensers (Kartell) were used.

The pheromone traps proved to be very effective and accurate for monitoring the flight of *Agriotes* adults at different localities; for example a few *A. litigiosus* males were captured at sites where no larvae of this species had been found, despite the fact extensive soil sampling had been conducted for several years previously. As for *A. ustulatus* it was observed a good correlation between last instar larvae population and the number of adults caugth in pheromone traps. For all the species the presence of a substantial population at a given site was quickly identified by pheromone traps. Trap captures permit and assure the determination of where and when high adult populations occur and then, evaluating the actual weather and soil conditions, it is possible to establish where there is a high risk of larval outbreak. If reliable information on the biology of the different *Agriotes* species are available the application of sex pheromone traps make it possible to determine the right moment to carry out field cultivations in order that egg and first instar mortality is enhanced, and to carry out chemical treatments to kill off adults before oviposition or to disrupt mating patterns.

Key words: Maize pests, *Agriotes* spp., trapping methods.

INTRODUCTION

Extensive studies carried out in North-eastern Italy have demonstrated that less than 5% of the fields planted with corn need to be treated with soil insecticides (Furlan, 1989, Furlan *et al.*, 1992). Despite this, at present most of the farmers use soil insecticide when planting mainly with the aim of controlling wireworms. Therefore, effective and inexpensive methods are needed to identify the soils that have high wireworm populations in order to treat only these, or to avoid cultivating maize on the same. *Agriotes* sex pheromones traps can effectively help us to attain this goal since they can easily give us useful information without making expensive and tiring surveys of the soil layers when searching for larvae. In fact they can monitor the only stage which lives outside the soil: the adult. In North-eastern Italy there are four *Agriotes* species capable of causing economic damage to corn crops: *Agriotes brevis* Candeze, *Agriotes sordidus* Illiger, *Agriotes litigiosus* Rossi, *Agriotes ustulatus* Schaller. The latter two mainly attack seeds and the first internodes; the former two, the most dangerous ones, seriously attack also already developed plants (up to 6-8 leaves) producing holes in the stem immediately beneath the soil line, just above or on the crown node. According to the features of the biological cycle that mainly influence the sex pheromone use, the mentioned *Agriotes* species can be divided into 2 groups:

A) Species with adults which do not overwinter including *A. ustulatus* and *A. litigiosus*

The adults belonging to these species do not overwinter, their swarming occurs in late Spring and summer and the eggs are laid few days after female hardening and darkening (Furlan, 1996). Egg laying takes few days.

B) Species with adults which do overwinter including *A. sordidus, A. brevis*

The adults overwinter and live several months (almost one year): from the Summer, when they form, until the next Spring they remain into a cell inside the soil; egg laying takes a long time (several months).Studies on the suitability of newly developed sex pheromone traps to implement IPM strategies against the species belonging to the 2 goups mentioned above have been carried out over a three year period (1995-1997) in the North-eastern regions of Italy.

MATERIALS AND METHODS

PHEROMONE TRAPS

1) Pheromone caps: these were produced at the Plant Protection Institute of Budapest; rubber and Kartell 730 vial dispenser were used; they were baited with the following compounds:
   Agriotes ustulatus: E,E-farnesyl acetate according to the indication of Kudryatsev *et al.*, 1993;
   Agriotes sordidus: geranyl-hexanoate
   Agriotes litigiosus: geranyl-isovalerate according the indication of Oleschenko, 1979, cited in Yatsynin *et al.*, 1980.
   Agriotes brevis: geranyl-butyrate and E,E-farnesyl butyrate;
   Compound dosages: different dosages were tested; in 1995 2 mg caps, in 1996 2 mg caps were compared to 0,6 and 10 mg caps, in 1997 10 mg caps were compared to 30 and 100 mg caps.
2) Traps: different traps were baited with the pheromone caps; in most fields the following types were used:
   a) STICKY: yellow sticky-traps 23 X 21,5 cm.
   b)BOTTLE:"funnel" traps made up from plastic water bottles,diameter 8 cm.
3) Height of the traps: 50 - 70 cm above the ground.
4) Position in the field: on the border of the field, or in the middle of field if the latter was planted with low growing crops (sugar-beet, soybean, meadow).
5) Distance between the traps: at least 50 meters.
6) Period of monitoring: May-September for *A. ustulatus* and *A. litigiosus*, March - August for the other species.
7) Replacement of the caps: every 15 days
8) Control: twice a week. All the specimens were removed at every control and identified. The sticky plates were replaced at least every 20 days.

LARVAL POPULATION EVALUATION

The larval population was estimated using two methods:

1) Soil sampling: 20-30 soil samples (diameter 12 cm and 25 to 60 cm deep according to the season) were collected per field (0,3 to 1 hectare) at random, using a manual soil sampler. The soil cores were then put into funnels, 26 cm in diameter, provided with a 0,5 cm mesh and a vial at the bottom according to the Tullgren method. The soil was allowed to dry for at least 30 days in a sheltered place and the larvae which fell into the vials placed at the bottom of the funnels were counted and identified.
2) Attractive traps: attractive traps were placed 3 m from each point where the soil cores had been taken. Each trap was made and used according to the description given by Chabert and Blot (1992) who modified that of Kirfman et al. (1986). They were manually observed after 15 days and the larvae found were counted and identified.

RESULTS

A) Species with adults which do not overwinter: *Agriotes ustulatus*, *Agriotes litigiosus*

The sex pheromone caps proved to be effective and accurate in monitoring adult presence and the swarming patterns of these species (Table 1 and 2, Figure 1) using 2 mg of active ingredient per cap; however, 10 mg and higher dosages proved to be better. For example a few *A. litigiosus* males were captured at sites where no larvae of this species had been found, despite the fact that extensive soil sampling had been conducted for several years previously. So, for both the species, the pheromone traps can permit:

1-The accurate identification of the presence of different species in different areas. This can allow us to forecast which crops might be damaged by wireworm, and when, according to the biology of the click beetle species without further surveys. For example in areas where *A. ustulatus* is the predominant species, corn planted late (in the beginning of May) cannot be seriously damaged even if there are high wireworm populations, because most of the larvae are in the premoulting and moulting (before pupae stage) phases (Furlan, 1996).

Table 1 -*Agriotes ustulatus* adults captured using sticky traps baited with 2 mg caps in Summer 1996 at different localities of the Veneto region characterized by different mature larvae population levels.

FARMER	BAGATELLA M.	BERTON D.	BONATO G.	BROLESE M. & S.	CARAVELLO R.	CARBONERA C.	DRIGO R.	FAGIANA	FURIN T.	GIACOMINI V.	GREGGIO L.	LUVISUTTO G.	MALVESTIO V.	MUTTA L.	PAVANELLO A.	PELLIZZARI V.	SCANTAMBURLO R.	TIENGO G.	VEDOVATO O.	ZANAZZO G.	ZINGALES F.LLI
LOCALITY	Chioggia	Venezia	Cavarzere	Cavarzere	Mirano	Caorle	Portogruaro	Eraclea	Portogruaro	Eraclea	Eraclea	Portogruaro	S. Maria di S.	Cavarzere	Cavarzere	Cavarzere	Mirano	Chioggia	Scorzè	Ceggia - Cessalto	San Donà di Piave
Population level	L	L	L	n.r.	n.r.	n.r.	n.r.	H	n.r.	H	L	n.r.	n.r.	n.r.	L	n.r.	n.r.	n.r.	n.r.	H	H
A. ustulatus	0	0	0	0	0	122	19	247	18	415	42	4	4	0	0	0	1	n.i.	3	323	153

L= no *A. ustulatus* larvae found using attractive traps and soil sampling in the field where the sex pheromone traps were used.

H = *A. ustulatus* larvae easily found using attractive traps and soil sampling in the fields where the sex pheromone traps were used

n.r.= not recorded

2 -<u>The precise evaluation of the adult population level and then the risk of future larval outbreaks.</u> A good correlation between last instar larval population and adult captures has been discovered: using 2 mg caps, the total number of *A. ustulatus* males per sticky trap ranged from 50 with a larval population of less than 1 specimens/sq.m. to about 5-600 with about 20 larvae/sq.m.; using 10 mg caps it ranged from 300 with a larval population of less than 5 specimens/sq.m. to more than 1500 with 20 larvae/sq. m. populations; very similar results were obtained with *A. litigiosus* populations. It is then possible to estimate the number of egg laying females and then the number of eggs (the average number of eggs laid by a female is 90 for *A. ustulatus* (Furlan, 1996), more than 120 for *A. litigiosus* (Furlan, unpublished data). If low adult populations are found (approximatevily less than 100 adults with 10 mg caps) no high larval populations are likely to be found the following Autumn, or in the

Spring two years later even if suitable soil conditions (moisture and food) occur during egg and first larval instar development. These 2 species in fact reach a size which is dangerous to the crops only during the Summer of the year subsequent to egg laying. A precise correlation between adult captures and the new larval population is to be ascertained with different soils, different crops and different weather conditions over the next few years.

Table 2 - *Agriotes litigiosus* adults captured using sticky traps baited with 2 mg caps in Summer 1996 at different localities of the Veneto region characterized by different mature larvae population levels

FARMER	ARMELLIN E.	BAGATELLA M.	BERTON D.	BONATO G.	BROLESE M. & S.	CARAVELLO R.	CARBONERA C.	DRIGO R.	FAGIANA	FURIN T.	GIACOMINI V.	GREGGIO L.	LUVISUTTO G.	MALVESTIO V.	MUTTA L.	PAVANELLO A.	PELLIZZARI V.	SCANTAMBURLO R.	TIENGO G.	VEDOVATO O.	ZANAZZO G.	ZINGALES F.LLI
LOCALITY	Jesolo	Chioggia	Venezia	Cavarzere	Cavarzere	Mirano	Caorle	Portogruaro	Eraclea	Portogruaro	Eraclea	Eraclea	Portogruaro	S. Maria di S.	Cavarzere	Cavarzere	Cavarzere	Mirano	Chioggia	Scorzè	Ceggia - Cessalto	San Donà di Piave
Population level	L	H	H	H	H	n.r.	n.r.	L	L	n.r.	L	L	L	n.r.	H	H	H	n.r.	H	n.r.	L	L
Males captured	35	419	44	602	131	1	0	1	5	0	4	16	0	0	279	302	87	0	595	0	1	2

L= no *A. litigiosus* larvae found using attractive traps and soil sampling in the fields where the sex pheromone traps were used.
H= *A. litigiosus* larvae easily found using attractive traps and soil sampling in the fields where the sex pheromone traps were used.
n.r-=not recorded

3-The identification of the period of adult swarming and the peak. For both species the beginning and the peak of flight were very precisely identified at the different localities (Figure 1).

Since male swarming begins only few days before that of females, it is possible to spray insecticides against the adults just before egg laying or to tillage the soil at the right moment with field cultivations in order to kill eggs or the newly hatched larvae, also destroying weeds to avoid larval feeding. In fact, newly hatched larvae are very sensitive to starvation (Furlan, 1996).

With only one treatment (Delthametrin) on sugar beet fields just after the peak of male swarming in 1995, it was possible to reduce *A. ustulatus* larval population in the Spring 2 years later by about 40% (unpublished data; trial carried out on 3 treated and 3 untreated plots of about 1,5 hectares each).

Since most of the adults of these two species swarmed during a short and well defined period, the disruption of mating patterns with the use of conspicuous quantities of sex pheromones can be considered possible both from a technical and an economical point of view.

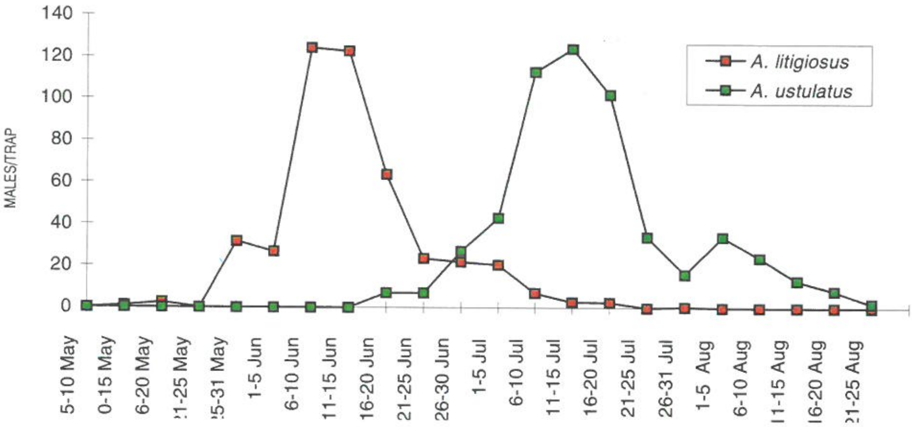

Figure 1 -*Agriotes ustulatus* and *Agriotes litigiosus* males captured using sticky traps baited with 10 mg caps in 1997 in the Province of Venice. Data average of 4 traps per each species.

B) Species with adults which do overwinter: *Agriotes sordidus, Agriotes brevis*

Six pheromone traps confirmed that males of these 2 species are active for a very long time (from March to August) and that they stay alive for almost one year. Conspicuous captures were in fact recorded in the Spring and Summer months (Figure 2). *A. sordidus* caps baited with 10 mg or more of Geranyl - hexanoate proved to be effective both in:

- Monitoring the presence of the species in different areas; specimems were captured in all the North-eastern regions where traps had been set up (Table 3).

Table 3 -*A. sordidus* males captured in 1997 using bottle traps baited with 10 mg caps in different localities of the Veneto region.

FARMER	BUOSO B.	BAGATELLA M.	BONATO G.	GALLO G.	GIARRETTA	GARZIERA G.	DRIGO R.	GUMIERO G.	FURIN T.	GREGGIO L.	MALVESTIO V.	MION G.	VALLE VECCHIA	ZINGALES	SCANTAMBURLO R.	BERTON	ZANAZZO G.
LOCALITY	Caorle	Chioggia	Cavarzere	Mirano	Cavarzere	Cavarzere	Portogruaro	Cinto Caomaggiore	Portogruaro	Eraclea	S. Maria di Sala	Mirano	Caorle	S. Donà di Piave	Mirano	Venezia	Ceggia - Cessalto
Males/ trap	27	243	163	66	159	77	16	256	22	123	14	8	97	40	152	2	34

- Describing swarming patterns and identifing the peak of male activity thus estimating the level of adult populations (Figure 2).

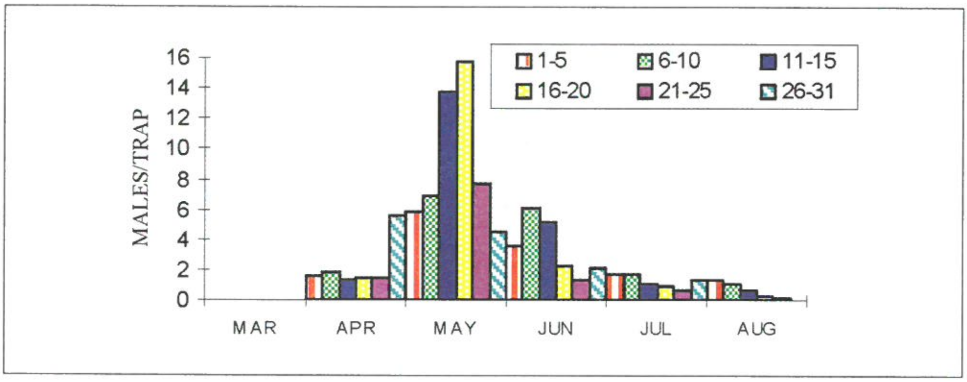

Fig. 2 – *Agriotes sordidus* males captured using bottle traps baited with 10 mg caps in 1997 in Veneto. Data average of 15 traps.

Since the effective sex pheromone compounds for *A. brevis* were found during the Spring of 1997 few reliable results are now available. At the moment we can maintain that *A. brevis* as well as *A. sordidus* males are sensible to sex pheromones for a long period as well as *A. sordidus* and that at least two peaks are present (April and late May). For both species the implementation of control strategies, based on sex pheromones, appear more difficult than for the first group. In fact the long period of adult activity means that it becomes expensive to carry out an effective population control by using mating disruption, as well as chemical treatments, against the adults. In any case, according to what is being observed with *A. sordidus*, the egg laying peak occurs about two months after the beginning of adult emergence from the soil, and just after the peak of male activity. The monitoring of male fligth makes it possible for us to concentrate agronomic and chemical strategies against these species during the best period.

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PLANT-PARASITIC NEMATODES ASSOCIATED WITH MAIZE IN PORTUGAL

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ABSTRACT

As a result of a survey in the portuguese maize-growing areas, 20 genera of plant-parasitic nematodes were found.The most widely distributed in country were *Pratylenchus, Helicotylenchus, Meloidogyne* and *Tylenchorhynchus s.l.*. The root-knot nematodes *Meloidogyne arenaria, M. incognita, M. javanica,* the reniform nematode *Rotylenchulus borealis* and several species of *Pratylenchus* were recovered from maize roots.

Control of plant-parasitic nematodes by crop rotations including maize is discussed.

Key-words: Portugal, plant-parasitic nematodes, maize, integrated pest management.

INTRODUCTION

Since its introduction in Portugal, in the XVI th century, maize has gradually conquered an important place for human consumption as well as for livestock feed. Either in small farms or occupying several hectares, maize is present everywhere from the North to the South of the country. The area devoted to maize production in Portugal was estimated in recent years to be 376 000 ha, with a mean yield of 6000t/ha.

Among the factors affecting the maize root development the action of plant-parasitic nematodes should be given due consideration. The first papers documenting the pathogenicity of nematodes on maize appeared only in the 1950's. Because polyspecific populations of nematodes usually are present in the fields, evaluation of loss by any given species is difficult.

Furthermore the interaction of nematodes with other soil-borne pathogens, such as fungi and bacteria may occur, resulting in complex diseases. In certain cases injury caused by specific nematodes have rather distinctive root symptoms and, unless they are mixed, one can with some degree of certainty identify damage by those nematodes. For example, species of root-lesion nematodes (*Pratylenchus* spp.) penetrate the root cortex and produce brown lesions in the sides and tips of the roots. Often several nematodes girdle and amputate the roots, which may produce many branches causing a brushy root effect. Species of root-knot nematodes (*Meloidogyne* spp.) usually produce a visible swelling on the parasitised roots.

In Portugal maize is grown in small farms submitted to very intensive land use, in which the soil is cultivated throughout the year. In these systems, maize is integrated in a crop rotation with horticultural crops such as potatoes, beans, lettuce and brassicas. It is also cultivated in extensive cropping systems, where maize precedes crops such as tobacco, sunflower or pepper. Although maize was introduced in Portugal almost four centuries ago, and the crop has attained great socio-economic importance, relatively little information on plant-parasitic nematodes associated with maize in the country is available.

The purpose of this study was to get information on plant–parasitic nematodes associated with maize in Portugal, in intensive as well as extensive cropping systems.

MATERIALS AND METHODS

A total of 430 soil and root samples were collected in maize-growing farms, from the North to the South of Portugal (Figure 1) of which 145 were in intensive cropping systems and 285 in extensive cropping systems. At least six root systems were collected from each field. Samples of roots were washed thoroughly, observed under a dissecting microscope for symptoms of root damage and afterwards incubated in tap water for 48 h. After the incubation period the nematodes were collected on a 325 mesh sieve, killed in water by gradual heating, treated with hot cotton blue lactophenol (0.0025%) and mounted in a drop of glycerine, on standard glass slides, following the wax-ring mounting method (De Maesener & d'Herde, 1963) and finally identified.

Each soil sample was a composite of 12 to 20 subsamples taken with a soil auger to a depth of 20-30 cm, in the rhizosphere of randomly selected plants. Nematodes were extracted from a 250 ml subsample of each sample by the centrifugal sugar-flotation technique (Caveness & Jansen, 1955), killed, fixed and mounted on glass slides as indicated above, and identified. When present in the root samples, females of *Meloidogyne* spp. were extracted, treated with hot cotton blue lactophenol (0.25%) for a few minutes, mounted on glass slides and identified. At least 12 perineal patterns of females extracted from each infected root system were observed.

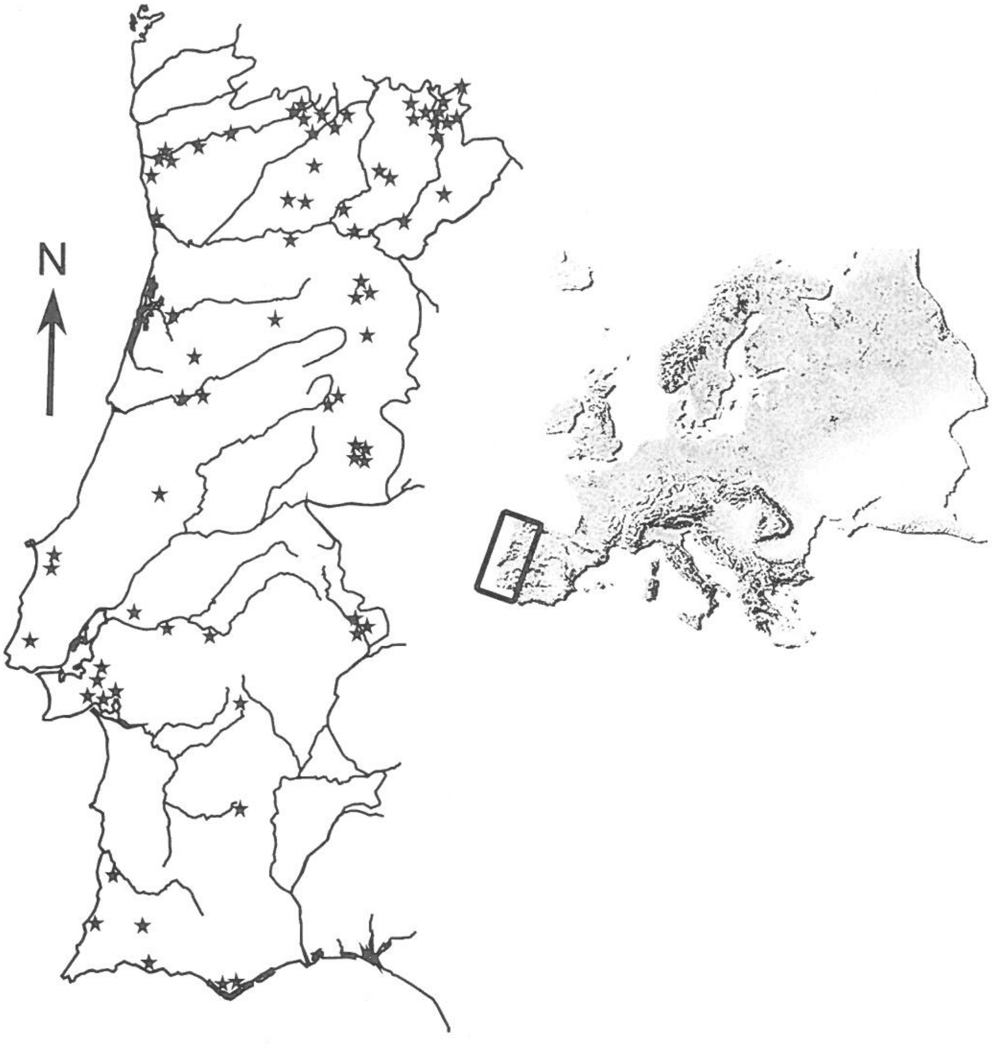

Figure 1 –Map of Portugal, showing its geographic position in Europe, and sites where root and soil samples were collected (★).

RESULTS

The frequency distribution of plant-parasitic nematode genera identified from soil and root samples collected in areas with intensive or extensive cropping systems integrating maize, is presented in Figure 2.

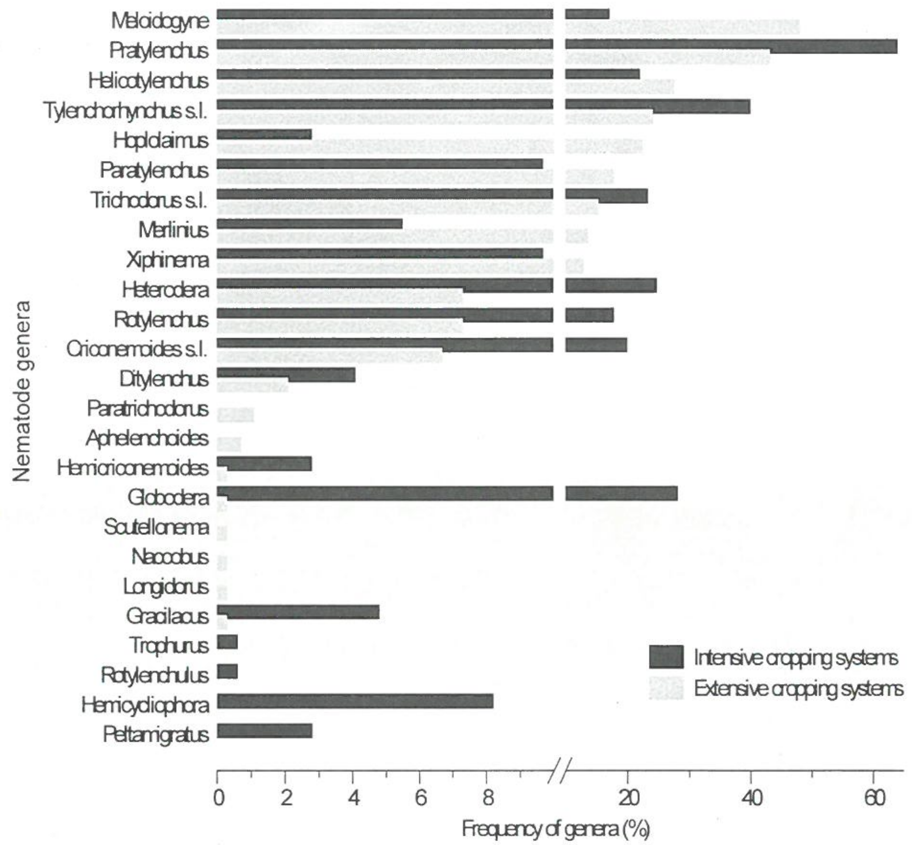

Figure 2 –Frequency distribution of plant parasitic nematode genera recovered from 145 soil and root samples collected from intensive cropping systems in the period 1979-90, and 285 samples collected from extensive cropping systems in the period 1984-91.

In farmlands submitted to intensive soil exploitation a total of 20 genera were identified in the 145 samples. Among the migratory endoparasites, root-lesion nematodes, namely *Pratylenchus crenatus*, *P. neglectus*, *P. penetrans*, and *P. thornei*, were found in 64% of the samples. Among the sedentary endoparasites, root-knot nematodes namely *Meloidogyne arenaria*, *M. hapla*, *M. incognita* (races 2 and 4), and *M. javanica* were found in 17% of the samples and *Rotylenchulus borealis* in 0.6% of them. In extensive cropping systems a total of 21 genera of plant-parasitic nematodes were identified in the 285 samples, with prevalence of root-lesion and root-knot nematodes, which were found in 48% and 43% of the samples respectively.

DISCUSSION AND CONCLUSIONS

Among the plant-parasitic nematode genera identified and as a consequence of their wide distribution, extensive host range, great reproductive potential and the damage they are known to cause, *Pratylenchus* spp. and *Meloidogyne* spp. must be ranked as very important root parasites. As the damage by plant-parasitic nematodes is proportional to their population densities (Seinhorst, 1965) in integrated crop protection schemes a range of measures over a complete rotation should be used to prevent populations increasing to, or decreasing them below the point where they threaten the next susceptible crop.

Although maize has frequently been included in crop rotations to control root-knot nematodes, many maize hybrids are good hosts and of limited value for control of *Meloidogyne* spp. (Windham & Williams, 1988). Resistance to *Pratylenchus* spp. *Zea mays* has been scarcely investigated, but sufficient information has been accumulated to show that resistance has promise as a useful control measure. Thomas (1980) found that commercial maize hybrids differed in their degree of susceptibility to root-lesion nematodes. A successful integrated pest control scheme requires detailed information on the host-parasite and environment inter-relationships. Cultural control of plant-parasitic nematodes would ideally include the use of tolerant or resistant cultivars in the crop rotation, soil management to decrease nematode survival, control of weeds and fallowing.

There is a need for more research regarding the susceptibility or resistance of the more common maize cultivars to the species of *Pratylenchus* and *Meloidogyne* occurring in Portugal, in order to prevent the build-up of nematode populations to damaging densities.

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THE CORN LEAF APHID *RHOPALOSIPHUM MAIDIS* Fitch.

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ABSTRACT

The Corn Leaf Aphid - *Rhopalosiphum maidis* Fitch - is spread on both spontaneous and cultivated Gramineae with a special preference for corn. It induces both direct damage to the plant and indirect as a vector carrying various diseases. It overwinters as a verginogenous larva on the roots of host plants. In Spring the larvae migrate to both spontaneous and cultivated Gramineae, chiefly barley, sorghum and corn. At a temperature of 15° C the lice become mature and at 23-24°C they perform very intense feeding.

As the aphid is apterous (wingless) it settles in the leaf cornet. On panicle emergence, the pest's intensive feeding on all plant organs induces serious physiological alteration, hindering corn cob development a.s.o.

In 1995 in Transylvania, Romania, different rates of damage were recorded on corn plots. This led us to establish a 5 grade scale to asses damage rates. Its highest grade, namely '5', stands for highest damage rate (aphids completely covering the whole plant, sterile or even missing corn cob, prematurely withered plant), while, the lowest grade, i.e. '0', denotes a plant free from aphid infestation.

Meanwhile, a fast diminishing of aphid population and attack was induced by intense breeding of natural beneficial species preying on aphids, such as *Coccinella septempunctata, Chrysopa spp.* etc.

The research we have carried out on samples of local populations, hybrids and varieties of corn have revealed a dissimilar behaviour that appears to be partly correlated with the maturation group and the implications of the biochemical components in the plant. The heaviest damage was recorded on early genotypes, on local varieties and populations.

Key words : Maize pests, *Rhopalosiphum maidis* Fitch.

INTRODUCTION

The Corn Leaf Aphid or the *green plant louse-Rhopalosiphum maidis* Fitch. - is spread all over the world and occurs on Gramineae; its favourite host is corn.

The damage it significantly causes occurs in the years that are favourable to both its direct breeding on the plant and its indirect proliferation as a vector agent for a series of diseases. Owing to its occurrence on most species of Gramineae, it is considered a cosmopolitan pest.

Its overwintering takes place as a veriginogenous larva on the roots of the Gramineae. As Spring comes, the larvae migrate to the leaves of different Gramineae where they start to feed on. The most damaged species are barley, sorghum and corn.

At temperatures over 15°C, the green lice are matured, whereas at about 24-25°C, which is regarded as the optimal temperature range, their activity becomes very intense. As a rule, the insect emerges in corn plots at the beginning of Summer and persists until frost. As a vector, it transmits leaf-mosaic diseases, both to corn and to other cultures of Gramineae.

The Corn Leaf Aphid is apterous and is typically located in the cornet of the rolled leaves; in its earlier growth stages it feeds on young plant tissues. Of no lesser importance are the winged females that are attracted by the very position of corn plants themselves. On corn emergence, the aphids set out to proliferate at fast pace. The high intensity of these larvae feeding on some parts of the plant induces their death.

As a sequel of a more or less intense infestation, the pollen decreases in quantity or even disappears completely.

The feeding of the aphids on plants engenders serious physiological alteration, either preventing the corn cob from developing or even hindering its emergence.

Thus on the most seriously infested plants, the pollination process is reduced and the number of grains dwindles subsequently. Seriously infested corn cobs remain undeveloped and sterile. The infestation of the panicle leads by any means to the infestation of the whole plant.

MATERIALS AND METHODS

The year of 1996 was a favourable year to aphid mass proliferation, which led us to carry out a research on a significant number of genotypes of corn (hybrids, varieties and populations), that have been, are and will be cultivated.

The rate of plant damage has been assessed by means of a 5-grade scale, where the highest grade (i.e. '5') was granted to plants reduced to complete sterility whose sterile cob was completely covered with aphids, whereas the lowest grade (i.e. '0') was attributed to plants free from any infestation.

We present here a 5 - grade scale of plant damage rate:

1. Few aphids (1 to 5) on plan panicle, stalk, leaves; no symptoms of harmful influence.
2. A medium load of aphids (6 to 20) on all plants organs; their presence does not visibly impair on plant vegetation and organ development.
3. A heavy load of aphids (20 to 40) on all plant organs ; both sides of plant leaves are covered with soot; both plant vegetation stage and its overall outlook are affected; maturation and corn cob development are advanced.
4. More than 40 aphids cover completely every organ of the plant; the plant displays a forced maturation. Corn cob growth is visibly impaired relative to non-infested plants.
5. The whole plant, from panicle to every organ, is completely covered with aphids and their secretions. The corn cob is either sterile or completely missing. The plant withers prematurely, shrinks and disappears.

RESULTS AND DISCUSSION

Meanwhile, an intense activity of pests in the *Coccinella* and *Chrysopa* genres has been noticed; these have thrived massively and rapidly, owing to the food abundance and environment conditions; they have lead quickly to a significant decrease of aphid attack and abated their effect (Table 1).

Table 1 -The behaviour of corn genotypes (hybrids, varieties and populations) to the attack of the corn leaf aphid (*Rhopalosiphum maidis* Fitch) and its correlation with maturity groups in 1996 at Turda, Romania

FAO maturity group	Number of genotypes	Frequency of plant damage	Frequency of high damage grades (4+5)	Frequency of medium damage rates	Average yield loss (%)
1 -200	15	26,3	13,2	3,1	7,3
2 - 300	28	28,2	2,7	2,1	2,8
3 - 400	10	27,0	0,8	1,6	1,9
4 - 500	9	18,4	0,1	1,13	0,6
5 - 600	8	17,7	0,0	1,1	0,1
> 600	2	22,5	0,0	1,6	1,12
Local varieties and populations	5	48,2	28,1	3,6	19,3

A series of conclusions have been drawn on the dissimilar behaviour of corn genotypes; some are based on mere observations, others have been carried out by means of specially designed research. Thus, some studies claim that later genotypes tend to become more intensely infested (Mc Collach 1921), or that those whose grain endosperm is yellow should be more resistant (Snelling *et al.* 1940) (Huber & Stringfield, 1942); other studies have credited panicle chemical composition or carotene contents to account for corn resistance (Coon, Mullor & Oran, 1948).

The data resulted from our observation reveal that in most cases there is a tight correlation between the way plants reacted to Corn Leaf Aphid infestation and their group of maturation. Damage rate and frequency are high with corn genotypes belonging to FAO 1-200 maturation group; as we go up the scale, the damage rate grows very significantly, reaching severe infestation forms with the FAO 3-400 groups (Table 1 and Table 2).

Table 2 -Genotype distribution by FAO maturity group depending on the rate of damage induced by *Rhopalosiphum maidis* Fitch.

	FAO Maturity Group						
Damage rate	1 -200	2 - 300	3 - 400	4 - 500	5 - 600	>600	Total
I. Very reduced	1 (1,3) *	3 (4,0)	1 (1,3)	6 (7,7)	8(13,5)	1 (1,3)	20 (28,1)
II. Reduced	2 (2,5)	16 (20,5)	7 (9,0)	3 (4,0)	-	1 (1,2)	29 (38,2)
III Medium	4 (5,1)	5 (4,6)	1 (1,3)	-		-	10 (11,0)
IV High	11 (14,1)	5 (4,6)	3 (4,1)	-		-	19 (22,7)
TOTAL	18 (23,0)	29 (33,7)	12 (15,6)	9 (11,7)	8 (13,5)	2 (2,5)	78 (100,0)

(...) - shown as percentage
* - classified according to annual damage rates observed on the genotypes we have studied

As the emergence of Corn Leaf Aphids coincided with conditions favourable to their breeding, season's high temperatures caused the early and very early forms of corn to swiftly go through the organogenesis phasis. However, this coincidence also fostered a stronger attack on these very forms. Besides, it is known that, on panicle emergence, the concentration of the chemical resistance factor (DAMBOA) starts to decrease, and along with it plant resistance declines as well. Observations carried out by several researchers have determined that the genotypes resistant to the European Corn Borer, *Ostrinia nubilalis* Hbn., are also resistant to Corn Leaf Aphids (*Rhopalosiphum maidis* Fitch).

More recent research has revealed that the onset of plant resistance is a complex matter, possibly controlled by several genes of dissimilar degrees of additivity and dominance (Pathokand Saxead, 1976) (Chang & Brewbaker, 1978); it has also revealed that corn resistance to *Rhopalosiphum maidis* Fitch. is conditioned by a single gene linked to the *locus* of the chromosome of he Rp gene which conditions resistance to blight.

The infestation rate of corn cultures proves the necessity of initiating a research programme for corn resistance to *Rhopalosiphum maidis* Fitch. which should include as compulsory stages: the detection of its resistance sources, the nature of its resistance and its transmitting. Each research stage should be carried out on artificially infested plots, so as to ensure result consistency.

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LOSSES CAUSED BY THE CORN BORERS *SESAMIA NONAGRIOIDES* (Lef.) AND *OSTRINIA NUBILALIS* HÜB. IN MAIZE - A CONTRIBUTION FOR THEIR QUANTIFICATION

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ABSTRACT

The utilisation of simple, easy to use and strict methods to quantify losses caused by pests are an important factor for the development of Integrated Pest Management (IPM) strategies.

In this paper is presented the methodology adopted for the determination of a descriptive model for the assessment of losses caused by the two species of corn borers in maize, *Sesamia nonagrioides* (Lef.) (*Lepidoptera*: *Noctuidae*) and *Ostrinia nubilalis* Hüb. (Lepidoptera: Pyralidae), at "Perímetro de Rega do Mira" (Alentejo/Portugal). This methodology involves three sequential steps: plant data organization in three homogeneous classes, using as discriminating criteria the ear length; determination of a production model, based on the class "non attacked plants"; and determination of a loss model (loss function). The model was applied to the data obtained from a survey, which provided a general information about the regional losses caused by those maize stem borers.

Key-words : Crop loss assessment, maize, *Ostrinia nubilalis, Sesamia nonagrioides,* regional loss.

OBJECTIVES

The maize stem borers are the most important pest problem on maize in almost all countries (Allemann,1979; Amaro,1982; Eizaguirre & Albajes,1989; ACTA,1990). The european corn borer *Ostrinia nubilalis* Hüb. is the main species in North America, North Africa and in Central and Northern Europe, but in the Mediterranean Region, and also in Africa, the species *Sesamia nonagrioides* (Lef.) causes heavy losses. Also at the "Perímetro de Rega do Mira", where maize is already an usual crop, occupying 41% of the total irrigated area, corn borers are largely distributed and the cause of important losses to the farmers.

There is a lot of information about the first species, including methodology for crop loss assessment, but the situation in the Mediterranean Region, where the two species overlap in time and space is very different, there are not appropriated methods available for such type of assessment.

In order to reduce this gap, a field research was developed having as final purpose the built of a model for estimating crop losses in maize caused by those species of corn borers. At the same time, it was intended to get information about the regional losses caused by those pests, in 1993 and the area of "Perímetro de Rega do Mira" under study.

MATERIALS AND METHODS

A) Loss assessment

The determination of the attack levels were based upon the natural infestation of maize fields. For such purpose, a number of complete and mature maize plants were selected, in order to obtain the largest experimental variation on attack intensity levels, and also a significant number of plants for each level. In this way, it was possible to obtain 10 infestation levels, from zero (no attack) to nine, considering the attack intensity (AI) parameter as the sum of the number of present larvae and pupae present (NLP) with the number of exit holes found (NH).

In each sampled plant it was determined the following variables: stalk length (SL), number of ears present (NE), ear length (EL), number of larvae and pupae of *O. nubilalis* present in the stalk and ears (NO), number of larvae and pupae of *S. nonagrioides* present in the stalk and ears (NS), the number of exit holes in the stalk (NH), weight of ears (WE), number of grains per ear (NGE), weight of the grains per ear (WG).

The method used was based on descriptions by Mexia (1985) and Amaro (1989), but it was necessary to modify and adapt this to the specific requirements of the species and culture, as described by Pereira (1994). The method requires three stages, with the following sequence: organization of the sampled plants in homogeneous classes; determination of a production's descriptive model; and determination of a descriptive model for the loss caused by the corn borers.

Sampled Plants Organization In Homogeneous Classes

For the analyse of the heterogeneity observed between the sampled plants, the Automatic Classification Method was used and the plant data were organised in three homogeneous classes, using as discriminating criteria the ear length. For the purpose of class organization, two situations were considered: 1st - SL, EL, NGE, WE and WG as "active" variables; 2nd-SL, EL and NGE as "active" and WE and WG as "illustrative", based on the fact that such variables could be influenced by NS and NO variables, at harvest.

Determination of a Production's Descriptive Model

The determination of the variable that explains better the plant's grain production was realised by testing all variables connected to the production (SL, EL, NGE, WE and WG), by successive linear regressions. Then an linear regression analysis was done, for the production of the plant groups "non-attacked" and "attacked"; two criteria were considered for the "attack" definition: a) NH, with 5 levels; b) AI, with 10 levels.

Determination of a Descriptive Model for Loss

The potential production (PPROD) for each sampled plants was calculated, using the production's descriptive model, and also by comparison with the real grain production obtained (RPROD=WG), the loss value for each plant (LOSS) was calculated [LOSS= PPROD - RPROD]. These values allowed the determination of the medium value for each attack level, which becames the loss estimation. Finally, with these values it was attempted, through successive linear regressions, the definition of a mathematic model that would translate, consistently, the existing relationship between the loss estimation and the corresponding attack level.

B) Regional Loss

To estimate the losses caused by the maize stem borers in the region, inspections were undertaken in 24 maize fields. These inspections consisted in the observation of 25 plants per field, with the determination of the percentage of plants attacked, the number of individuals for each pest species present and the respective developmental stage.

RESULTS

A) - Loss assessment

Sampled Plants Organization In Homogeneous Classes

In both scenarios considered, it was verified that the correlation coefficients for the studied variables were high enough (always more than 0.60),although it was highest when all the variables were taken in consideration as "actives". Nevertheless, the relative arrangement of data was more uniform when the system with only three axis was considered (2nd scenario: three "active" variables and two "illustrative" ones); this can be shown by the histogram in Table 1.

Table 1 - Histogram relative to data organization of data, using the axis system

Situation	**1st - all the active variable**	**2nd - "active" & illustrative variables**
Active variable	Histogram	Histogram
SL	******************	****************
EL	*****	********
NGE	***	****
WE	*	-
WG		-

** represents the data distribution in each axis considering its relative proportion.

By the decomposition of the intra and inter-classes inertia (Table 2), it was possible to conclude about the existence of a reasonable discrimination from the centres of gravity of each class (SME - stalk with medium ears; SLE - stalk with large ears; SSE - stalk with small ears), and that the classes homogeneity is satisfactory, indicated by the low-values of inter-classes inertia.

Table 2 - Decomposition of intra and inter-classes inertia.

Inertia	**1st scenario - all "active" variables**		**2nd scenario - "active" & "illustrative" variables**	
	Inertia	N° plants	Inertia	N° plants
Intra-classes				
*SME	0.1101	116	0.1506	136
*SLE	0.0857	68	0.0828	57
*SSE	0.1237	93	0.1457	84
Inter-classes	0.6769		0.6210	
Total	**0.9964**	**277**	**1.000**	**277**

Because both results supported the plant's organization into three classes, the next step was the analysis of the plants arrangement and production by classes (Table 3). The results obtained showed that the definition of SL, EL and NGE as "active" variables and WE and WG as "illustrative" variables conducted into a more homogeneous and robust classes organization.

Table 3 - Distribution percentages of plants and production for the three plant classes.

Classification Type	Class	Nº plants	% of total nº of plants	% of total production
All variables "active"	SME	116	42	42
	SLE	68	24	39
	SSE	93	34	19
"Active" & "illustrative" variables	SME	136	49	50
	SLE	57	21	32
	SSE	84	30	18

Determination of the Production's Descriptive Model

Two criteria were used to test which variable explains better the production, both with SL, EL and NGE all as independent variables: I - with WG as a dependent variable; II - with WE as a dependent variable. For both models, a diversity of linear regressions were tested, and it was shown that the best results (biggest R^2 values) were obtained in model I: with the three variables simultaneously (R^2=0.80); with NGE and EL (R^2=0.76); and only with NGE (R^2=0.66).

Because the amount of explanation due to all the variables do not seem enough to justify the increase in work, only the two following hypothesis were considered: H1) with SL and NGE; H2) only with NGE. In Table 4 are presented the production functions of "non-attacked plants" group, for the two hypothesis and also for the two attack criteria previously defined.

Table 4 - Production functions obtained for the "non-attacked plants" group.

Type of variable used			Equation to describe	Value of	R^2	
Attack	Dependant	Independent	Production Model	t const.	(%)	OBS.
NH	WG	NGE	WG = - 9.01 + 0.41 NGE	-0.99	80.3	
			WG = 0.39 NGE		80.3	const. = 0
	WG	NGE, EL	WG=-103.2+0.32NGE+6.68EL	-5.78	85.4	
AI	WG	NGE	WG=23.4+0.36NGE	1.40	85.8	
			WG=0.41NGE		85.1	const. = 0
	WG	NGE,EL	WG=-50.8+5.28EL+0.28NGE	-1.38	88.1	

Determination of a Descriptive Model for Loss

As a result of the R^2 values obtained for the different production functions (Table 4) it was shown that the descriptive model from loss should be defined using the following production functions:

- WG=23.4+0.36NGE and
- WG=-50.8+5.28SL+0.28NGE.

The loss from each plant was calculated in grams and in percentage of potential production (PPROD), in accordance with the following:

LOSS g = PPROD - RPROD or LOSS% = (PPROD - RPROD)/PPROD x 100, with RPROD as real grain production obtained .

For each AI level, the medium loss value was determined (Table 5); the production function using EL and NGE variables results in lesser PPROD values and, consequently, also in lesser loss values.

Table 5 - Loss medium values for each intensity attack level (AI).

Attack Level	Production Function WG=23.4+0.36NGE				Production Function WG = –50.6+5.28EL+0.28NGE			
AI	RPROD	PPROD	LOSS g	LOSS %	RPROD	PPROD	LOSS g	LOSS %
0	203 .3	203.54	0.24	0.12	203.3	203.12	-0.18	-0.09
1	183.0	188.7	5.74	3.04	183.0	186.36	3.36	1.80
2	163.4	172.9	9.45	5.47	163.4	169.82	6.42	3.78
3	108.4	143.35	34.85	24.33	108.4	133.67	25.27	18.91
4	112.5	146.50	34.00	23.21	112.5	137.77	25.27	18.34
5	95.7	135.31	39.61	29.27	95.7	130.98	34.98	26.77
6	92.1	140.36	48.26	34.38	92.1	130.38	38.28	29.36
7	79.5	137.84	58.34	42.32	79.5	125.25	45.75	36.53
8	74.2	136.03	61.83	45.45	74.2	121.74	47.54	39.05
9	67.9	133.51	65.61	49.14	67.9	126.11	58.21	46.16

Because the production functions were obtained from the values of the "non-attacked plants" group with AI=0, and since at least 80% of these plants were included in the SLE class, the potential production (PPROD) calculated in excess and also the assessed losses were higher than the real figures, therefore, the use of the production function with EL and NGE variables in loss evaluation can contribute to this error's reduction.

The loss estimates by plant were the basis for the regression analysis study on the existence of a close relationship between the loss and the corresponding attack levels. As the results in Table 6 show, it is well known that the percentage of variance accounted (R^2) is very similar.

Table 6 - Loss functions equations.

Production Function: WG =23.4+0.36NGE		**Production Function: WG =-50.6+5.28EL+0.28NGE**	
Loss Function	R^2 (%)	Loss Function	R^2 (%)
LOSS g= 1.12 + 7.71 AI	95.3	LOSS g= -0.97 + 6.55 AI	96.7
LOSS g= 7.88 AI	95.8	LOSS g= 6.39 AI	97.0
LOSS%= -0.47 + 5.81AI	95.9	LOSS%= -1.95 + 5.34 AI	97.0
LOSS%= 5.74 AI	96.3	LOSS%= 5.03 AI	97.0

In Figure 1 and 2 are explained the loss functions determined for each production function considered in this study, with constant=0, which allows us to improve the R^2 values at the decimal level (Table 6).

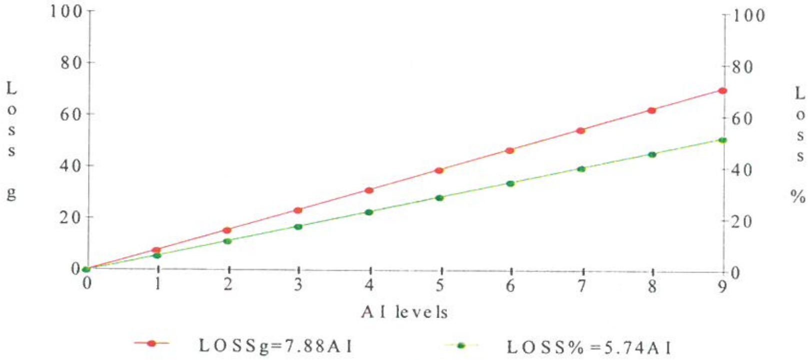

Figure 1 - Loss function based on the production function WG=23.4+0.36NGE.

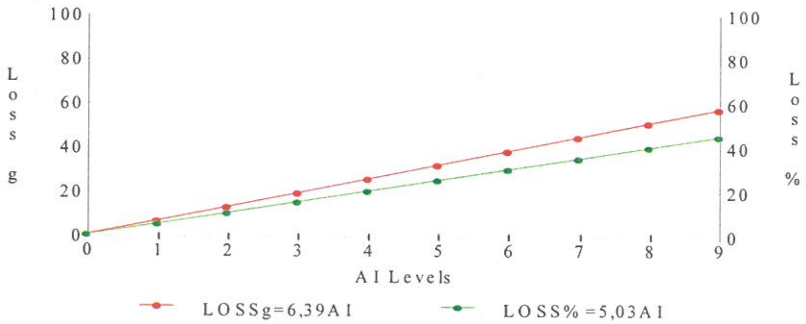

Figure 2 - Loss function based on production function WG=50.6+5.28EL+0.28NGE.

B) Regional Loss

In Table 7 are presented the results obtained with the inspections done to the maize fields.

Table 7 - Results of inspections of maize plants from local fields.

Total		Attacked Plants		Number of pests		
Fields	Plants	Nº	%	*Sesamia nonagrioides*	*Ostrinia nubilalis*	Plant/ Medium
24	600	114	19	66	17	0.73

To estimate the regional losses, it was determined the AI/plant medium value for each field inspected, and, by application of the loss function LOSS % = 5.03 AI, the corresponding losses per field. The regional losses value, expressed in percentage of potential production, is the arithmetic mean of the values calculated for each field: REGIONAL LOSS = 3.65% PPROD

DISCUSSION

The determination of a loss assessment model for the two species of maize stem borers, allowed to verify that, in spite of the sample heterogeneity, it was possible to organise the plants into three homogeneous classes, in accordance with the stalk length. Even so, in both scenarios considered, it was verified that almost all the "non-attacked plants" appears in the class "Stalk with large ears" (SLE), that immediately draws attention to the excessive determination of potential plant production using this methodology.The plant classes, determined with SL, EL and NGE as "active" variables and WE and WG as "illustrative" variables, show different total loss percentages, depending upon the production function considered (Table 8).

Table 8 - Percentage contribution from each plant class to the total loss sample.

Production Function	Class 1 (SME)		Class 2 (SLE)		Class 3 (SSE)	
	% Loss	% Sample	% Loss	% Sample	% Loss	% Sample
WG = 23.4+0.36 NGE	45.9	49	8.4	24	45.8	34
WG=-50.6+5.28EL+0.28NGE	53.6	49	6.2	24	40.2	34

As verified by the analysis of Table 8, Class 1 (SME) represents the largest sample share and also the largest contribution to the final loss, in either considered situation. The SLE plants have, due to the fact that this class includes 80% of all plants without any type of attack (AI=0), a reduced contribution to the final loss, especially in the case where the production function considered includes EL and NGE as independent variables.

The improvement of almost two units in R^2 value, obtained with the introduction of the variable EL in the production function (Table 6), seem not to justify the increased work necessary for the determination of this variable values, in all the sampled plants. The loss functions expressed in percentages (Table 6) seem similar to the functions determined by Bode & Calvin (1990). But are higher than the relation defined by Patch *et al.* (1951) (3% loss/larva),which has been frequently used in following works.

The results obtained with the loss function are promising, but more field work and testing need to be carried out in future years based on a larger sample dimension, since the one reported here represents the losses occurring with specific well-determined conditions.

The estimated regional loss that was calculated is significantly lower than the level of 15-20% found by Figueiredo (1993) for the *S. nonagrioides* species, as a national loss level, which indicates the wide range of results that can be obtained in such kind of studies on the losses caused by maize stem borers in irrigated corn.

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IMPORTANCE OF THE SECOND GENERATION OF ECB (*OSTRINIA NUBILALIS* HBN.) IN THE PRODUCTION OF MAIZE AS A SECOND CROP

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ABSTRACT

The bivoltine type of ECB (*Ostrinia nubilalis* Hbn.) has been prevalent in the Yugoslavian Corn Belt since 1973. Since then, the infestation intensity by both generations has been studied in various genotypes of maize of different growing seasons planted on regular planting dates, and according to the obtained results the degree of infested plants by ECB over generations was calculated.

This present study encompasses investigations exclusively on the second generation of ECB in Zemun Polje in stubble crop planting of maize grown under irrigation conditions in 1992 and 1993.

The effects of three essential factors in maize productions were observed:

❶ Factor A - Tillage regimes at seedbed preparation: A1 - conventional tillage (stubble ploughing at 25 cm along with harrowing and finalization), A2 - reduced tillage (only two harrowings at 12-15 cm) and A3 - no tillage, i.e. mulching with direct planting.

❷ Factor B - Fertilizing with nitrogen: B1 - control without fertilization, B2 - a lower rate of 80 kg N ha^{-1} and B3 - a higher rate of 120 kg N ha^{-1} and KB2 - rate with no weed control by herbicides.

❸ Factor C - Maize genotypes: C1 - hybrid ZPTC 112 and C2 - hybrid ZPTC 196, both hybrids of FAO maturity group 100/200.

The infestation intensity by the second ECB generation (degree of infested plants) and the rate of plant damage (tolerance evaluation) were estimated, grain yield was determined, while the number of larvae per plant was registered in 1993.

Statistically processed data were mutually compared for the conventional tillage regimes and for nitrogen rates with the non-fertilized variant.

Obtained results indicate that the reduced tillage at seedbed preparation for stubble maize planting (after wheat) with the adequate nitrogen rate, can result in the infestation decrease by the second ECB generation, in the plant tolerance increase, as well as in the grain yield increase.

Reduced soil tillage, especially direct maize planting, led to the significant infestation decrease by the second ECB generation. At the same time, plant tolerance did not change much, while the number of larvae significantly reduced and grain yield increased.

Nitrogen fertilizing led to the significant increase of the plant infestation by the second ECB generation, plant tolerance decrease, the increase of the number of larvae per plant, and especially to the grain yield increase.

The higher nitrogen rate (120 kg ha^{-1}) in the direct planting, probably higher than necessary nitrogen amounts in relation to available phosphorus and potassium, was followed by a two–fold higher percentage of infested plants (24.2%: 12.8%), i.e. 12.2% and by the plant tolerance decrease of 3.6 (4.3 : 4.0).

Maize weed infestation stubble crop planting led to a certain increase of the percentage of infested plants (42.8% : 35.0%) and a decrease of plant tolerance to damages caused by larvae of the second ECB generation (5.1: 4.6).

The hybrid ZPTC 196, with more developed plants, was more infested than the hybrid ZPTC 112, and it was also more yielding (by 20.6%). The percentage of infested plants, plant tolerance evaluation, number of larvae per plant and grain yield amounted to 40.7%: 35.2%, 4.96: 4.62, 0.508: 0.472 and 4.003: 3.320 (t ha^{-1}), respectively.

According to all analysed results it can be concluded that the direct planting with the application of 80 kg N ha^{-1} and weed control by herbicides is the optimal one for the maize production in the stubble crop planting, under irrigation conditions, from the aspects of plant infestation by ECB and tolerance, i.e. plant tolerance to the second generation, as well as from the aspects of grain yield.

Key words: *Ostrinia nubilalis*, second generation, maize, nitrogen, tillage regime.

INTRODUCTION

The production of maize as a second crop is possible in the Yugoslavian Corn Belt due to the sufficient total heat units, as well as to a wide scope of early maturity hybrids. The silage, sweet corn and production of maize for grain could be of an excellent quality if irrigation is applied as a cropping practice. Furthermore, the mentioned types of maize are suitable feed for the second generation ECB larvae.

The objective of this study was:

-To evaluate the percentage of attacked plants by the second ECB generation, in the production of maize as a second crop under irrigation conditions.

-To study the intensity of attack and degree of plant damage from the second ECB generation on two early maturity hybrids in three tillage regimes, three fertilizing rates with nitrogen and without weed control, as well as the grain yield production.

MATERIALS AND METHODS

The study was performed under irrigation on the experimental field of the Maize Research Institute, Zemun Polje, in the vicinity of Belgrade during the second half of the growing seasons of 1992 and 1993. Planting with density of 82,236 plants ha^{-1} was performed twice, on the 1st and 3rd July. Soil moisture at the depth up to 50 cm was monitored after waterings. There were 9 and 8 irrigations in the intervals of 6 to 11–13 days, respectively.

The next factors were observed:

A-Tillage regimes at seedbed preparation
- A1- Conventional tillage (stubble ploughing at 25 cm, harrowing and finalization)
- A2- Reduced tillage (only two harrowings at 12 – 15 cm)
- A3- No tillage, mulching with direct maize planting at the beginning of July.

B - Nitrogen fertilizing
- B1- Check with NO fertilization
- B2- Lower rate 80 kg N ha^{-1}
- B3- Higher rate 120 kg N ha^{-1}
- KB2 - 80 kg N ha^{-1}, with no weed control.

C - Maize genotypes
- C1- Hybrid ZPTC 112 (FAO 100)
- C2- Hybrid ZPTC 196 (FAO 100 - 200).

The visual assessment of the rate of plant damage and the intensity of plant attack by the second ECB generation were done in mid October. The rate of plant damage was assessed on the 1-10 scale (1 = resistant, 10 = susceptible), Hadžistević (1969). Besides the visual assessment, plant dissection was performed in 1993. Thirty plants per each treatment (3 x 10/replication) were dissected.

The data on general damage rating, percentage of attacked plants, number of larvae $plant^{-1}$ and grain yield were calculated as an average for each investigated factor and treatment and processed by the analysis of variance, using experiment model number 13: randomized complete block design for factor A, with factor B as a split plot on A and factor C as a split plot on B.

The monitoring of the moth flights was done on the light trap, located within the meteorological station, nearby the experimental field.

WEATHER CONDITIONS

Weather conditions, amount of precipitation and mean monthly temperature in comparison to the corresponding long-term average are presented in Table 1.

Table 1 - Meteorological data for Zemun Polje in 1992 and 1993

Precipitation (mm)

Year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Σ
LTA	38.8	35.8	37.8	48.0	66.3	79.7	63.6	50.2	45.3	37.2	47.1	52.3	602
1992	1.9	21.9	11.8	59.7	11.9	37.3	57.9	50.5	19.3	38.0	53.5	36.8	400
1993	21.9	18.5	49.0	13.7	17.8	32.6	54.2	42.0	47.4	9.9	61.2	78.2	446
$\overline{X}$	**11.9**	**20.2**	**30.4**	**36.7**	**14.8**	**35.0**	**56.0**	**46.2**	**33.4**	**24.0**	**57.4**	**57.5**	**423**

Mean monthly temperatures (°C)

Year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	$\overline{X}$
LTA	-0.8	1.5	5.9	11.3	16.5	19.7	21.4	20.9	17.2	11.8	5.9	1.8	17.8
1992	0.2	3.1	7.7	13.0	18.9	21.5	23.6	27.5	19.2	13.0	7.6	0.6	20.6
1993	-0.7	-1.7	4.6	12.0	20.5	22.5	23.6	24.4	18.8	14.9	3.0	4.8	20.3
$\overline{X}$	**-0.2**	**0.7**	**6.2**	**12.5**	**19.7**	**22.0**	**23.6**	**26.0**	**19.0**	**14.0**	**5.3**	**2.7**	**20.4**

LTA = Long Term Average

The mean monthly air temperature MMT for 1992 and 1993 was higher than the average for the 1953-1989 period. MMT for a six-month growing season (April – September) was 20,6°C and 20,3°C in 1992 and 1993, respectively, with the increase of 2,8°C and 2,5°C. The highest departure from LTA was in August 1992, when MMT amounted to 27,5°C, with the increase of 6,6°C and 31,6%.

Monthly precipitations for July, August and September were regularly lower or at the level of long term average (LTA). Both years were extremely dry with precipitation of 400 mm and 446 mm. Precipitation deficiency was 202 mm and 156 mm in 1992 and 1993, respectively (on the average, 179 mm or 29,7% less than the average).

FLIGHT OF THE ECB MOTH

Under agroecological conditions of Yugoslavia, ECB has been having two generations, or at least two flights per year with prevalent number of the moth in the second flight, since 1973, Glumac and Ruškuc (1979), Bača and Hadžistević (1989).

Up to the 60's when hemp was grown on significant areas, this crop was a main source of the second generation. According to Nagy and Szentkiraly (1993), 8 – 100%, and 0 – 10% adults for the second flight originated from the non-diapausing larvae of ECB population from hemp, and maize, respectively. These authors still doubt the existence of the entire second generation of ECB in Hungary. Table 2 presents the data on the ECB flight in comparison to LTA from 1973 to 1991.

Table 2 -Total number of trapped ECB moths, the 1st : 2nd flight and female: male ratios at Zemun Polje 1992 and 1993

Year	First generation		Second generation		Total	N° of	N° of	Maximum flight	
	N°	%	N°	%	Number	Females	Males	1 st gen.	2nd gen
LTA	458	18.6	2005	81.4	2463	1500	963	06/24	08/16
1992	849	20.6	3271	79.4	4120	2909	1211	06/12	08/10
1993	640	24.0	2029	76.0	2671	1385	1286	06/21	08/09

In spite of the extremely high number of 3271 second generation moths, trapped in 1992, the percentage of attacked plants was only 15.3%.

The percentage of attacked plants in 1993 was 60.7%, when the number of the trapped moths was 2020, the same as a long term average (2005). Very high air temperatures during the second flight of ECB in comparison to the LTA and significantly lower precipitation amount, especially in 1992 in the second half of the growing season, had a negative influence on the mating of the moths, fecundity of the female and survival of the second generation larvae.

RESULTS AND DISCUSSION

The data on plant infestation by the second ECB generation of maize, grown as a second crop, are presented over years, investigation factors and applied criteria in Tables 3, 4, 5, 6.

Table 3- Influence of tillage regimes, fertilizing with nitrogen and maize genotypes on plant tolerance and attack by the second ECB generation

Treatments	General Damage Rating			Attacked Plants (%)		
	1992	1993	$\overline{X}$	1992	1993	$\overline{X}$
A1 C1 B1	6.0	3.6	4.80	26.7	64.5	45.6
A1 C2 B1	5.1	4.3	4.70	20.0	82.2	51.1
A2 C1 B1	6.5	4.0	5.25	13.3	37.8	25.6
A2 C2 B1	7.0	2.9	4.95	13.3	62.2	37.8
A3 C1 B1	5.2	2.5	3.85	13.3	15.6	14.4
A3 C2 B1	2.2	4.6	3.40	8.9	11.1	10.0
B1 Check Ø N ha^{-1}	**5.3**	**3.6**	**4.49**	**15.9**	**45.6**	**30.8**
A1 C1 B2	6.0	4.3	5.15	13.3	75.5	44.4
A1 C2 B2	5.0	4.4	4.70	13.3	80.0	46.6
A2 C1 B2	5.5	4.0	4.75	0.0	82.2	41.1
A2 C2 B2	6.7	3.9	5.30	20.0	84.4	52.2
A3 C1 B2	5.7	1.0	3.35	0.0	6.7	3.4
A3 C2 B2	4.8	4.4	4.60	11.1	33.3	22.2
B2 80kg N ha^{-1}	**5.6**	**3.7**	**4.64**	**9.6**	**60.4**	**35.0**
A1 C1 B3	5.3	3.9	4.60	15.6	93.3**	54.4
A1 C2 B3	5.5	4.6	5.05	35.6	93.3**	64.4
A2 C1 B3	7.0	3.7	5.35	6.7	71.1	38.9
A2 C2 B3	7.5	4.5	6.00**	13.3	93.3**	53.3
A3 C1 B3	4.4	4.3	4.35	16.7	51.1	33.9
A3 C2 B3	4.6	3.8	4.20	11.1	17.8	14.4
B3 120kgN ha^{-1}	**5.7**	**4.1**	**4.92**	**16.5**	**70.0**	**43.2**
A1 C1 KB2	6.4	3.1	4.75	33.3	73.3	53.3
A1 C2 KB2	6.6	4.6	5.60	53.3**	80.0	66.6
A2 C1 KB2	3.0	3.9	3.45	3.3*	80.0	41.6
A2 C2 KB2	6.3	5.1**	5.70	0.0	91.1	45.6
A3 C1 KB2	7.6**	4.1	5.85	15.6	35.5	25.6
A3 C2 KB2	7.0	3.6	5.30	8.9	40.0	24.4
80 kg N ha^{-1} Ø W.C.	**6.2**	**4.1**	**5.10**	**19.1**	**66.6**	**42.8**

Based on the analysis of variance, significant differences were registered, as it follows:

- Plant damage rating (PDR) for the years: LSD = 0.86 at 0.05 and 1.69 at 0.01. Coefficient of variation = 23.23%

- Percentage of attacked plants (PAP): LSD_C = 12.02 at 0.05 and 23.61 at 0.01. Coefficient of variation = 23.09%.

- Yield for factor B, nitrogen fertilization: LSD = 0.243 at alpha 0.05 and 0.348 at 0.01; LSD_C = 0.365 at 0.05 and 0.718 at 0.01 ; LSD_{AC} = 0.290 at 0.05. Coefficient of variation = 7.28 %.

A3 B2 and the lower nitrogen rate (80 kg N ha^{-1}) are the most suitable combination of the tillage regimes and nitrogen fertilization for ZPTC 112 with the lowest values of PDR and PAP (3,35 and 3.4%, respectively). The corresponding data for the hybrid ZPTC 196 are A3 B1 with no nitrogen fertilization and PDR = 3,40, and PAP = 12,2 %.

Reduced soil tillage, especially direct maize planting, led to the significant infestation decrease by the second ECB generation. At the same time, plant tolerance did not change much.

Nitrogen fertilizing led to a significant increase of plant infestation by the second ECB generation, plant tolerance decrease, and especially to the grain yields increase.

Table 4 - Plant damage rating and percentage of attacked plants by the second ECB generation according to tillage regimes and fertilizers rate

Variants Tillage / Fertil	B1 Check Nitrogen = 0 ha^{-1}	B2 80 kg N ha^{-1}	B3 120kg N ha^{-1}	KB2 Ø W.C 80 kg N ha^{-1}	$\overline{X}$
Plant Damage Rating (PDR)					
Conventional	4.75	4.92	4.82	5.18	**4.92**
Reduced	5.10	5.02	5.68 *	4.58	**5.10**
No tillage	3.62 **	3.98	4.28	5.58	**4.36**
$\overline{X}$	**4.49**	**4.64**	**4.92**	**5.11**	**4.79**
Percentage of Attacked Plants (PAP)					
Conventional	48.4	45.3	59.4 *	60.0 *	**53.2 ***
Reduced	31.7	46.6	46.1	43.6	**42.0**
No tillage	12.2 **	12.8 **	24.2	25.0	**18.6 ****
$\overline{X}$	**30.8**	**35.0**	**43.2**	**42.8**	**38.0**

* and ** the lowest and the highest values, respectively

The variant A3 B1 for both hybrids is the best combination of the tillage regimes and nitrogen fertilization according to the values of PDR (3.62 and 3.98) and PAP (12.2% and 12.8%).

In the absence of weed control PAP was at the level of higher nitrogen rate (25.0%:24.2), but PDR was much higher (5.58: 4.28).

Table 5 - Average number of larvae per plant in 1993

Variant	B1 Check N ha^{-1} = 0	B2 80 kg N/ ha^{-1}	B3 120 kg N ha^{-1}	KB2 Ø W.C 80 kg N ha^{-1}	$\overline{X}$
A1 C1	0.5000	1.3000	1.0000	0.9000	0.9250
A1 C2	0.3667	1.1000	1.1667	0.9333	0.8917
Conventional	**0.4333**	**1.2000**	**1.0833**	**0.9167**	**0.9084**
A2 C1	0.1667	0.4333	0.3667	0.3000	0.3167
A2 C2	0.3667	0.5000	0.3333	0.3667	0.3917
Reduced	**0.2667**	**0.4667**	**0.3500**	**0.3333**	**0.3542**
A3 C1	0.1667	0.1333	0.1667	0.2333	0.1750
A3 C2	0.0667	0.1000	0.4333	0.3667	0.2417
No tillage	**0.1167**	**0.1167**	**0.3000**	**0.3000**	**0.2084**
$\overline{X}$	**0.2722**	**0.5944**	**0.5778**	**0.5167**	**0.4903**

Based on both, the PAP and PDR, in 1993, the lowest number of ECB larvae per plant was in the variants A3 B1 and A3 B2 with direct planting without nitrogen and with 80 kg N ha^{-1}.

The average number of overwintering ECB second generation larvae calculated per area amounted to 36 772 ha^{-1} (0.4903 x 75,000).

If the same reaction had occurred in the production of sweet corn as a second crop after wheat in order to get the lowest attack and the mallest number of larvae per plant, the direct planting and fertilizing with lower dosage of nitrogen, would have been recommended.

Grain yield (Table 6) ranged from 2 970 kg ha^{-1} in A1xB1 variant with conventional tillage without nitrogen application to 4558 kg ha^{-1} in the A3 B3 variant with no tillage i.e. with direct planting and higher nitrogen rate (120 kg ha^{-1}).

On the base of the analysis of variance there was highly significant differences among the rates of nitrogen (B) and between the hybrids (C) and the interaction among tillage regimes (A) and hybrids (C).

The ZPTC 196 of FAO 200 was more yielding proving that each yielding potential of each later maturity group is higher by approximately 0.7 t ha^{-1}.

Table 6 - Grain yields (kg ha^{-1} with 14% grain moisture) according to tillage regime and nitrogen rate, Zemun Polje 1992 and 1993

Variants Tillage/Fertil.	B1 Check Nitrogen = 0	B2 80 kg N ha-1	B3 120 kg N ha-1	KB2 Ø W.C 80 kg N ha-1	$\overline{X}$
A1	2 970 *	3 595	4 024	3 409	**3 500**
A2	3 248	3 857	4 379	3 157	**3 660**
A3	3 189	4 100	4 558 *	3 450	**3 824**
ZPTC 112	2 913	3 451	3 881	3 033	**3 320**
ZPTC 196	3 357	4 250	4 760	3 644	**4 003**
$\overline{X}$	**3 136**	**3 851**	**4 320**	**3 339**	**3 662**

CONCLUSIONS

The level of plant damage and intensity of attacked plants of two maize hybrids was determined in this study on the ECB second generation.

The obtained results on the investigated agronomic practices, indicate that reduced tillage and especially no tillage at seedbed preparation for stubble maize planting (after wheat) with the adequate nitrogen rate, resulted in the infestation decrease by the second ECB generation, in the plant tolerance increase, in the decrease of the larvae number per plant, as well as in the significant grain yield increase.

According to all analysed results on PDR, PAP, number of larvae per plant and grain yield, it can be concluded that the direct planting with the application of 80 kg N ha^{-1} and weed control by herbicides is optimal for the maize production in stubble crop planting, under irrigation conditions.

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EVALUATION OF ECONOMIC THRESHOLD AND TOLERANCE THRESHOLD FOR *SESAMIA NONAGRIOIDES* (Lef.) AND *OSTRINIA NUBILALIS* (Hub.) IN CORN

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ABSTRACT

An Integrated Pests Management strategy require the application of different control measures, chosen and applied according, not only to ecological and toxicological aspects, but also injury thresholds. To set such a strategy presupposes the profound knowledge about injury thresholds of pests and diseases.

Based on essays made in Alentejo (1992 and 1993) to study the losses caused by corn borers (*Sesamia nonagrioides* Lef. and *Ostrinia nubilalis* Hub.), it is presented, as an example, the methodology and the calculus for the evaluation of the economic threshold and tolerance threshold for those pests.

Key-words: Maize stem borers, economic threshold, tolerance threshold.

INTRODUTION

In Integrated Pest Management (IPM) the control of pests requires technologies which satisfy economical, toxicological and ecological exigencies having in account the relation costs/benefits due to those methods of control. The definition of this kind of strategy demands, therefore, deep knowledge about pests levels and the correspondent losses. Knowing this relationship and the acceptable losses level is very important.

In IPM there are several concepts about the relation between pests levels and the correspondent losses, the reason why it is important to know their meaning and the used methodologies in their evaluations. In entomology the concept usually adopted is the economic threshold, which corresponds to the pests level from which control measures should be applied in order to prevent possible losses, higher than the costs, of the control measures, and the undesirable consequences due to them. The tolerance threshold is the reduction of production (in percentage) from which it is profitable to control pests (Amaro & Baggiolini, 1982; Aguiar, 1992; Portugal *et al*, 1995).

The calculation of the economic threshold involves the existence of a descriptive model of losses and the knowledge of the control costs. In this paper we present the economic and tolerance thresholds of the maize stem borers *Sesamia nonagrioides* Lef. and *Ostrinia nubilalis* Hub in corn using only one method of control: the application of insecticides.

METHODOLOGY

The losses model used in this study is a descriptive model, the linear model, calculated by Pereira (1994), in researches made in Alentejo.

This model is presented by the following formula:

$$Y = -1.95 + 5.34\ AI$$

Being

Y - Production losses, in percentage, caused by the maize stem borers

AI - Pests level

The r^2 was of 97% (Pereira, 1994).

Taking in account the above mentioned model, the economic threshold was calculated using the following formula, pointed by Fernandes (1994):

$$d = \frac{C}{b \times E \times P \times Pp}$$

Being

d - Pests level from which it is profitable the control

C - Costs of the control measures

P - Unit price of production

Pp - Potential production paid to the productor

E - Efficiency of the control measures (between 0-1)

b - Linear model parameter

Following the same criteria, the tolerance threshold was calculated. To evaluated the tolerance threshold it is used the formula indicated by Fernandes (1994), as follows:

$$Y = \frac{C}{ExPxPp} x100$$

Being

Y- The tolerable reduction of production, in percentage, caused by maize stem borers

C, *P*, *Pp*, and *E* already mentioned

RESULTS AND DISCUSSION

Tables 1 and 2 show, respectively, the values of control costs (C), the other parameters already referred, as well as their explanation.

Table 1 - Evaluation of the control costs

Parameter	Calculations
Situation 1 - Endosulfan (380 g/l)	
Costs of insecticide (C1)	Concentration - 500ml/hl Dose - 5 l Price (*l*)- 2400 PTE C1 -5 x 2400 = 12000 PTE
Workmanship (C2)	Daily - 6000 PTE Hours/ha - 0.75 C2 - 0.75 x 6000 = 4500 PTE
Equipment cost (C3)	Petrol/ha - 6 l Petrol price (l) - 73 PTE Amortization rate - 700/ hour Hours/ha - 0.75 C3 - 6 x 73 + 0.75 x 700 = 963 PTE
C (costs of control)	C1+C2+C3 = 17463 PTE
Situation 2 - Lambda-cialotrin (50 g/l)	
Costs of insecticide (C1)	Dose - 400 ml/ha Price (*l*)- 9600 PTE C1 - 0.4 x 9600 = 3840 PTE
Workmanship (C2)	C2 - 0.75 x 6000 = 4500
Equipment cost (C3)	C3 - 6 x 73 + 0.75 x 700 = 963 PTE
C (costs of control)	C1+C2+C3 = 9303 PTE

In order to evaluate the parameter C (control costs), it was considered the use of two different insecticides (endosulfan and lambda cialotrin), both indicated for maize and having an equal way of application and similar efficiency. All the evaluations are based in hectare and the costs are in escudos (PTE).

Table 2 -Parameter values used in formulas to evaluate economic threshold and tolerance threshold.

Parameter	**Value**	**Observations**
P	43 PTE /Kg	Price in 1994
Pp	6000 Kg/ha	Medium value of zone of essays and according to the productors information's
E	0.65	65% of efficiency for both insecticides was considered. It is difficult to combat the borers when they are inside the plant.
b	5.43	Value achieved by Pereira (1994). This value must be divided for 100 to be used in the formula.

With the formulas presented before and the values presented in Table 1 and 2, it was evaluated, for both insecticides used in the control of the maize stem borers, the economic threshold and the tolerance threshold.Table 3 shows the values of the two injury thresholds.

Table 3 -Values of the economic threshold and tolerance threshold of maize stem borers with data obtained in 1994 in Alentejo.

Injury thresholds *Insecticides*	**Economic threshold** *Larvae and pupae/plant*	**Tolerance threshold** %
Situation 1 - Endosulfan (380g/l)	1.95	10.4
Situation 2 - Lambda cialotrin (50/l)	1	5.5

The results show that the economic threshold to maize stem borers can present values between 1.95 larvae and pupae/plant, when endosulfan is used, and 1 larvae and pupae/plant, when lambda- cialotrin is used.

The tolerance threshold presents a similar variation, with the different mentioned products.

Attention must be paid to the fact that, when using endosulfan, it is allowed a number of maize stem borers two times superior than when lambda cialotrin is used. The results show clearly that, according to the costs of the control, different values of economic threshold and tolerance threshold are achieved. The higher the cost of control is, the higher both thresholds will be. The obtained economic threshold with lambda cialotrin (the cheaper insecticide) is superior to the indicated in Switzerland, for *Ostrinia nubilalis* (0.3-0.5 larvae/plant) mentioned by Bigler *et al* (1990). Therefore, the thresholds obtained are similar to those indicated by Redford (1976) and Amaro (1981) (1-2 larvae/plant and >1 larvae/plant, respectively) in France.

These results prove, once again, the importance of the insecticide used in the control, in the value of the economic threshold and in the tolerance threshold. This importance is bigger when the products have different efficiencies. In spite of this differentiation has not been demonstrated in this work, due to the fact that it was considered equal efficiency for both products (endosulfan and lambda cialotrin), it can be deducible, however, from the formula used in the evaluations of the economic threshold.

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CHANGES IN THE PEST STATUS WITHIN MAIZE INSECT ASSEMBLAGES IN THE CARPATHIAN BASIN

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ABSTRACT

The maize is the most important crop plant in the Carpathian Basin. Within this region North Serbia (Vojvodina), Croatia, Hungary and even West Romania (Transylvania) have important areas with maize. The importance of animals attacking maize might be very variable during the centuries, however, among pests mainly the European corn borer (*Ostrinia nubilalis* Hbn.) proved to be disastrous especially in the southern half of the Carpathian Basin in the 19-20. centuries. During the 20th century the European corn borer has remained nearly a continuous, but variable pest of the maize, however, damages were seldom catastrophic. Over the last two decades some changes were found in the pest status of more harmful species (*O. nubilalis, H. armigera, D. v. virgifera*) within phytophagous insect assemblage associated with maize in the Carpathian Basin. The new situation can be explained by climatic fluctuation and partly by land-use.

Generally new situation began to arose from the fifties, when a conspicuous second flight of the European corn borer was detected, which should be considered as a possibility for a real second generation. Until the 90's only scattered and uncertain data were available in this respect. In late summer of 1987-89 and 1995 several egg clusters and newly hatched and even L_2-L_5 larvae were detected in maize stands in South Hungary. It turned out, that a prolonged warmer period until autumn is enough to complete the larval development in the southern part of the Carpathian Basin.

Until now, there have been no economic damages caused by the 2nd generation larvae on industrial corn fields, however, damages may be expected on late growing sweet corn.

Unexpected invasions of the cotton bollworm (*Helicoverpa armigera* Hbn.) represent another new and severe situation on maize in the Carpathian Basin. Sporadical appearances were noticed since some decades in Hungary, but, in 1986 and especially in 1993-94 strong invasions and damages were found in southern and even in central Hungary, too. New data showed a possible partial overwintering of the pupae as a result of increasing number of warm and droughty years associated with mild winters between 1980-1993. In years with suitable climate pattern further damages of the cotton bollworm are also expected on maize.

The appearance of the Western corn rootworm (*Diabrotica v. virgifera* LeConte) in the southern region of the Carpathian Basin was the result of a „successful" introduction and colonisation of this north-American insect pest in 1992. According to the rate of

dispersal between 1993-1996, it seems very likely that no climatic and/or ecological barriers exist for *Diabrotica* in the Carpathian Basin at present.

Occasional developments of the second generation of the European corn borer, the new, stronger invasions of the cotton bollworm and the accidental, but powerful colonisation and spreading of the *Diabrotica* has created a new situation of the insect pest status on maize in the Carpathian Basin and it requests more international cooperative research efforts and control practice.

Key words: Maize pests, Carpathian basin.

INTRODUCTION

The insect pest assemblage of maize is characteristic of a given geographical region. The pest complex of the Hungarian maize fields (which is nearly similar in the surrounding countries, as well) may be seen in a simplified table, in which the insect pests are arranged by their importance and by their seasonal appearance (Table 1). Beside the European corn borer, from time to time *Oscinella frit* L.,*Tanymecus dilaticollis* Gyll., *Agrotis segetum* Den. et Schiff., *A. ipsilon* Hufn., more seldom other noctuids and aphids proved to be somewhat important pest of maize, causing variable loss periodically.

Table 1 -The insect pests on maize in Hungary arranged by their approximate importance and seasonality

Damage level	Spring May	Early-Summer June-July	Late-Summer August-September
High	*Agriotes*, etc. *Tanymecus*	*Diabrotica ?* *Helicoverpa*	
Medium		*Ostrinia* (1st G.) *noctuids*	
Low	*Oscinella*	aphids Oulema Phyllotreta	*Ostrinia* (2nd G.?) aphids (R. *padi*) mites (T. *urticae*)

Abreviations: G: generation; ?: the future of the pest is unpredictable.

Here there is no space and time to analyse the importance of each species. Our main aim is to focus on those insect pests, which became crucially significant in the last decade. The insect pest assemblage of the maize has suffered important ecological changes during the last decades, which caused parallel modifications in the economical evaluation, as well.

The phenomena to be discussed here, are as follows:

1-The number of flights and possible generations per year of the ECB *(Ostrinia nubilalis* Hbn.) within the Carpathian Basin;

2-The growing importance of the cotton bollworm (*Helicoverpa armigera* Hbn., CBW) in Hungary;

3-The spread and colonisations of the western corn rootworm (*Diabrotica v. virgifera* LeConte, WCR) in Europa.

THE SITUATION OF THE EUROPEAN CORN BORER (ECB)

The maize is the most important crop plant in the Carpathian Basin and within this region Vojvodina (North Serbia), Croatia, Hungary and even Transylvania and somewhat South Slovakia have the most intensive corn growing areas. The importance of animals attacking maize might have been very variable during centuries, however, among the harmful maize insect pests, particularly the ECB proved to be sometimes disastrous, especially in the southern half of the Carpathian Basin. Devastating outbreaks were registered e.g., around the turn of this century, especially in the 1890s, when maize fields suffered almost a total crop loss in a great part of the Pannonian Plain (Jablonowski 1898). The damages caused by the ECB were fluctuating around the economical level through the 20th century, so, there were no need (except in some specific, sporadic cases) for regular chemical control. Over several decades recommended control methods against the ECB were the correct handling of the dry corn stalk (fodder, burning the stalk material until 15th of May, ploughing the chopped stalk into the soil).

A new era has started from the 1950s, when a conspicuous and nearly continuously appearing second (Summer) flight of the ECB moths was detected in Hungary (Nagy 1961, 1993), however, the starting period of this second flight is still not known accurately. This change in the phenology of the flight might have happened between 1930 and 1953 in Hungary, because prior to this period no more than 1-2 % of the populations showed a second flight (Nagy and Szentkirályi 1993). Sáringer (1973) found no differences among responses of the Hungarian ECB populations originating from different localities of the country to the photo- and thermoperiodic effects. After 1959 by the settlement of Hungarian agricultural light trap network the long-term patterns of both flights of ECB could have been monitored countrywide. By these patterns a change was detected in the proportion of flights mainly in the Southern area of corn belts since 1972: the number of individuals captured during the 2nd flights was consequently higher than in the 1st ones (Figure 1). According to the Figure 2 over the last three decades Hungary had become a transitive zone for ECB populations having one (Northern areas), or two (Central and Southern areas) flights per season.

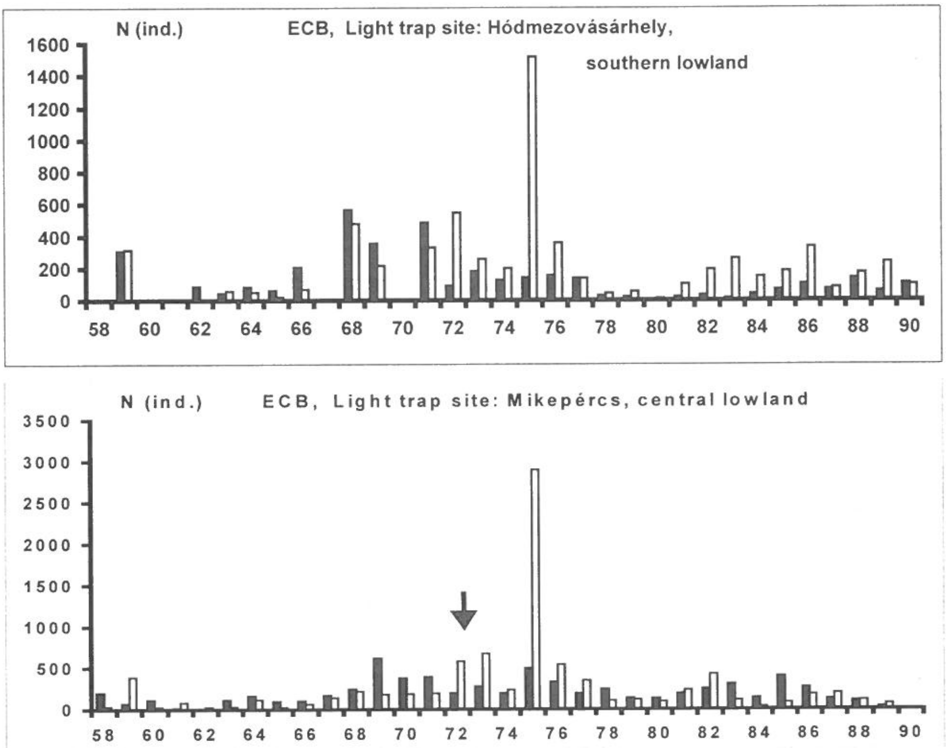

Figure 1 – Long-term yearly fluctuation pattern of the first and second flight of the European Corn Borer at two light trap stations within the Hungarian corn belt (N:number of captured individuals, arrows sign the beginning of change in the ratio of the two flights, black column 1st flight, white column: 2 nd flight).

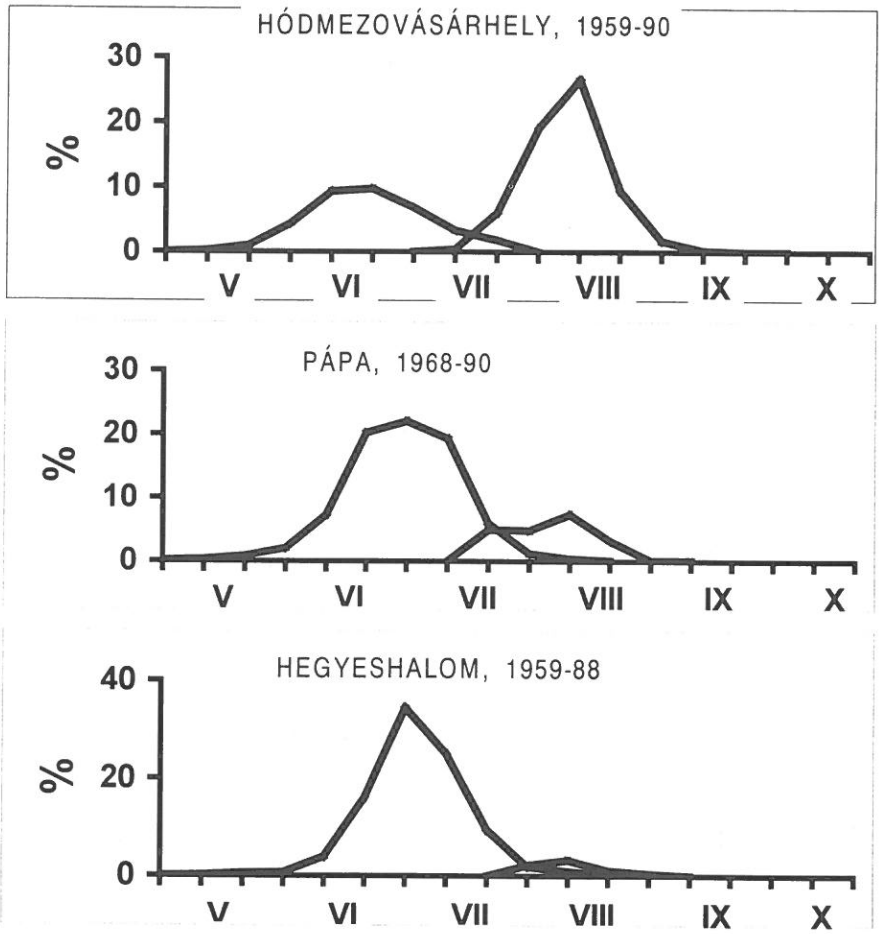

Fig. 2- Differences in the mean seasonal pattern of the European Corn Borer flights based on light- trapping in Hungary in a S-N transection. The latitudinal change in the ratio of the 1st to 2nd flight indicates a possible transitive zone of voltinism in Hungary. (Hódmezõvásárhely:Southern station, Pápa: Central station, Hegyeshalom: Northern station).

In spite of the high number of the moths from the second flight, there were only scattered and unconvincing data related to the existence of a real and complete second generation in Hungary (Nagy 1993, Nagy and

Szentkirályi 1993). For the first time in Hungary detailed examinations showed the existence of second generation ECB larvae in the late 1980s. These investigations were carried out in the southern part of Hungary (at the Station of Plant Hygiene and Soil Protection, Szekszárd), where the differentiation of developmental stages was made periodically by measurement of the head capsules of the ECB larvae sampled from maize plants taken in every week from the fields.

Figure 3 shows the gradually appearing new, second generation larval populations (L_1-L_5) in late August and early September. This phenomenon was found in the previous years (1987, 1988) as well.

By our surveys these second generation larvae could complete their development and reached the last stage (L_5) until October. During a control survey in 1995 we found again egg clusters, hatching larvae and even L_2 and L_3 on the 19th of September. There was no doubt that these larvae were belonged to the second generation, as well.

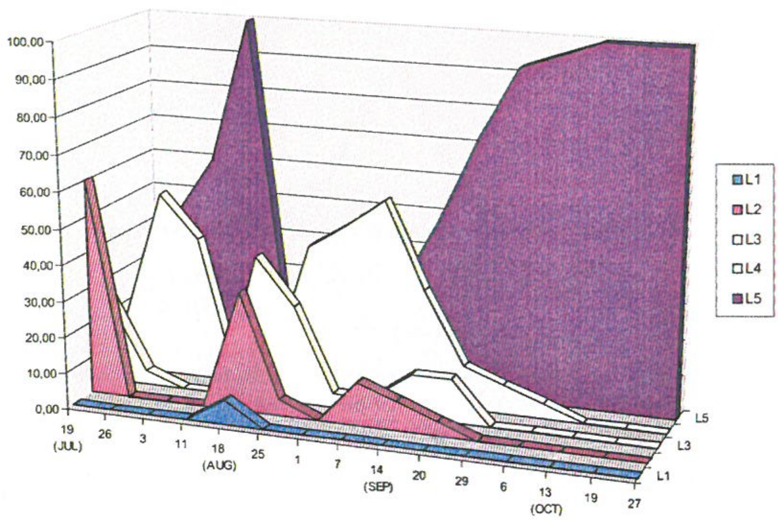

Figure 3 -Temporal distribution of larval stages of 2nd generation European Corn Borer population in Southern Hungary (site: Nagytormás, 1989).

Based on the above mentioned facts, it may be concluded that at least in Central and Southern Hungary, the second generation of the ECB is already present, however the size of the population, the regularity of the flight and the life history are not properly known. It seems likely that the further development of these larvae depends on the prevailing temperature and suitable food sources in the late Summer and early Autumn.

Scattered experiences show an increasing pressure on the second generation by parasites, especially by the egg-parasite *Trichogramma* (Nagy 1984). Our observations in South Hungary, cited above, were also demonstrated a high level of egg parasitization (91.5%) by *Trichogramma* sp. between 19-22. of September. This high mortality might be one of the main obstacle against a stronger second generation in Hungary. Related to the appearance of the second generation in this period, we should mention that in the last 15 years there were a series of hot and droughty years in Hungary. The increasing occurrences of the second generation might also correspond with this climatic change.

With full knowledge of the facts, the situation of the voltinism of the ECB in the Carpathian Basin may be summarised as follows: Baca *et al.* (1993) mentions two regularly recorded generations related to the Beograd-Zemun district ("Serbian corn belt"), however, he stated that the 2nd generation was low or negligible before 1972 (Baca 1984). The dominance of the second flight was clearly shown in a long-term light-trapping at Zemun between 1973 and 1985 (Baca and Hadzistevic 1989) as it was also detected in Hungary (Figure 1). In the Central part of Transylvania (Turda) pheromone trap catches have proved the existence of one flight between 1990 and 1993 (Muresan and Mustea 1993). However, the area of Banat (SW from Transylvania) lays in an intermediate position between Vojvodina and South Hungary, therefore, the second flight ought to be present in Banat, too. In Hungary, nowadays there is a great fluctuation in the ratio of the two flights (Figures 1-2). The most accentuated and predominantly regular second flight may be found in the "warmer" Southern half of the country, however, in particular cases the second flight may be traced even in the "cooler" Northern part of the country, too (Nagy 1961, Mészáros 1969).

"The second generation was observed for the first time (1983!) in the Southern districts of Slovakia" (Longauerova 1989); this statement surely relates to an occasional second flight only and not to a real second generation.

It is regretful that, we do not have recent data, but as far as the presence of a real second generation, the situation in Eastern Croatia may be very similar to that of the Zemun district. In Southern Hungary the occasional occurrence of the complete 2nd generation of ECB has been detected since the mid-eighties. We can draw the conclusion: recently the 2nd generation ECB larvae do not cause any economic damages in maize fields in Hungary.

COTTON BOLLWORM (CBW)

The increasing data for the existence of a second generation of the ECB and the more and more frequent appearances of the CBW in Hungary seem to be analogous phenomena. In contrary to the ECB, the occurrence, seasonality and host plant pattern of the CBW is relatively well-studied in Hungary. The spatial immigration pattern of this species detected by light trapping is presented in Figure 4, on which can be see the immigration to the Southern part of Hungary.

It was also demonstrated, that most of the outbreaks and appearances were connected mainly with the droughty and warm Summers (Szeõke and Dulinafka 1987, Szabóky and Szentkirályi 1995) (Figure 5). The increasing number of mild Winters of the last decades may contribute to the successful pupal overwintering in Hungary (Szeõke 1995, Vörös pers. communication, Kozár 1997), which was not observed here previously. In Hungary the deleterious outbreaks of CBW have occurred after strong invasions of adults from the Mediterranean region. Therefore, the population movements of the Mediterranean stocks already have to be taken into consideration in time.

In spite of the CBW polyphagy, maize should be considered a host preferred plant in Hungary and consequently the problem of plant protection on maize fields will be increased by pest invasions and outbreaks. This pressure to the plant protection seems to be much higher in the Central and Southern parts of Hungary, where the maize is the most important crop ("Hungarian corn belt"). As a Mediterranean invasive species, the CBW can cause similar or even more serious damages in Vojvodina and Banat, but the possibility of an extensive colonisation is almost negligible to Slovakia and

Transylvania provided that the ecological and genetical characteristics of this species will not change in the near future.

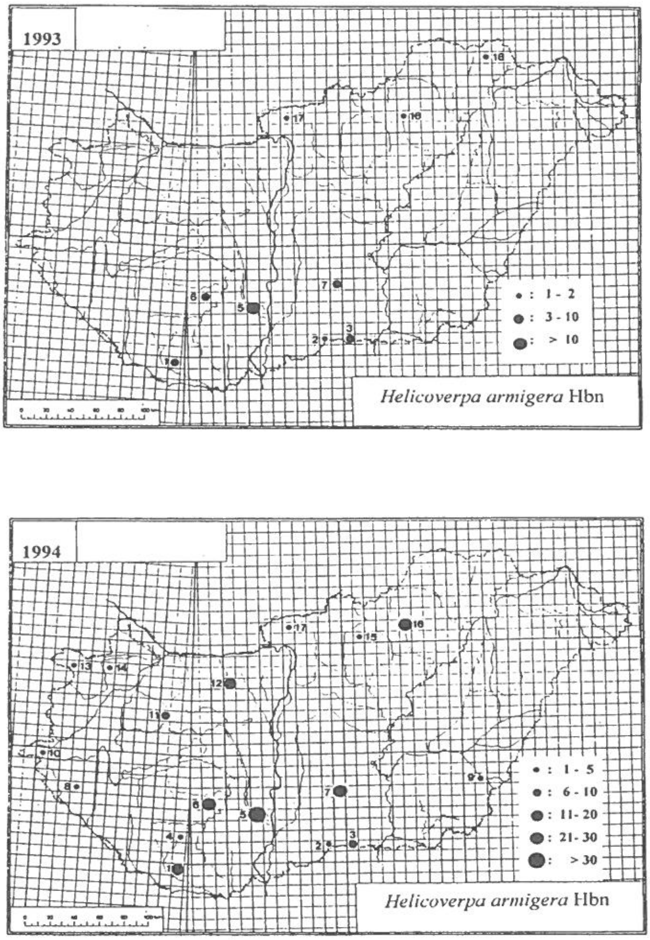

Figure 4 -Spatial distribution of *Helicoverpa armigera* adults captured by light traps in Hungary (1993-94). (The numbers mean the trap sites, the diameter of circles change by the range of total number individuals captured) (after Szabóky and Szentkirályi 1995).

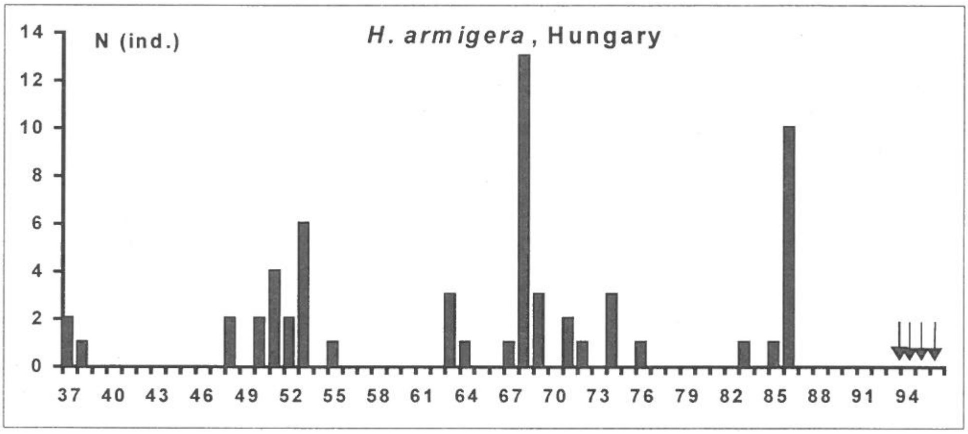

Figure 5 -Long-term occurrence pattern of *Helicoverpa armigera* in the Carpathian Basin based on total number of adults collected since 1931 in Hungary (Arrows sign the years characterised with very high levels of population) (after Szeõke and Dulinafka 1987, modified).

WESTERN CORN ROOTWORM (WCR)

The WCR, the "amerikai kukoricabogár" (= "American corn beetle" is the name of this insect in the Hungarian plant protection practice), is an entirely new and not indigenous invader in Europe.

The geographical position of the place of introduction (near to Beograd) represents suitable position for dispersion and for new colonisations nearly to every directions (Baca *et al.* 1995, Camprag *et al.* 1995). The WCR had expanded its range of distribution to Hungary (1995) and 35 new points of dispersion were detected in 1996 (Ilovai *et al.* 1997) and the first damages were also noticed.

There is no doubt, that the appearance of WCR will hemper the plant protection practice of the maize in the Carpathian Basin, as well.

There have been tremendous experiences accumulated in relation to *Diabrotica* spp. in North America (e.g. Levine and Oloumi-Sadeghi 1991), however, it is too early to predict the future position of the WCR in Central and Southeastern-Europe. In any case, detailed analysis of climatic and other factors proves, that there are no serious obstacles to the dispersion even to the main corn growing districts of Germany (Baufeld *et al.* 1996).

This statement seems to be more valid to Hungary, where maize fields form even more continuous, nearly unbroken " green carpet". We have the feeling that it should be concentrate the research work mainly to the ecological aspects which may help to dismantle problems connected with the WCR in Europe.

CONCLUSIONS

The last two decades brought quite new elements into the plant protection of the maize, which is one of the main crops of the Carpathian Basin. The detectable occurrences of the second brood of ECB, the increasing number of invasions and outbreaks of the CBW, the successful spreading and colonisations of the WCR, these all represent entirely new situations and new members of the insect assemblage of the maize fields in the Carpathian Basin. These spatio-temporal changes mean a new situation in the ecology of this insect pest assembly and will generate new and, in some times, severe problems both in the research work and plant protection practice.

In serious cases, evoked by the CBW and WCR, even chemical treatments should be used, but, considering the high level of parasitization of the egg clusters of the ECB in August and September, it is obvious that this natural control must be supposed even artificially, too.

In the case of the WCR we have to remember Stanley Beck's words (1987) written in connection with the introduction of the ECB to America: "The twentieth-century invasion by the insect provided a powerful impetus for intense pursuit of the biological knowledge essential to the containment of its agricultural threat".

It seems likely, that the changes (climate change, land-use change) which have been accumulating in the Southern and Central regions of the Carpathian Basin, are highly suitable for the insect pests discussed above. The geographical dispersal of these maize insects, require an accurate, co-ordinated research and practical work among at least four countries in this Southern edge of the Carpathian Basin. We hope, that after consolidation of political and economical situation in this crucial area, the possibility of a closer co-operation will be fruitful both at the research level and at practical, grounds, as well.

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THE MIGRATORY CAPACITY OF *TRICHOGRAMMA DENDROLIMI* IN CABBAGE AND MAIZE FIELDS

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ABSTRACT

The migratory capacity of *Trichogramma dendrolimi* was determined both in a cabbage field against *Mamastra brassicea* L. (natural conditions) and in a maize field where no natural infestation of *Ostrinia nubilalis* Hüb. was detected (artificial conditions), against *Sitotroga Cerealella*.

In the cabbage field conditions the average migratory capacity recorded in first treatment, with *T. dendrolimi* reared on eggs of *Sitotroga cerealella* O. under gamma light radiation, was 28.8% and 77.3% at a distance from the center of 7.2 m and 0.80 m, respectively. In the second treatment, with *T dendrolimi* reared in eggs of *Sitotroga cerealella* without gamma light radiation, the, migratory capacity ranged from 18,7% to 71,6% at distances from the center of 7.2 m and 0.8 m, respectively

In the maize field conditions, the average migratory capacity in the first treatment differed from 19,6% to 81,6%, while in the second treatment it varied from 14,0% to 64,5%. Parasitizing percent both in the cabbage and maize fields, was significantly higher in the first treatment than in the second.

Coefficients of paired correlations were always sufficiently high both in the first (R=-0.980) and second treatment (R=-0.979) in the cabbage field , as well as in the maize field for the first (R=-0,980) and the second (R=-0,850) treatments.

Key words: *Trichogramma dendrolimi,* migratory capacity.

INTRODUCTION

The migratory capacity of *Trichogramma* from the releasing centre and the ovi-parasitizing of its host can be determined by its endogenous and exogenous factors.

According to Grinberg, S. (1991) and Muresan,F. and Mustea, D. (1995) the perceptible effect of *Trichogramma* over the population of insects can be noticed at a circular area with a radius of 3-5m from the releasing point.

One efficient methodology to raise and increase the activity of *Trichogramma* consists in collecting a bulk of bio-materials and raising it on egges of *Sitotroga cerealella O. sterilized* with gamma light (Knipling and Guire, 1968; Martson and Erte, 1969). The resulting effects of this methodology can be accounted for a 2-3 fold quantity increase and for a 16-17 % increased looking capacity (quality) at the field conditions (Gravilita, L. 1995; Gravilita, L. 1995).

MATERIALS AND METHODS

The migratory capacity of *Trichogramma dendrolimi* was determined both in cabbage field against *Mamestra brassicae* (natural conditions) and in a maize field where no natural infestation of *Ostrinia nubilalis* was detected (artificial conditions), against *Sitotroga cerealella.*

The experiments, carried out both in the cabbage and maize fields, were performed two times, with two treatments and two replications. In the first treatment *T. dendrolimi* was reared on eggs of grain moth treated with gamma light while no gamma light radiation was used in the second treatment.

Data were collected on the amount of parasitized host eggs, at different distances (0.8–2.4–4.0–5.6–7.2 m) and in different directions (North, South, East and West) from the releasing point. Based on these data, regression lines and coefficients of pair correlation between ovi-parasitizing rate of its host and flight distances of *T. dendrolimi*, were determined.

No *Trichogramma* was released in the standard (control) fields of maize and cabbage. Recording was conducted in the cabbage field in natural conditions.

RESULTS AND DISCUSSION

Research in migratory capacity was determined both in maize and cabbage fields.

In maize, the obtained results on the migratory capacity are shown in Tables 1 and 2. The releases of *T. dendrolini*, under these somehow artificial conditions, were performed following a design of two treatments in eggs of grain moth. In the first treatment, *T. dendrolini* was raised in eggs treated with gamma light, while in the second treatment it was raised in untreated eggs.

It was found that a distance of 0.8 m from the releasing centre of *Trichogramma*, the parasitizing rate accounted for 81.6 % for the first treatment and 645.5 % for the second treatment. Following the same pattern, those values were, respectively for the 1st and 2nd treatments: 43.2 % and 26.5 %, for a distance of 2.4 m, 29.5% and 23.3% for a distance of 4.0 m, 22.6 % and 18.1 % for a distance of 5.6 m, and 19.6 % and 14.0% for 7.2 m.

In cabbage (natural conditions) the obtained results on the releasing of *T. dendrolimi* against the cabbage cutworm, are shown in Tables 3 and 4. The same pattern of two treatments was followed, with *Trichogramma* reared in eggs of grain moth treated with gamma light (1^{st} treatment) and with *Trichogramma* grown in untreated eggs (2^{nd} treatment).

While the records show significant differences of the ovi-parasitizing rates along the different distances from the releasing points, no significance was detected in what different directions are concerned.

Table 1 - Migratory capacity of *T. dendrolimi* in maize (*Trichogramma* reared in gamma lighted eggs of grain moth)

N	Distance (m)	Parasitizing (%)				
		North	South	East	West	Averange
1	0.8	74.2	90.5	82.5	79.2	**81.6 ± 2.1**
2	2.4	46.0	46.0	40.2	40.4	**43.2 ± 1.4**
3	4.0	32.7	29.3	27.2	27.5	**29.5 ± 1.8**
4	5.6	22.3	23.2	21.5	21.0	**22.6 ± 1.1**
5	7.2	19.6	18.3	19.3	17.1	**19.6+1.2/39.2+7.5**

Table 2 - Migratory capacity of *T. dendrolimi* in maize (*Trichogramma* reared in eggs of grain moth without gamma light)

N	Distance (m)	Parasitizing (%)				
		North	South	East	West	Averange
1	0.8	63.2	70.2	60.7	63.2	**64.5 ± 1.9**
2	2.4	25.7	27.0	25.3	28.5	**26.5 ± 2.1**
3	4.0	23.0	23.0	23.0	23.3	**23.3 ± 1.7**
4	5.6	16.1	17.5	18.8	17.2	**18.1 ± 1.1**
5	7.2	13.6	13.7	13.5	13.0	**14.0+1.6/29.2+6**

Table 3 - Migratory capacity *T. dendrolimi* in cabbage (*Trichogramma* reared in gamma lighted eggs of grain moth).

N	Distance (m)	Parasitizing (%)				
		North	South	East	West	Averange
1	0.8	75.0	77.5	80.6	76.0	**77,3 ± 1.47**
2	2.4	65.2	67.7	66.0	64.1	**65.7 ± 3.0**
3	4.0	55.7	58.2	59.6	55.5	**57.2 ± 3.0**
4	5.6	38.6	41.2	36.1	37.5	**39.3 ± 2.0**
5	7.2	26.1	28.5	33.2	27.7	**28.8+1.5/53.4+8.8**

Table 4 - Migratory capacity *T. dendrolimi* in cabbage (*Trichogramma* reared in eggs of grain moth without gamma light)

N	Distance (m)	Parasitizing (%)				
		North	South	East	West	Averange
1	0.8	68.6	75.7	73.4	34.4	**71.6 ± 3.0**
2	2.4	49.2	56.0	52.5	52.0	**52.6 ± 3.4**
3	4.0	35.6	38.1	38.2	39.4	**38.1 ± 2.4**
4	5.6	27.9	28.3	27.2	25.6	**26.3 ± 1.1**
5	7.2	18.7	19.2	18.8	18.2	**18.7+4/41.4+9.4**

It was found that a distance of 0.8 m from the releasing centre, the parasitizing percentage accounted for 77.3% in the first treatment and 71.6 % in the second treatment. Following up the same sequence, those values were, respectively for the first and the second treatments: 65.7 % and 52.6 % for a distance of 2.4 m, 57.2 % and 38.1% for 4.0 m, 39.3 % and 26.3 % for 5.6 m, and 28.8 % and 18.7% for a distance of 7.2 m. No parasitizing rate was detected in the control plot.

Data show that, either in the maize or in the cabbage situations, the parasitizing rate changes as the distance from the *Trichogramma* releasing point progresses. This leads to conclude about the need of increasing the number of releasing points, since it would allow the raising of the parasitizing rate.

Sufficiently high paired correlations were obtained from the data collected in the cabbage field, either for the first (R=-0.980) and the second (R=-0.979) treatments (Figure 1).

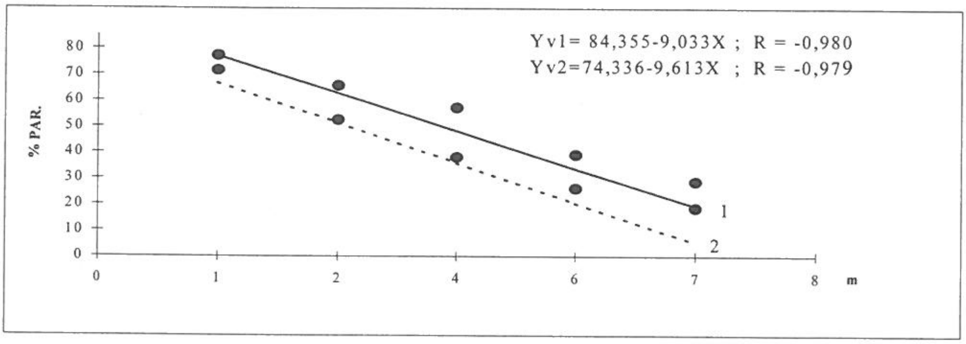

Figure 1- Regression line depending on the percent of ovi-parasitizing of *Mamestra brassicae* by *T. dendrolimi* from distance of fly in a cabbage field.

Also, the degree of the regression lines shows a direct relation between the ovi-parasitizing rate of *Mamestra brassicae* L. and the distance from the releasing point, flown by *T. dendrolini.*

On the other hand, the degree of the regression lines of ovi-parasitizing *Sitotroga cerealella* in maize (artificial conditons) (Figure 2), shows the same direct relation between parasitizing rates and distances from thr releasing point. Here again, sufficiently high paired correlation coefficients were obtained both for the first (R=-0.890) and second (R=-0.850) treatments.

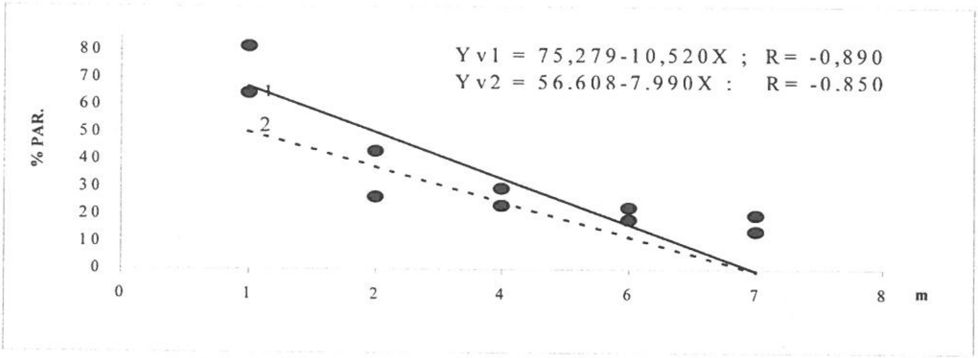

Figure 2- Regression line depending on the percent of ovi-parasitizing of *Sitotroga cerealella* by the *T. dendrolimi* over distances in a maize field.

The migratory capacity of *T. dendrolimi* (measured by its parasitizing rate) after its releasing in the field, has shown to be higher in the first treatment, both in maize and cabbage.

The quality of the migratory capacity of *T. dendrolimi* is influenced by climatic factors like temperature, air moisture, wind speed, leaf area and others.

The average temperature in this experiment was 26-28°C, the air moisture 65-70% and the leaf area was considered high in size.

CONCLUSIONS

1 -In the cabbage field the average migratory capacity of *T. dendrolimi* was high, both in the first treatment where it was reared in gamma lighted eggs of *Sitotroga cerealella,* and in the second treatment without gamma light, ranging from 28.8% to 77.3% and from 18.7% to 71.6% respectively.

2 -In the maize field, the average migratory capacity varied from19.6% to 81.6% in the first treatment and from 14.0% to 64.5% in the second.

3 -The degree of the regression lines of ovi-parasitization of *Sitotroga cerealella* egggs (artificial conditions) in maize and *Mamestra brassicae* eggs (natural conditions) in cabbage, show a direct correlation between the parasitizing rate and the distance from the releasing point of *Trichogramma dendrolimi.*

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SOME RESULTS REGARDING THE INFLUENCE OF THE GROWING SUBSTRATE OF SUBSTITUTION HOST - *EPHESTIA KUEHNIELLA* Zell. AND OF *TRICHOGRAMMA* spp.

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ABSTRACT

In this paper the results from a research conducted at Agricultural Research Station of TURDA from 1995-96 regarding the growth of *Ephastia kuehniella* Zell on different substrates (maize flour, triticale flour, wheaten flour, spring barley flour and oat flour) are presented.

In laboratory conditions (T = 16-21 °C, H = 50-50%), the highest number of adults was obtained on maize flour (1014.0/growing units), triticale flour (732.0/g.u.) and wheaten flour (677.0/g.u.). On these substrates the occupancy level was between 75,5 - 100% and the exploitation time of the growing block was 15-20 days.

At T =21,5 °C, H=70-75% and 14 hours/day light conditions the highest degrees by *Trichogramma maydis* on *Ephastia kuehniella* eggs were obtained on triticale and maize flour (96,9 and 95,7% respectively), and the same pattern was observed concerning the hatching from *Ephastia kuehniella* eggs: 94,8 - 96,3% and 80,1 - 94,4% respectively on triticale and maize flour groundings.

Key words : *Ephestia Kuehniella* Zell., *Trichogramma* spp., Growing substrate, Parasitizing degree, Hatching degree

INTRODUCTION

To obtain *Trichogramma* spp. with good qualitative attributes and in enough quantity, it is very important to choose the host.

Research on this area was done by many researchers (Ciochia, 1982; Jeanne Daumal, 1985; Haritinia Manolescu, 1987; Sandru, 1993) who growed *Ephestia kuehniella* Zell. on different substrates: wheaten flour with 2% dry yeast, wheaten semolina, maize semolina, wheaten embryo, etc.

Begining with 1995, at Agricultural Research Station, Turda, research was done regarding the influence of some growing substrates concerning : the number of *Ephestia kuehniella* adults and couples obtained, the *Ephestia kuehniella* prolificacy, the parasitizing of *Ephestia kuehniella* eggs by *Trichogramma* spp. and the hatching of this entomophagan.

MATERIALS AND METHODS

During 1995-1996, in laboratory conditions at T = 16-21°C, H = 50-70% and continuous semiobscurity, *Ephestia kuehniella* was obtained on different substrates: maize flour, triticale flour, Spring barley flour, oat flour, in 6 repetitions. The *Ephestia kuehniella* eggs obtained were applied on cardboard booklet (60x100 cm) and introduced in glass tubes near *Trichogramma* spp., in 3 repetitions.

Some observations were done concerning: the number of *Ephestia kuehniella* adults and couples obtained on each substrate, the food consumption of *Ephestia kuehniella* larvae, the prolificacy and deveplopment degree of larvae, the parasiting degree of *Ephestia kuehniella* eggs by *Trichogramma* spp. and hatching of these parasites.

The statistical calculations for these elements were done with analysis of variance, the Duncan test and the correlations in the classic model.

RESULTS AND DISCUSSION

1. In the above mentioned conditions, the adults obtained on moth flour started after 40 - 50 days from sowing with eggs on the growing substrate. Regarding the adult number of obtained adults, the biggest values were obtained on maize flour which reflects the superiority of this substrate as it was shown by the analysis of variance or Duncan test. The triticale and wheat flour showed similar results, but different from those on maize flour. The most reduced number of adults was obtained on Spring barley flour and especially on oat flour (Table 1).

2. The greatest amount of consumed food was on maize flour, sensibly equal to triticale flour, as it was demonstrated by analysis of variance and Duncan test. Wheaten flour was, however, the favorite, but its amount was significantly lower than the other two.

3. Regarding the troffic space represented by the alveols number/growing block occupied by the moth larvae, this was enough uniformly occupied on the three substrates: 100% on maize flour, 82% on triticale flour and 75% on wheaten flour. On Spring barley and oat flour the alveoli number

occupation/growing block, was lower between 6,3 - 46,0%.

Table 1 - The delimitation of trophic space and the amount of consumed food (g) by *Ephestia kuehniella* Zell. larvae, obtained on different growing substrates (A.R.S. TURDA, 1995 - 1996)

Growing substrate	Average number adults obtained/G.U.				The amount of consumed food (g) from 500 g/G.U.				Alveols ocuppied by larvae from 572/G.B.			
	absolute value	%	signfic.	Duncan test	absolute value	%	signfic.	Duncan test	absolute value	%	signfic.	Ducan test
Maize flour	1014,0	100,0	witness	a	431,0	100,0	witness	a	545,0	100,0	witness	a
Triticale flour	732,0	72,0	***	b	414,0	96,0	-	ab	374,0	82,0	***	b
Wheaten flour	677,0	66,7	***	b	392,0	91,1	*	b	339,0	75,0	***	b
Spring barley flour	385,0	38,0	***	d	349,0	81,0	***	d	212,0	46,0	***	d
Oat flour	54,0	5,0	***		100,0	23,0	***		29,0	6,3	***	
D.L. (P 5%)	63,07	6,21			35,35	8,21			38,32	8,43		
D.L. (P 1%)	85,71	8,44			48,04	11,15			52,07	11,46		
D.L. (P 0,1%)	116,19	11,45			65,13	15,12			70,59	15,53		

G.U. = Growing Unit
G.B. = Growing Block

4. The highest number of couples (male/female) /100 adults, was obtained on the maize flour (Table 2). However, comparatively with this, the triticale flour, although significantly lower, still ensures a relative enough number of couples/100 adults, being possible to replace it in obtaining moth flour in the laboratory. The third place was occupied by the wheaten flour which ensured less couples than the other two. Regarding the food consumed/larva, it can be seen that the greatest consumption was recorded on maize and triticale flour (0,58 and 0,57 g/larvae), which are statistically similar.

Table 2 -The number of obtained couples/100 adults and the amount of consumed food (g)/ *Ephestia Kuehniella* Zell. larva (A.R.S. TURDA, 1995 - 1996)

Growing substrate	Average number of couples /100 adults				The amount of consumed food (g) / larva			
	absolute value	%	signific.	Duncan test	absolute value	%	signific.	Duncan test
Maize flour	38,0	100,0	Witness	a	0,58	100,0	witness	a
Triticale flour	33,0	88,0	***	b	0,57	97,0	-	a
Wheaten flour	30,0	78,0	***	c	0,51	86,9	-	ac
Spring barley flour	23,0	61,0	***	d	0,45	76,0	**	c
Oat flour	12,0	32,0	***		0,41	70,2	**	c
D.L. (P 5%)	2,05	5,38			0,09	16,22		
D.L. (P 1%)	2,78	7,31			0,13	22,05		
D.L. (P 0,1%)	3,77	9,91			0,17	29,89		

5. Regarding the biometrical measurements of the moth larvae, it was observed that in what concerns the length of the larvae, they are not significantly different no matter the growing substrate, excepting the oat flour where very significant negative differences were recorded (Table 3). The greatest weight of moth larvae was obtained on triticale flour, with a plus of weight significantly different (16,2%) when compared with those obtained on the other substrates (maize, wheaten and Spring barley flour).

Table 3 -The biometrical measurements of *Ephestia Kuehniella* Zell. larvae, obtained on different growing substrates (A.R.S. TURDA, 1995 - 1996)

Growing substrate	The larvae lenght (cm)				The larvae weight (g)			
	Average (cm)	%	signific.	Duncan test	Average (g)	%	signific.	Duncan test
Maize flour	1,033	100,0	witness	ab	0,013	100,0	witness	b
Triticale flour	1,067	103,2	-	a	0,015	116,2	**	a
Wheaten flour	1,000	96,7	-	ab	0,014	101,7	-	b
Spring barley flour	0,933	90,3	-	b	0,012	99,4	-	
Oat flour	0,783	75,8	***		0,011	82,5	**	
D.L.(P 5%)	0,113	10,912			0,002	11,613		
D.L.(P 1%)	0,153	14,828			0,002	15,780		
D.L. (0,1%)	0,208	20,102			0,003	21,392		

6. Considering the prolificacy of moth flour obtained on the same substrates, it was observed that the highest number of viable eggs was obtained at the couples proceeded on maize flour (Table 4). From the statistical analysis it came out that the second place was occupied by couples raised on triticale flour and in third place on wheaten flour.

Table 4 -The *Ephestia Kuehniella* Zell. prolificacy obtained on different growing substrates in laboratory conditions (A.R.S. TURDA, 1995 - 1996)

Growing substrate	Average number of viable eggs/couple			
	absolute value	%	signific.	Duncan test
Maize flour	370,0	100,0	witness	a
Triticale flour	286,0	77,3	**	b
Wheaten flour	191,0	51,5	***	c
Spring barley flour	113,0	30,5	***	d
Oat flour	4,0	1,1	***	
D.L. (P 5%)	52,60	14,21		
D.L. (P 1%)	71,47	19,31		
D.L. (P 0,1%)	96,90	26,17		

7. From the observation regarding the moth flour eggs obtained on the best substrates and parasitized by *Trichogramma* spp, it was concluded that the growing substrate of the moth did influence the parasitizing agent (Figure 1). In fact, the three species of *Trichogramma* parasitized a high percentage of eggs from moths obtained on maize and triticale flour (92,0 - 97,7%).

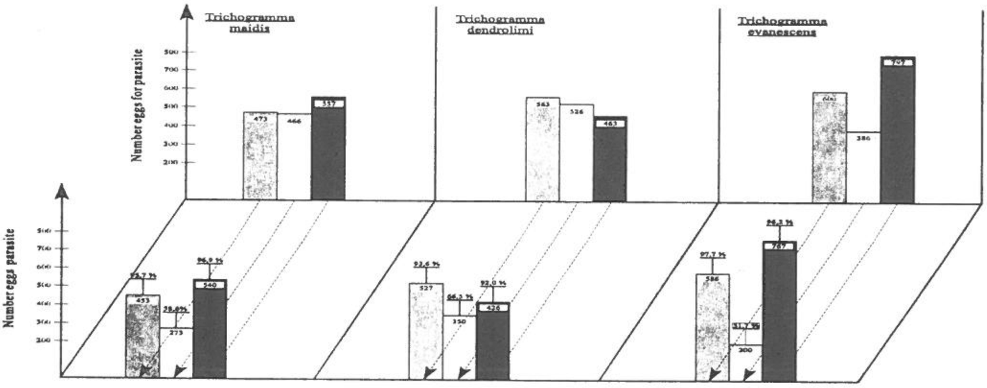
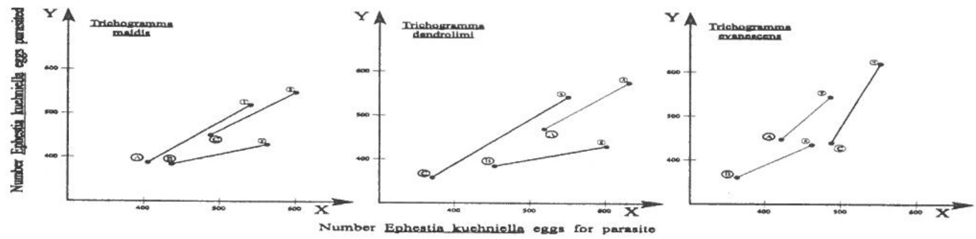


Fig. 1. The parasiting degree of Ephestia kuehniella eggs obtained on diferents substrates, by Trichogramma spp. in laboratory conditions (A.R.S. Turda, 1996)

8. The correlation between number of eggs per moth obtained on the same substrates and their parasitation by the three species of *Trichogramma* was, in all cases, significantly very high, as it is shown by the correlation coefficient values (r = 0,99***) (Fig. 2).



① y = 0,95 + 0,85x ; r = 0,999***
② y = -2,06 + 0,59x ; r = 0,994**
③ y = 0,46 + 0,96x ; r = 0,998***

④ y = 0,64 + 0,93x ; r = 0,999***
⑤ y = 2,17 + 0,65x ; r = 0,976**
⑥ y = 4,32 + 0,95x ; r = 0,996***

⑦ y = -0,01 + 0,97x ; r = 0,999***
⑧ y = -2,56 + 0,52x ; r = 0,992**
⑨ y = -0,12 + 0,98x ; r = 0,999***

Ⓐ - Maize fluor (1;4;7) Ⓑ - Wheathen fluor (2;5;8) Ⓒ - Tritical Fluor (3;6;9)

Fig. 2. The regression equations for the relations between the number of Ephestia kuehniella eggs, obtained on different substrates and the parasiting degree of these by Trichogramm spp. in laboratory conditions (A.R.S. Turda, 1996)

9. The adults hatching of *Trichogramma* spp (Figure 3) obtained on triticale respectively. This was shown by the performed linear regression in which the correlation coefficient was significantly high (r =0,99***) for all species of *Trichogramma* (Figure 4).

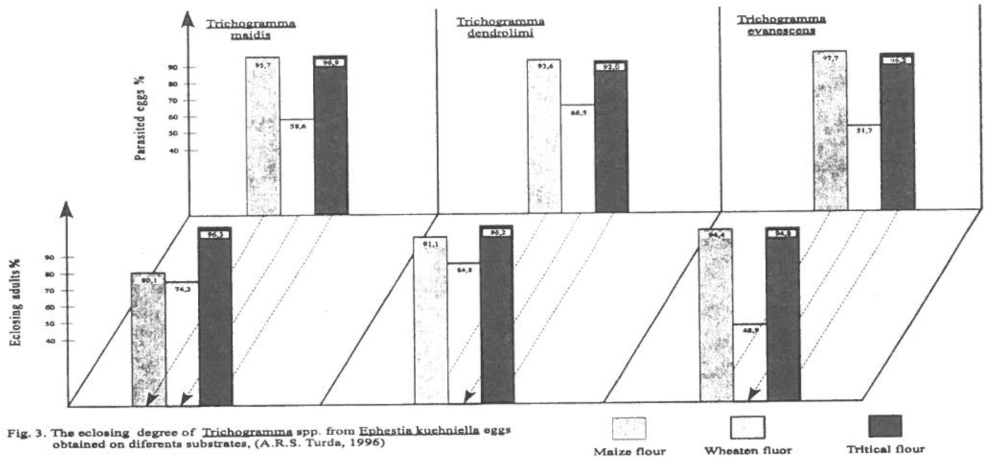
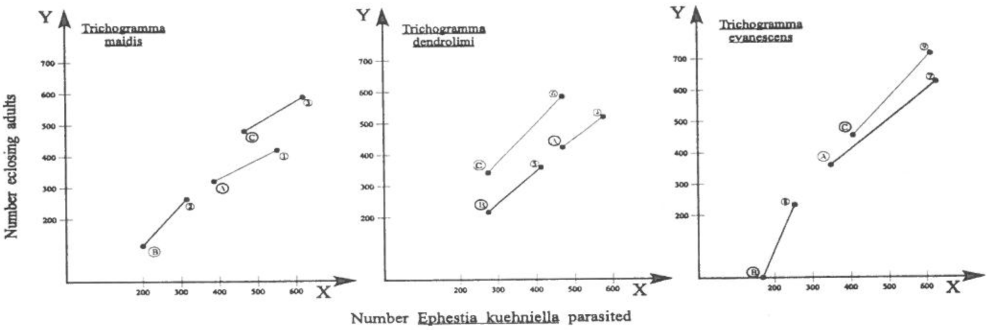


Fig. 3. The eclosing degree of Trichogramma spp. from Ephestia kuehniella eggs obtained on diferents substrates, (A.R.S. Turda, 1996)



① y = 2,16 + 0,79x ; r = 0,980***
② y = -0.51 + 0,74x ; r = 0,942**
③ y = 0,55 + 0,96x ; r = 0,999***

④ y = 2,44 + 0,90x ; r = 0,997***
⑤ y = -6,22 + 0,87x ; r = 0,990**
⑥ y = -0,64 + 0,97x ; r = 0,999***

⑦ y = 0,27 + 0,94x ; r = 0,999***
⑧ y = 4,92 + 0,43x ; r = 0,963**
⑨ y = 0,28 + 0,96x ; r = 0,998***

Ⓐ - Maize fluor (1;4;7) Ⓑ - Wheathen fluor (2;5;8) Ⓒ - Tritical Fluor (3;6;9)

Fig. 4. The regression equations for the relations between the number of Ephestia kuehniella eggs parasited by Trichogramma spp. and the adults eclosing (A.R.S. Turda, 1996)

CONCLUSIONS

1. The growing substrates with the greatest number of adults of moth flour (*Ephestia kuehniella* Zell) were maize flour (1014,0 adults/G.V.), triticale flour (732,0 adults/G.V.) and wheaten flour (677,0 adults/G.V.). The occupation level of alveols feeding from the growing block on the same substrates was between 75,0 - 100,0% and the exploitation time of growing block was 15-20 days at T= 16-21 °C and H= 70-75%.

2. Regarding the development degree of moth larvae, no significant differences were observed between maize and wheaten flour, but sensible significant positive differences on triticale flour. The prolificacy and food consumed/larva were both higher on maize flour followed by triticale and wheaten flour.

3. The parasitizing of the moth eggs by *Trichogramma* spp was somehow influenced by the substrate grounding. *Trichogramma maydis* parasited the moth eggs which were obtained on triticale and maize flour in a high percentage (96,9 and 95,7%).

4. The hatching of adults *Trichogramma* spp was influenced by the temperature, the humidity during the developmental period, and the growing substrate of the moth. The three *Trichogramma* species hatched in a higher proportion from the eggs obtained on the triticale and maize flour (between 94,8 - 96,3% and 80,1 - 94,4% respectively).

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THE EFFECTIVENESS OF INUNDATIVE RELEASE OF *TRICHOGRAMMA EVANESCENS* WESTWOOD (*HYMENOPTERA*; *TRICHOGRAMMATIDAE*) AGAINST *OSTRINIA NUBILALIS* HUBNER (*LEPIDOPTERA*; *PYRALIDAE*) IN TURKEY

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ABSTRACT

This study was carried out to determine the biological control possibilities of the main pest of maize, *Ostrinia nubilalis* Hübner with inundative releases of egg parasitoid *Trichogramma evanescens* Westwood in Adana/Turkey during the years of 1993-1994.

O.nubilalis, *Ephestia küehniella* Zeller (*Lepidoptera*; *Pyralidae*) and *T. evanescens* were reared in a climatic room under constant temperature (25±1°C), relative humidity (65±10%) and appropriate light regime for the three species.

In 1993, *T. evanescens* was applied in two releases with one week interval, at the beginning of the oviposition period of third generation of *O. nubilalis* in the second crop maize. A total of 80.000 parasitoid per 5 days were released in this experiment, egg parasitism was 80.93 % and the reduction of infested plants was 57.14 %. In 1994, in order to determine the appropriate number of releases to control *O. nubilalis*, three releases of *T. evanescens* were conducted. The egg parasitism was respectively 48.68 % in the first, 71.49 % in the second and 81.29 % in the third releases. In addition, the reduction of infested plants was 25.00 %, 54.16 % and 70.83 % respectively.

As a result of this experiment it was concluded that three releases were more effective than two releases. Because of this, for biological control of *O. nubilalis* with *T. evanescens* in Çukurova conditions, three releases of T. *evanescens* are recommended.

Key words - Maize, *Ostrinia nubilalis, Trichogramma evanescens,* inundative releases.

INTRODUCTION

In the world, most of the countries have used natural enemies against the European Corn Borer *Ostrinia nubilalis* due to the need to establish a permanent control programme and to diminish the negative effects of insecticides in the surrounding environments by means of lowering the insecticide levels. Especially egg parasitoid *Trichogramma* species was used in the biological control of *Lepidopterous* pests (Clausen, 1978; Hassan *et al.*, 1978; Neuffer, 1982; Romig *et al.*, 1985; Voegele, 1986; Heyde, 1990) in different countries. The advantages of using natural enemies in biological control, compared to chemical pesticides, include the following: (a) no toxic residues on plants and soil, (b) no complex equipments required for application, (c) no requirement of pest resistance, (d) it is effective on insecticides if used suitably, (e) no negative side-effects on the environment and on natural enemies, (f) easy to rear and release in large numbers, (g) lower in cost than chemical control.

The first attempt to control *O. nubilalis* with *T. evanescens* has been reported from France in 1975 (Voegele *et al.* 1975), and later from Switzerland (Suter and Babler, 1976; Suter and Ramser, 1978; Krehbiel and Wittwer, 1979), from Germany (Hassan *et al.*, 1978; Hassan and Heil, 1980; Neuffer, 1980; Hassan *et al.*, 1986), from Austria (Berger, 1984) and from other Western European countries (Ivanov, 1982).

In Turkey, *O. nubilalis* is the most harmful pest to the second crop maize. Before 1985 the most extensive recommended measure to control corn borer was chemical application. However, the control was quite often unsatisfactory due to under dosage, infrequent use of insecticides or wrong timing. At the time of application, the maize is full-grown and aerial application is needed, which often makes chemical control of corn borer difficult and expensive. Also, among the natural enemies of corn borer, *T. evanescens* is the most important and efficient in a sense that it widely accompanies the distribution of *O. nubilalis* in the East Mediterranean Region of Turkey (Kayapinar, 1991). For the reasons stated above, this study was carried out to determine the effectiveness of *T. evanescens* on holding the *O.nubilalis* population under economic threshold by inundative releasing of this egg parasitoid.

MATERIALS AND METHODS

This study was conducted in laboratory and field experiments.

Laboratory experiments

O. nubilalis, *E. küehniella* and *T. evanescens* were reared continously in a climatic room under constant temperature (25 ±1°C), relative humidity (65 ±10%) and appropriate light regime for the three species. Also, *T. evanescens* was reared on eggs of *O.nubilalis* in order to prevent the parasite from quality deterioration

Field experiments

In 1993, three maize fields, sown between 23-25 June, were selected in the second crop maize fields of the Faculty of Agriculture in Adana. Each experimental plot was about half hectare in size. The first field was the released parcel, the second one was the control parcel and the third one the insecticides used parcel. The distance between the treated and untreated control plots was 200m.

The adult population of the pest was observed by means of Robinson light trap which was located near the experimental plots to determine the releasing time.

When the first adult of *O. nubilalis* was captured (27.07.1993), previous egg masses were counted at 20 different points by totally controlling 5 out of 100 plants.

T.evanescens was applied in two releases with one week interval (27.07.1993 and 02.08.1993) at the beginning of the oviposition period of the third generation of *O. nubilalis* in the second crop maize. A set of 40000 parasites twice per 5 days were released (Hassan *et al.*, 1986; Stein, 1987). The distance between the two releasing points varied from 7-10m (Tran *et al.*, 1986; Cheng *et al.*, 1986). Different development stages of parasitoids from 40 points were released in 8 releasing bags per day (8000 parasitoids/day). Each releasing bag designed by Hassan and Heil (1980) included approximately 1000 *E. küehniella* eggs. In addition, they included a stripe of 22x18 cm on which about 1 000 *E. küehniella* eggs were glued, a part similar in size (that was) bent to 90 degrees to protect the eggs from predators and rain, and also a square part with an opening of 22x 3.5 cm hung to a plant leaf.

In 1994, the experiment was conducted to hold the pest population under economic threshold and to determine the appropriate number of releases in second crop maize which was sown between of 29-31 May. For this purpose, four maize fields were selected. Each experimental plot was about half hectare in size. The distance between the treated and untreated control plots was 500 m and the releasing time was determined by light traps. After capturing the first adult of the pest (08.07.1994) and a previous counting has been performed, the first release (09.07.1994) was accomplished at the beginning of the oviposition period of the second generation *O. nubilalis*. The second and third releases were made against the third generation *O. nubilalis* in intervals of 7 to 10 days (30.07.1994 and 08.08.1994). The first parcel was treated once, the second parcel was treated twice and the third one was treated three times, by releasing around 40 000, 80 000 and 120 000 parasitoids respectively. As mentioned above, the previous counting and periodic counting after releasing the egg parasitoid, were conducted weekly in all experimental plots till harvest time.

The effect of this biological control method was determined by assesing parasitization of egg masses and reducing infested plants among 100 plants per field (Bigler, 1986). Moreover, the efficiency of the parasites was evaluated at harvesting time by counting the number of damaged plants for all the experiments carried out in two years. 100 plants per plot taken at random were cut and examined and 1000 kernels weighted after harvest. Infestation rate was counted by means of Abbott formula.

RESULTS AND DISCUSSION

In the 1993, 4 days after releasing the parasitoid, the parasitism was 61.92 % and after 11 days it increased to 79.82%. This high parasitism lasted till the end of the season, when it was found to be 100%.

In the untreated control parcel, 246 egg masses (which included a total number of 4372 eggs) were counted and 2692 eggs were found to be parasitized (61.57%) (Table 1). The cause of high parasitism in untreated control parcel was similar to the treated parcel and the parasitoid had a high passive spread due to wind effect.

Table 1-The parasitization rate of *T.evanescens* on the eggs of *O.nubilalis* in released, control and insecticides used parcels in Adana in 1993.

Field plots	Number of eggs	Number of parasitized eggs	Parasitization rate (%)
Released parcel	4753	3470	80.93
Control parcel	4372	2692	61.57
Insecticides used parcel	580	62	10.68

In the parcel where insecticides were used, 15 egg masses which included 287 eggs were not parasitized in the previous counting. After 4 days, the first eggs of *O. nubilalis* were observed (31.07.1993) and the first application was carried out by a dosage of 100cc Cyfluthrin (Baythroid). On 29.08.1993, the second application was carried out against the corn earworm (*Helicoverpa armigera* Hbn.) by a dosage of 200cc Cylahotrin (Karate). At the end of the season, it was determined that 62 out of 580 (37 egg masses) were parasitized (10.68%) (Table 1).

As a result, in 1993, the percentages of parasitism among the parcels were found to be as it follows: 80.93% parasitism in the released, 61.57% in the control, and 10.68 % in the insecticides used parcel (Figure 1, Table 1).

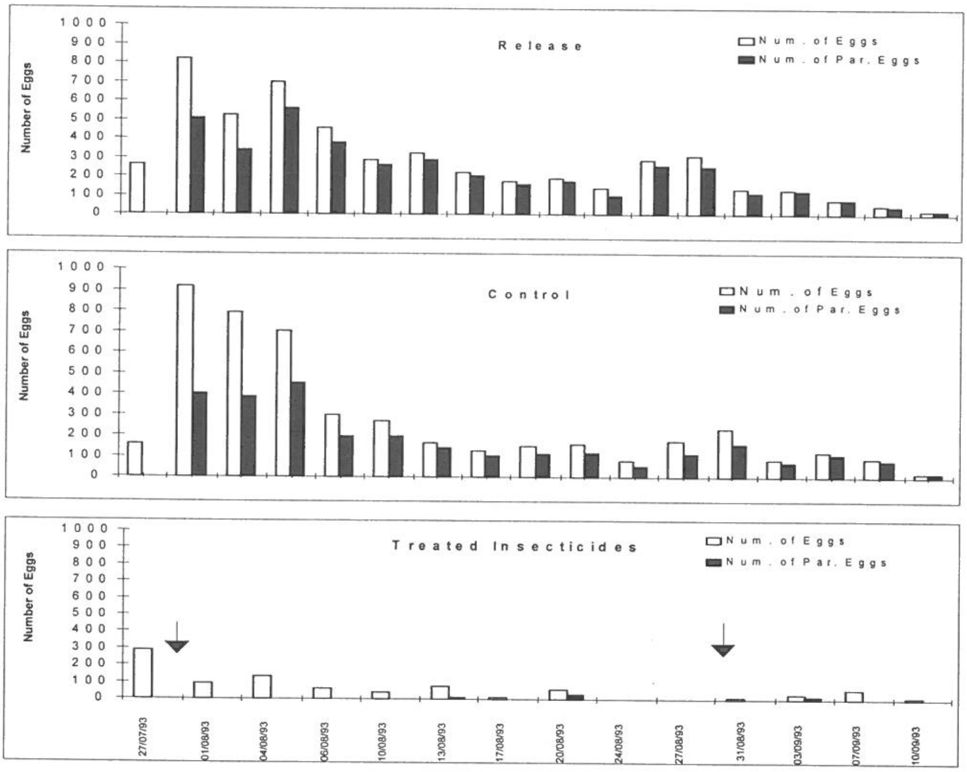

Figure 1- The parasitization rate of *T. evanescenson* on the eggs of *O.nubilalis* in the released, the control and the insecticides used parcels in Adana in 1993 (27.07.1993 = Previous counting).

The reduction of the number of infested plants in released parcel in comparison to the control was 57.14%, and 67.85% in comparison to insecticides used parcel. When the yield taken from harvested maize is compared, the average grain weight was increased by 804 kg/ha, 747 kg/ha and 817 kg/ha respectively in the released, control and insecticides used parcel. In addition, the average 1000 kernels weight was 364gr in the released, 327gr in the control and 352gr in the insecticides used parcel respectively.

In 1994, it was found that the effect of parasitoid on the parcel was 48.68% in the first release, 71.49% in the second and 81.29% in the third respectively, and in the control parcel this rate was 16.87% (Table 2, Fig. 2).

Table 2- The parasitization rate of *T.evanescens* on the eggs of *O.nubilalis* in the parcel that was released one, two, three times and the control parcel in Adana in 1994.

Number of releases	Number of eggs	Number of parasitized Eggs	Parasitization rate (%)
One release	267	130	48.68
Two releases	421	301	71.49
Three releases	310	252	81.29
Control	391	66	16.87

The reduction in the number of infested plants was 25.00%, 54.16% and 70.83% respectively (Table 3).

Table 3 - Data at harvest in 1993 and 1994.

DATA AT HARVEST	1993			1994			
	Release	Control	Insecticides	One release	Two releases	Three releases	Control
Production kg/ha	804	746	817	656	751	880	604
1000 kernels weight (gr)	364.0	327.0	352.0	298.2	313.9	319.9	271.5
Num. of infested plants (%)	12	28	9	18	11	7	24
Num. of larvae (%)	10	24	9	17	10	5	23
The reduction of the number of infested plants (%)	57.14	-	67.85	25.00	54.16	70.83	-
The reduction of the number of larvae (%)	58.33	-	62.50	26.08	56.52	78.26	-

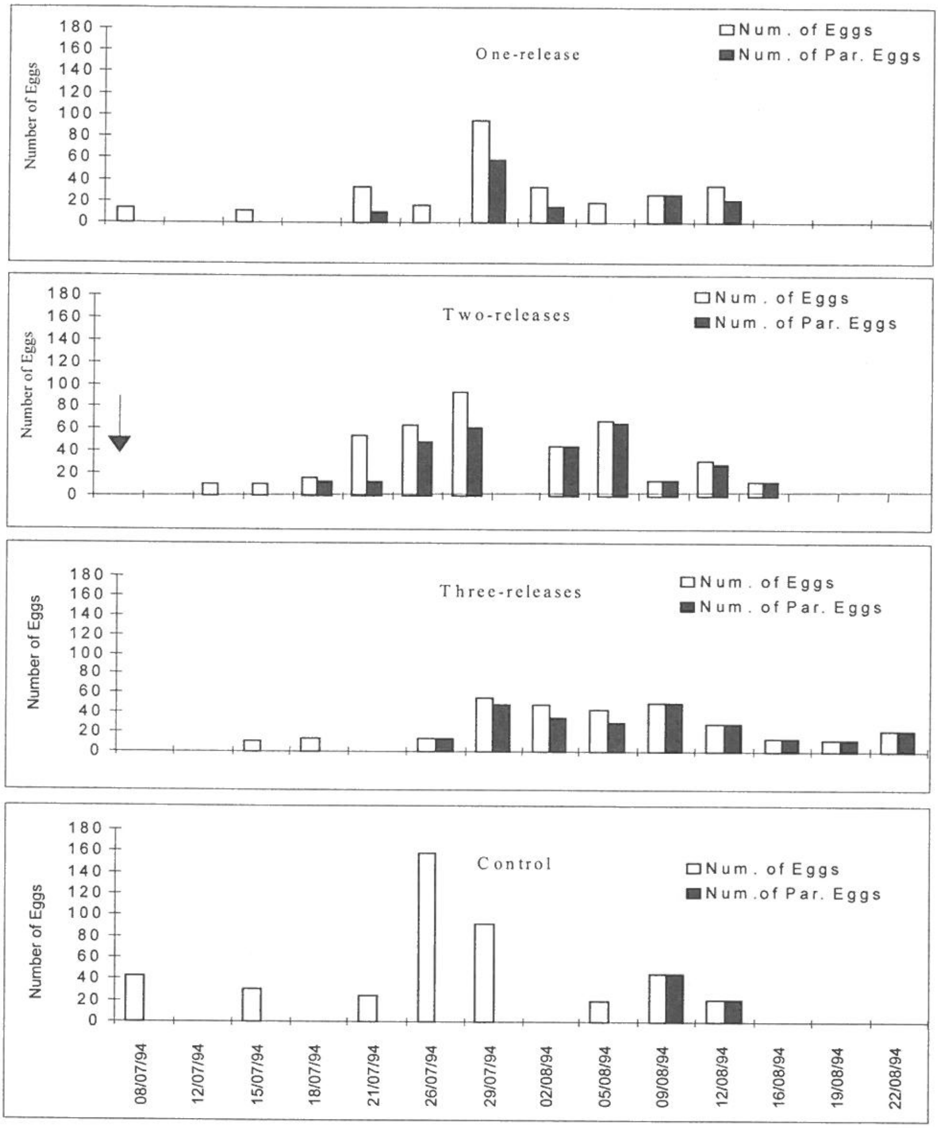

Figure 2 - The parasitization rate of *T. evanescens on* eggs of *O.nubilalis* in the parcel that was released one, two, three times and the control parcel in Adana in 1994 (08.07.1994 = Previous counting).

When comparing the yield taken from harvested maize, the average grain weight was increased by 656 kg/ha, 751 kg/ha and 880 kg/ha on the parcel where releasing was made once, twice and three times respectively and 604 kg/ha in control. Also, the average1000 kernels weight was 298.2 gr, 313.9gr, 319.9gr on the parcel where releasing was made one, two and three times respectively and 271.5 gr in the control.

Having in account the values of "infestation rate", "yield" and "1000 kernels weight", it was found that three releases would be sufficient to hold the population of O.nubilalis. It should be noticed that the highest yield was taken from the parcel with three releases.

Furthermore, in 1994, the parasitization rate increased in a direct proportion with the number of releases, having in account that it would depend on the period of adult flight activity, weather conditions, plant phenology, releasing time and the age of the parasitoids. Similar results were found by Neuffer (1982) and Tran et al (1986). Hassan (1981) reported that the reduction in the number of larvae was 75% by releasing of parasitoids at three times. Sweden (Krehbiel and Wittwer, 1979), Bulgaria (Karadjov, 1982), France (Raynaud and Crouzed, 1985), Austria (Rawensberg and Berger, 1986), Switzerland and France (Heyde, 1991) reported similar results.

ZUSAMMENFASSUNG

Wirksamkeit wiederholter Freilassungen von *Trichogramma evanescens* Westwood (*Hymenoptera; Trichgrammatidae*) gegenüber *Ostrinia nubilalis* Hübner (*Lepidoptera*; *Pyralidae*) in der Türkei

Ziel der vorliegenden Untersuchung war es, die Möglichkeiten zur biologischen Bekämpfung des Hauptschädlings im Mais, *Ostrinia nubilalis* Hübner durch wiederholte Freilassung des Parasitoiden *Trichogramm evanescence* Westwood in Adana/Türkei in den Jahren 1993 ß 1994 zu untersuchen.

O.nubilalis, *Ephesitia küehniella* Zeller (*Lepidoptera*; *Pyrilidae*) und *T. evanescens* wurden bei einer Dauertemperatur von 25 °C, einer relativen Luftfeuctigkeit von 65 ± 10 % und je nach Art angepassten Beleuchtungsdauer in Klimakammern gezüchtet.

Im Jahre 1993 wurde *T. evanescens* zweimal in wöchentlichen Abständen zu Beginn der Eiablage der dritten Generation von *O. nubilalis* während der zweiten Vegetationsperiode des Mais freigelassen. Insgesamt wurden 80.000 Parasitoide je 0,5 ha freigelassen. In diesem Versuch betrug die Parasitierungsrate der Eier 80,93 % und der Anteil der infizierten Pflanzen konnte um 57,14 % reduziert werden. Um die angemessene

Anzahl von Parasitoidfreilassungen zur Bekämpfung von *O. nubilalis* zu bestimmen, wurden im Jahre 1994 drei Freilassungen von *T. evanescens* durchgeführt. Der Anteil parasitierter Eier betrug 48,68 %; 71,49 %; und 81,29 % bei ein-, zwei- bzw. dreimaliger Freilassung des Parasitoiden. Zudem wurde der Anteil infizierter Pflanzen in Abhängigkeit von der Anzahl der Freilassungen um 25,00 %; 45, 16 % bzw. 70,83 % reduziert.

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PRESENCE OF *LYDELLA THOMPSONI* HERT. ON ECB (*OSTRINIA NUBILALIS* Hbn.) LARVAE IN IWGO TRIALS AT HARVEST

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ABSTRACT

Lydella thompsoni Hert (*Diptera, Tachinidae*) (LT) is a well known endoparasite of European Corn Borer (ECB) larvae in our country and it has a greater or smaller impact on this pest larval population reduction. The first international studies on this species in Yugoslavia began in the 1920's, when the International Laboratory for ECB Studies was founded in Zagreb.

The present study encompassed the observance of the number of ECB larvae in maize inbred line stalks, as well as the presence of LT pupae in the IWGO trials at and after harvest. The trials were set up and carried out in the experimental field of the Maize Research Institute, Zemun Polje, for 20 years (1977-1996). The "A" variant plants were used for artificial infestation with egg masses (2+2 each) in the egg black head stage.

Plant dissection was performed during September in the fields and then finished off in the laboratory. It was carried out during the Winter months - from October to February. The number of alive and especially dead ECB larvae, then the number of LT pupae (cocoons and exuviae) were registered. The dissection period affected, to a certain extent, the alive to dead larvae ratio. A higher number of alive *vs* dead individuals was found by the early *vs* late dissection, respectively. Parasitism rates were evaluated on the basis of the total number of ECB larvae and the sum of the number of LT cocoons and pupae. As neither alive nor dead overwintering larvae were dissected, the obtained values on the total parasitism rates were probably a bit lower than the actual ones.

The obtained results on the number of larvae per plant over studied years indicate that the average number of individuals feeding on and damaging maize inbred line plants ranged from 3.0 and 3.1 in 1982 and 1983, respectively, to 13.7 in 1987. The average number of larvae per plant amounted to 7.0 for the 20-year period.

The share of alive larvae ranged from 68.0% in 1981 to 98.0% in 1987, i.e. it amounted to 89.6% on the average. The larvae died from entomopathogen micro-organisms presumably were not infested by *L. thompsoni*.

The total reduction of ECB larvae population abundance during July, August and September ranged from 0.01% in 1987 (the highest average number of ECB plant^{-1} amounted to 13.7) to 13.14% in 1978 (5.5 larvae plant^{-1}). The average Summer parasitism rates amounted to 3.35%.

According to the 20-year investigation, the parasitism rates based on conditionally agreed categories, amounted to:

- 0.01 - 5.00% (low rates) over 16 years,

- 5.01 - 10.00% (average rates) only in 1979 (9.24%) and
- over 10.00% (high rates) over three yeras (12.20% in 1981 and 1982 and 13.14% in 1978).

The correlation analysis of results on the average number of larvae per plant at harvest and the parasitism rates (r=-0.529*) show that the Summer parasitism rates significantly affected the reduction of ECB larval abundance.

Key words: *Lydella thompsoni, Ostrinia nubilalis*, maize inbreds, parasitism.

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INTRODUCTION

European Corn Borer (ECB), *Ostrinia nubilalis* Hbn., has a great number of natural enemies which mostly parasitize as larvae, eggs and pupae. Over 100 species were registered only among parasites including *Lydella thompsoni* Hrt. (LT) and predators (Čamprag, 1994). According to the same author, the greatest significance in the ECB population reduction is observed in egg parasites of the genus *Trichogramma,* which infests over 90% of eggs.

Lydella thompsoni Hrt. (*Diptera; Tachinidae*) is the most important endoparasite in the reduction of ECB larvae in Yugoslavia and it is followed by *Campoplex alcae, Eulophus viridulus* and *Horogenes punctorius*. Its female hatches the larvae in the tunnels made by ECB larvae. LT larvae penetrate into ECB larve and at the end of the development live the dead host, cocooning in its vicinity. According to Jarwist and York (1997) the fourth larval stage is the most favourable for parasitizing.

The earliest international studies on ECB and its entomophage, referring also to *L. thompsoni*, were performed in Yugoslavia during the 1920's when the International Laboratory for ECB Studies was established in Zagreb (Manojlović *et al.* 1994).

The international studies within the IWGO Programme were initiated in 1969 in ten European and American countries. Later on India, the

Philippines, Egypt, China, Portugal, Greece, Israel and Turkey joined in. The data presented in this study were collected during the 20-year period (1977-1996). The data gathering began in 1973, just a few years upon the determination of an ECB bivoltine type, which also could affect the *L. thompsoni* polulation (Bača and Hadžistević, 1989). The aim of the present study was to determine and quantify the significance of the LT Summer population in the number reduction of EC larvae.

MATERIAL AND METHODS

The number of investigated maize inbred lines within the IWGO trials ranged from 12 genotypes in 1995 and 1996 to 76 in 1984. This number varied annually from 176 to 9213 plants. The total of 13,111 plants were dissected, then 93,997 ECB larvae, 2403 LT pupae + cocoons and 2460 exuviae of ECB pupae were collected. The IWGO trials were set up in the experimental field of the Maize Research Institute, Zemun Polje, in Zemun Polje, near Belgrade. The light trap and meteorological station were located in the vicinity of the experimental plot. The "A" type plants were used for dissection. Artificial infestation with 2+2 egg masses, performed on these plants, was synchronised with the second half and the end of the flight of moths of the first generation, i.e. in the third decade of June and the first decade of July.

Stalk dissection of the earliest maturing maize inbred lines was performed during September, mostly in the field. Stalks of later maturing maize inbreds were dissected from October to February, before the diapause of the LT overwintering generation was broken. The number of alive and dead ECB larvae were separately registered during the stalk dissection. The dead larvae were not dissected. The dissection period had a certain effect on the ratio of alive to dead larvae. A higher number of alive larvae was found during the earlier dissection.

RESULTS AND DISCUSSION

The results obtained on the average number of fully grown (FG) ECB larave (L) per plant, percent of their parasitization from the Summer LT population, percent of exuviae (Ex) of bivoltine type of ECB population and percent of the total reduction in the abundance of the grown larvae over years and categories are shown in the Tables 1, 2 and 3.

Table 1 - The number of ECB larvae per plant, parasitization by *Lydella thompsoni* Hrt. and percent of bivoltine pest population during the 1977-1996 period in Zemun Polje

Year of testing	Larvae /Plant	LT/ECB Larvae	Eg ECB / Larvae	% of L Reduct.	Year of testing	Larvae/ Plant	LT/ECB Larvae	Eg ECB /Larvae	% of L Reduct.
1977	9,10	3,62	0,67	4,29	1987	13,87	0,01	0,20	0,21
1978	5,49	13,14	1,13	14,27	1988	8,69	0,93	0,59	1,52
1979	7,13	9,24	23,96	33,21	1989	6,96	2,26	0,02	2,29
1980	10,73	0,55	0,25	0,80	1990	9,97	1,96	0,07	2,02
1981	3,30	12,20	1,81	14,01	1991	8,20	0,74	0,06	0,80
1982	2,98	12,20	0,00	12,20	1992	4,32	0,69	1,03	1,72
1983	3,14	1,84	0,26	2,10	1993	3,34	1,60	0,14	1,75
1984	8,72	1,03	0,01	1,04	1994	5,61	1,22	18,52	19,74
1985	11,29	0,23	1,22	1,45	1995	4,05	2,10	0,28	2,38
1986	7,61	0,80	0,61	1,41	1996	5,85	0,68	52,82	53,50
20-year average						7,17	3,35	5,18	8,54

The average number of FG larvae from 2 + 2 egg masses (60-80 eggs) feeding on maize inbred line plants and harming them, was 7.17 per plant. This number ranged from only 2.98, and 3.1 per plant in 1982 and 1983, respectively to 13.87 per plant in 1987. Based on achieved results it is noticeable that 10% of ECB larvae survived under agroecological conditions of Zemun Polje during the 20-year period in relation to the number of egg masses found on artificially infested plants.

The role of *Lydella thompsoni*, as one of the principal entomopathogen agents in reduction of the ECB larvae number, ranks first without doubt. It varied signficantly, from barely recordable, below 1% during the eight investiagion years, to over 10% in the three investigation years.

There are no literature data on ECB larvae parasitization on maize inbred lines artificially infested with egg masses. Investigations were carried out in the natural population of ECB larvae on maize or some alternative hosts. There are several Yugoslav researchers working on studies of *Lydella thompsoni* and other parasites of ECB: Hergula (1929, 1930/31); Vukasović (1953), Jovanić (1961); Bjegović and Lazarević (1963), Bjegović (1970); Manojlović (1984, 1985 and 1989); Bača *et al.* (1990 and 1994); Kaitović and Bača (1995). Each of these authors indicated a notable role of *Lydella thompsoni* in the reduction of abundance of the natural population of ECB larvae.

Lydella thompsoni, a solitary parasite of ECB larvae, overwinter as a second degree diapausing larva. Increased temperatures easily break the diapause which is not synchronised with the breakage of the ECB larvae diapause.

According to Tsitsipis *et al.* (1993) and Galichet (1985) in certain parts of Greece and France there are other four species of the order *Lepidoptera: Archanara geminipuncta, A. spargani,, A. dissoluta* and *Nonogria typhae* which are complementary hosts of Spring generations of *Lydella thompsoni*, beside the principal ones such as ECB and *Sesamia nonagriodies*. Complementary hosts of LT have not yet been studied in Yugoslavia, but it is sure that they have to exist and have a significant effect on the abundance of Spring, and therefore on Summer population of *Lydella thompsoni*.

The level of the LT Summer population of about 3.35% of ECB FG larvae parasitization amounted to 14.311 per hectare and was calculated as follows: 60.000 plants ha^{-1}, the average number of larvae was 7.17 $plant^{-1}$ → 60.000 x 7.17 = 430,200 ECB larvae ha^{-1} x 3.35/100 = 14,412 LT ha^{-1}. This number indicates a high level of the parasitic population in relation to the abundance of the natural ECB population.

Table 2 presents the average number of FG larvae per plant during the dissection period classified according to the number of infested plants with egg masses, their average number per plant, percent of alive larvae at harvest and the average percent of parasitization by LT.

Table 2 - Categories of ECB survival per egg mass and parasitism rates

Category of Parasitization	Frequency (No of Years)	No of FG ECB Larvae/Plant	% of Alive Larvae	% of Parasitism Rate
< 1 L/Egg mass	5	3.34	84.2	5.99
1–2 L/Egg mass	7	6.13	85.3	4.00
2-3 L/Egg mass	7	9.53	91.6	1.29
> 3 L/Egg mass	1	13.87	98.0	0.01

The obtained values on the total parasitism rate of ECB larvae by *L. thompsoni* are lower than the actual one, as neither alive nor dead FG larvae were dissected. They are lower by the percentage of parasitism rates of overwintering LT populations and for a part of total parasitization of dead

FG larvae caused by entomopathogen micro-organisms. According to Manojlović (1984), the total parasitism rate of ECB larvae in the region of Bačka Planka, 130 km Northwest away from Belgrade, varied from 18.74% (1976) to 30.225 % (1975). Parasitization by LT varied from 13.10% (70% of the total parasitism rate) in 1976 to 24.70% (82% of the total parasitism rate) in 1975. According to the same author (Manojlović, 1985), the role of Summer populations of this *tachinidae* in reduction of ECB abundance is approximately equal to the role of the overwintering generation.

Table 2 shows that the increase of the number of ECB larvae per egg mass was followed by the decrease of both the parasitism rate by LT and the mortality rate by entomopathogen micro-organisms. There were 5.59% of LT and 84.2% of alive larvae in the case of less than one FG larva per egg mass, while the parasitism rate by LT amounted to 0.01% and percentage of alive larvae amounted to 98.0% in the case of more than three FG larvae per egg mass. According to the presented results, the favourable conditions for LT are at the same time favourable for entomopathogen micro-organisms.

The percent of parasitization percentage of ECB by LT are grouped into four categories presented in Table 3.

Table 3 - Categories of ECB parasitization by the Summer population of *Lydella thompsoni*

Category of Parasitization	Frequency (No of Years)	No of FG ECB Larvae/Plant	% of Alive Larvae	% of Parasitism Rate
0.01 – 1.0 %	8	8.8	89.9	0.56
1.01 – 5.0 %	8	6.4	92.1	1.95
5.01 – 10.0 %	1	7.1	77.6	9. 24
Over 10.0%	3	3.9	74.7	12.60
Minimum		**3.0**	**68.0**	**0.01**
Maximum		**13.7**	**98.0**	**13.14**
Average		**7.2**	**89.6**	**3.35**

A very low parasitization (0.01-1.0%) by a high population of ECB larvae obtained by artificial infestation with four egg masses per plant and low parasitization (1.01 – 5.0 %) by LT were registered during the 16-year investigation. Medium high and high parasitization were registered in only four investigation years. Parasitization over 10% was registered in only three years and the average number of ECB larvae decreased to 3.9 per plant, while the alive/dead larvae relation amounted to 74.7% / 25.3%. The increase of parasitization by the Summer population of LT resulted in the increase of the percentage of dead larvae (at harvest) by entomopathogen micro-organisms.

CONCLUSION

Based on the obtained results the following can be concluded:

- Approximately 10% of ECB larvae out of the total number of eggs found on artificially infested plants reached the full grown stage.
- ECB larvae mortality under conditions of Zemun Polje, caused by the effects of the Summer LT population, is a very significant factor in this pest population reduction. The correlation analysis of the average FG ECB larvae per plant and the parasitism rate by the Summer population of *Lydella thomsponi* ($r = -0.529^{**}$) points to its highly significant role on reduction of the population level of ECB larvae.
- If the obtained results on the parasitism rate of ECB larvae by the Summer LT population are multiplied by 2, according to the results of Manojlović (1985), which point to the approximately similar significance of both Summer and Winter population, then the total parasitism rate of ECB larvae by *Lydella thomsponi* amounted to 26% in 1976. The obtained 20-year results are in accordance with the results of the cited authors.

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EFFECT OF SOME CHEMICAL AND MICROBIAL INSECTICIDES, AND PLANTING DATES OF MAIZE ON LARGE SUGAR-CANE WORM INFESTATION AND THE ASSOCIATED PREDATORS

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ABSTRACT

The effectiveness of 3 Bt-formulations, 3 IGR and 2 insecticides and their mixtures against *Sesamia cretica* (L.) in maize field was determined. In addition, the rate of the pest infestation was evaluated in relation to the planting date. Data revealed that the Bt. formulation SAN 415 I was the most toxic compound against the pest, since it exhibited infestation reduction of 55.43%. On the other hand the least effective compound was Sumicidin with infestation reduction of 16.90%. With the Bt. formulations viz. SAN 415 I, Thuricide HP, and Dipel treatments, a high yield of maize production and a low infestation of *Sesamia cretica* were achieved. In addition, they were less harmful to the associated predators. Data also indicated that the early dates of maize planting (sown on 20th April and 5th May) resulted in high infestation of *S. .cretica*, whereas the maize planting sowed on 20th and 5th June were lightly infested.

In conclusion SAN 415 I, Thuricide HP and Dipel should be recommended to control *S. cretica*. June is the favorable time for planting maize in Upper Egypt.

Key words: Maize pests, *Sesamia cretica*, planting dates.

INTRODUCTION

Maize (*Zea mays* L.) is the main food for some 100 million people. The crop is especially important to many developing countries in Africa, Asia and Latin America where it serves as a human subsistence crop. In Egypt, it is the most dominant summer cereal crop and the area under cultivation already reached 1.95 million feddans. Many varieties of insects attack roots, stem, ears and foliage of the maize plants. The large sugar-cane worm, *Sesamia cretica* Led. is one of the major insect pests of maize in Africa (Chiang, 1978) and in Assiut (El-Naggar, 1967). The number of egg-masses of *S. cretica* laid on maize plants and the intensity of their infestation were varied greatly according to the planting date (Willcocks, 1925; Ahmed and Kirah, 1960; El-Sherif, 1965; El-Sadany, 1965 and El-Naggar, 1967).

In the last few years, some authors recorded an increase in tolerance of many insects in field against organophosphate, carbamate and pyrethroid insecticides. Under such conditions, other compounds of other groups that have biological importance, have been introducted to play a basic role in pest management. An important class of insecticides, the benzoylphenyl urea was found to inhibit chitin synthesis and interfere with cuticle formation, leading to abnormal endocuticular deposition and death.

Integrated pest control, by minimizing the use of chemical pesticides and using biological agents, promises to reduce pest losses to maize on a continuing basis and at the lowest cost. Some of these agents such as *B. thuringiensis* have shown much potential and offer an excellent alternative to chemical pesticides for IPC program. *B. thuringiensi,,* as a bacterial pathogen infecting a wide range of insect pests, is the most common microbial insecticide in use today (Walker, 1979).

The present study was conducted to evaluate the following:

1- Effect of certain chemical and microbial insecticides against large sugar-cane worm on maize, the associated predators and the yield.
2- Effect of planting dates of maize on intensity of infestation by *S. cretica* and the yield.

MATERIALS AND METHODS

1- <u>Effect of chemical and microbial insecticides:</u>

This work was carried out at the farm of Experimental Station of the Faculty of Agriculture, University of Assiut. An area of about 3/4 feddan was divided into 75 plots of 42 m^2 each in 1995 season. The plots were planted with maize cultivar "diploid hybrid 215" on April 20, 1995.

The maize plants were sprayed with the tested compounds 3 weeks after planting (Table 1). The tested compounds were applied using a knapsack sprayer. Split plot design was adopted in the experiment and each treatment was replicated four times.

Table 1- Chemical and microbial insecticides applied against large sugar-cane worm in maize and the rates of application.

Trade name	Chemical or scientific name	Rate of application/ feddan
1-Dipel (16000 Iu/mg)	*Bacillus thuringiensis* var. *kurstaki*	500 Kg
2-Thuricide HP (16000 Iu/mg)	*Bacillus thuringiensis* var. *kurstaki*	500 Kg
3-SAN 415 I (27000 IU/mg)	*Bacillus thuringiensis* var. *kurstaki*	500 Kg
4- XRD 473 EC 5%	1-(3,5-dichloro-4-(1,1,2,2-tetrafluoroethoxy phenyl) -3-(2,6-difluorobenzoyl) urea	1.0 L
5- Diflubenzuron WP 25% (Dimiline-TH 6040)	1-(4-chlorophenyl)-3-(2,6-difluorobenzoyl) urea	1.0 Kg
6- Chlorfluazuron EC 5% (IKI 7899)	1-(3,5 dichloro-4-(3-chloro-5-trifluoromethyl-2-pyridyloxy phenyl)-3-(2,6-difluorobenzoyl) urea	1.0 L
7- Th. + XRD		500 g + 0.2 L
8- Th. + Dim.		500 g + 0.2 kg
9- Th. + IKI		500 g + 0.2 L
10-Monocrotophos (Nuvacron) EC 40%	Dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate	1.5 L
11- Fenvalerate (Sumicidin) EC 20%	(RS)- -cyano-3-phenoxybenzyl	0.6 L
12- Th. + Nuv		500 g + 0.3 L
13- Th. + Sum.		500 g + 0.12 L

The numbers of infested maize plants with the large sugar-cane worm and the numbers of the associated predators on 10 plants in each plot were recorded. The counts were made after 2, 7 and 14 days from spraying. Analysis of variance was used and the averages were compared using LSD (0.05). The percentage of pest infestation and the number of associated predator were used as a criterion to evaluate the effectiveness of the tested compounds.

2- Effect of planting dates:

The experiments were carried out at Assiut University Experimental Farm in 1994 and 1995 seasons. Six maize plantations were sown on 20th April, 5th May, 20th May, 5th June, 20th June and 10th July, each replicated ten times.

The maize cultivar "diploid hybrid 215", being the common variety of maize grown in Assiut locality, was used for the experiment. The area of the experiment (3/4 feddan) was divided into 75 plots. Each plot, measuring 1/100 feddan (6x7 metres) contained 9 rows. Sampling was accomplished by examining 10 randomized plants from each plot. The percentages of infestation were determined by finding out the number of infested plants, either containing eggs, larvae, pupae or just showing symptoms of attack, and calculating their ratio to the total number of plants in the sample.

RESULTS AND DISCUSSION

1 - Effect of certain chemical and microbial insecticides on large sugar-cane worm infestation, the associated predators and yield of maize.

Data are summarized in Tables 2 and 3 and illustrated in Figs 1 and 2.

The insect pest of major importance and dominance after 3 weeks from the planting of maize was *Sesamia cretica* Led. The associated predators were *Coccinella undecimpunctata* L., *Orius* spp. (*Orius laevigatus* and *Orius albidipennis* Reut.), *Scymnus syriacus* Mars. and true spiders.

Data revealed that the Bt.-formulation SAN 415 I was the most toxic compound against the large sugar-cane worm, since it exhibited a reduction of 55.43%. This is in agreement with results obtained by Navon (1989), who found that *B. thuringiensis* HD-1-S. 80 was an active compound against *Sesamia nonagrioides* larvae. The least effective compounds were Sumicidin and IKI 7899 exhibiting a reduction of 16.90 and 21.12%, respectively.

The growth regulators compounds XRD 473 and Dimiline were effective against *S. cretica* and with these treatments the level of the pest infestation has been reduced. Similar results were obtained by Larue (1984).

Data also indicated that plants in control plots harbored significantly more pests than the treated plants. This inevitably led to a significant reduction in the yield of control plots. Data also showed that the highest yield amounts were found in the plots treated with Bt.-formulations, SAN 415 I and Thuricide HP and the maize yield amounts per plot (1/100 feddan) were 33.25 and 29.50 kg with increasing rates of 73.10 and 52.37%, respectively. The lowest ones were recorded in untreated plots (19.36 kg/plot). The maize yield increased moderately by using the tested chemical insecticides Dimiline TH 6040, IKI 7899, XRD 473, Nuvacron and Sumicidin in controlling the insect pest.

Table 2 -Effect of chemical and microbial insecticide on large sugar-cane worm and the associated predators on maize.

Treatments	Pest infestation (%) and number of predators 2, 7 and 14 days after treatment/100 plants														
	S. cretica. spp			*Coccinella* spp			*Scymnus* spp.			*Orius* spp			Spider		
	2	7	14	2	7	14	2	7	14	2	7	14	2	7	14
Dipel	10.75	11.50	10.50	31.00	32.25	49.25	7.25	16.75	13.00	6.00	10.75	17.25	12.25	19.25	27.00
Thuricide HP	9.75	10.25	8.00	30.75	32.25	44.75	7.25	13.50	14.25	5.25	10.25	17.75	12.25	18.25	29.25
SAN 415 I	7.25	8.50	8.00	34.25	36.50	50.00	8.75	11.25	11.75	6.25	11.25	16.00	11.75	17.00	25.75
XRD 473	10.50	9.25	11.00	29.50	26.50	31.25	7.50	3.25	8.25	6.75	3.75	7.50	11.25	18.25	26.75
Dimiline	13.25	10.50	14.25	35.25	30.00	38.00	8.75	4.75	10.25	5.75	4.25	2.75	11.75	20.00	25.00
IKI 7899	12.50	14.25	15.25	32.50	28.25	31.50	6.25	5.25	11.75	7.00	4.75	13.25	10.25	17.00	26.75
Th. + XRD	8.00	9.75	11.50	23.25	25.50	39.25	3.25	4.00	10.25	6.00	9.75	12.25	5.25	16.25	26.75
Th. + Dim.	10.25	9.25	11.25	20.25	22.50	42.25	4.00	4.75	12.75	6.00	7.25	15.75	8.00	18.75	27.25
Th. + IKI	9.50	10.25	11.75	20.50	21.25	43.50	3.00	3.50	13.25	5.75	8.25	12.25	7.00	19.75	26.00
Nuvacron	11.25	14.25	15.00	9.75	18.25	28.75	1.75	3.75	7.75	2.00	3.50	8.00	4.75	6.25	17.75
Sumicidin	13.25	15.00	16.00	12.25	20.75	31.25	2.25	5.25	9.25	4.25	6.25	11.75	4.75	7.75	18.00
Th. + Nuv.	7.00	9.25	10.25	19.75	30.00	41.75	6.75	8.25	11.50	5.75	10.75	13.25	8.00	15.25	23.00
Th. + Sum.	8. 50	11.50	9.50	23.50	31.00	47.25	6.00	8.75	10.75	6.25	11.25	16.25	8.50	17.75	25.25
Control	12.75	18.75	21.75	38.50	49.75	53.25	8.25	15.25	14.25	6.75	11.00	19.75	12.25	19.75	27.25

SAN 415 I was the safest tested compound against the beneficial natural enemies *Coccinella* (14.62% reduction), while Nuvacron and Sumicidin were the most harmful compounds, since they exhibited reductions of 59.9 and 54.6%, respectively. Dipel, Thuricide HP and SAN 415 I were the safest compounds against *Scymnus* sp. and *Orius* spp. and with an individual reduction of 1.98, 7.31, 15.89 and 9.36, 11.36, 10.72% of predators, respectively. Nuvacron and Sumicidin were the most harmful tested compounds against the two predators and they exhibited reduction of 64.94, 55.64 and 64, 40.72%, respectively.

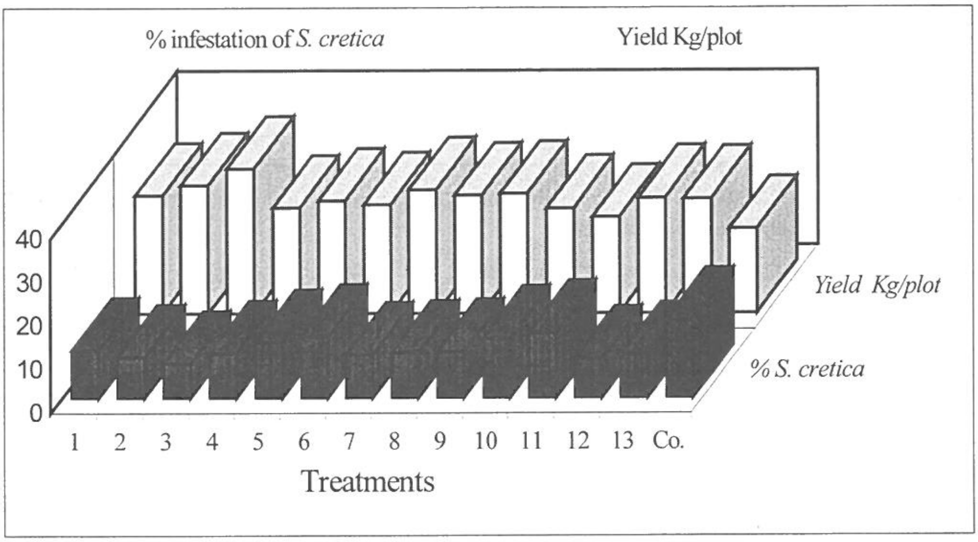

Figure 1- The infestation percentage of *S. cretica* and maize yield as affected by some chemical and microbial insecticides.

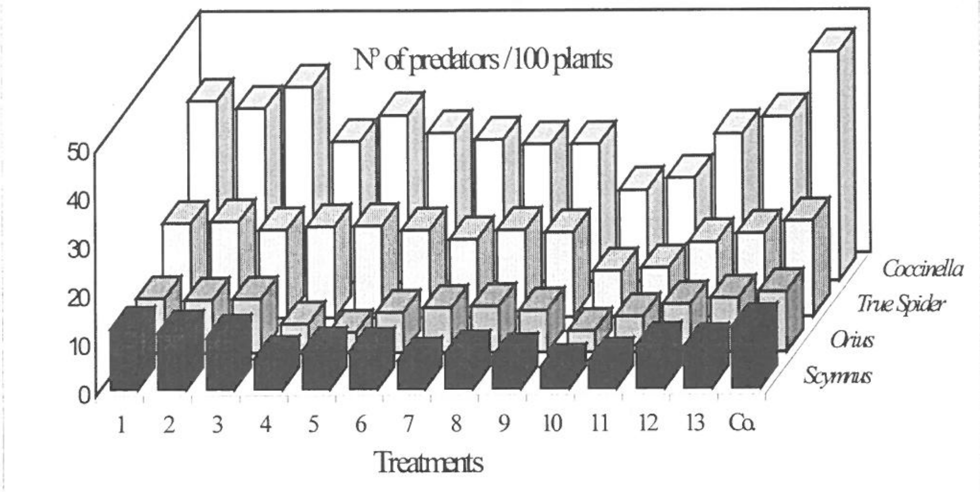

Figure 2 – The mean numbers of predators associated with *S. cretica* as affected by some chemical and biological insecticides on maize.

Table 3. - Effect of chemical and microbial insecticides on mean of % infestation of large sugar-cane worm, the associated predators and yield of maize.

Treatments	Mean of % pest infestation and number of the predators of three observations (2,7 and 14 days after spraying) /100 plants					Yield kg/plot
	S. cretica spp.	*Coccinella* spp	*Scymnus* spp	*Orius* spp	True spider	
Dipel	10.91 bcdef	37.50 bc	12.33 a	11.33 ab	19.50 ab	27.16 ab
Thuricide HP	9.33 ef	35.91 bcd	11.66 a	11.08 ab	19.91 a	29.50 ab
SAN 415 I	7.91 f	40.25 ab	10.58 ab	11.16 ab	18.16 ab	33.25 a
XRD 473	10.16 cdef	29.08 de	6.33 cde	6.00 de	18.75 ab	24.33 bc
Dimiline	12.66 bcde	34.41 bcde	7.91 cd	4.25 e	18.91 ab	25.83 abc
IKI 7899	14.00 abc	30.75 cde	7.75 cd	8.33 cd	18.00 ab	25.00 bc
Th. + XRD	9.75 def	29.33 de	5.83 de	9.33 bc	16.08 ab	28.41 ab
Th. + Dim.	10.25 cdef	28.33 e	7.16 cd	9.66 bc	18.00 ab	27.16 ab
Th. + IKI	10.50 cdef	28.41 e	6.58 cde	8.75 bc	17.58 ab	27.50 ab
Nuvacron	13.50 bcd	18.91 f	4.41 e	4.50 e	9.58 c	24.16 bc
Sumicidin	14.75 ab	21.41 f	5.58 de	7.41 cd	10.16 c	22.16 bc
Th. + Nuv.	8.83 ef	30.50 de	8.83 bc	9.91 abc	15.41 b	26.50 abc
Th. + Sum.	9.83 def	33.91 bcde	8.50 bc	11.25 ab	17.16 ab	26.33 abc
Control	**17.75 a**	**47.16 a**	**12.58 a**	**12.50 a**	**19.75 a**	**19.36 c**

Means followed by the same letter (s) in a vertical row are not significantly different at 5%.

The combination of Bt-formulation Thuricide HP with XRD 473, Dimiline TH 6040, IKI 7899, Nuvacron or Sumicidin at the field recommended rate of Thuricide HP with 20% of the field recommended rates of the other chemical compounds, resulted unsignificant difference in yield and effectiveness against *S. cretica*, while it lead to increase the harmful effect of the Bt-formulation against *Scymnus*, *Coccinella* (with Dimilin TH 6040 and IKI 7899), and the true spider (with Nuvacron).

The combination effects may involve many and different mechanisms such as simple incompatability of the microorganism and the pesticide, prevention of food uptake by the insect, hormoligosis in the insect or the microorganism, physiological stress on the insect by the insecticide, and detoxification of the insecticide by the microorganism (Benz, 1971).

The statistical analysis of yield data, the infestation of the insect pest and the associated predators on maize plants revealed that Bt-formulations exhibited a higher yield and reduced *S. cretica* infestation with least harmful effect on its natural enemies and this leads to quite an effect against the pest when combined with resident natural enemies (Falcon, 1971).

2 - Effect of planting dates of on the intensity of infestation by *S. cretica* and the yield.

Data was summarized in Table 4 and illustrated in Figure 3. It was found that the effect of planting dates on the borer infestation percentages and the yield of maize was as it follows:

The early maize planting (sown on 20th April and 5th May) received the highest infestation of *S. cretica* (% infestations were 17.71 % and 13.56% in the 1994 season and 19.47 % and 14.27 % in the 1995 season, respectively), whereas the plantings sown on 10th July and 20th May were moderately infested (% infestation were 3.77 % and 4.97 % in the 1994 season and 4.03 % and 6 % in the 1995 season, respectively).

The maize plantings sown on 20th and 5th June were lightly infested (% infestation were 1.78 % and 2.24 % in the 1994 season and 2.15 % and 3.4 % in the 1995 season, respectively).

Similar results were obtained by Ahmed and Kirah (1960), who found that at Bahtim, the number of egg-masses of *S. cretica* laid on maize plants varied greatly according to the planting date and they concluded that April plantings received the greatest number of *S. cretica* egg masses.

El-Sherif (1965) pointed out that the "early Summer" maize plantings sown during March and April were usually subjected to higher rates of infestation by *Sesamia cretica*, whereas the "late Summer" maize plantings (sown in May and early June) tend to be less infested by *S. cretica*. These same findings were generally confirmed by Willcocks (1925), El-Sadany (1965) and El-Naggar (1967).

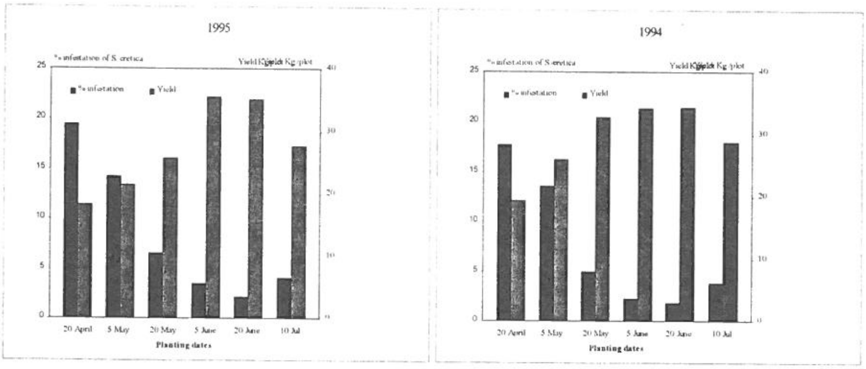

Figure 3 – Averange percentages of *S. cretica* infestation in the sucessive planting dates of maize and the yield.

Table 4 -The fluctuations in the percentage infestation of *S. cretica* in the 6 successive planting dates of maize and the yield during 1994 and 1995 seasons.

Planting date	**Percentage of infestation at different ages in weeks**								**Yield kg/plot**	
	2		3		4		Mean			
	1994	1995	1994	1995	1994	1995	1994	1995	1994	1995
20 April	12.66	14.06	18.81	20.98	21.66	23.38	17.71 a	19.47 a	19.36 c	18.25 c
5 May	13.49	12.75	14.73	15.08	12.47	14.98	13.56 b	14.27 b	25.91 be	21.50 bc
20 May	2.49	3.69	4.06	5.18	8.38	10.78	4.97 c	6.55 c	32.70 ab	25.75 b
5 June	1.82	2.14	3.16	3.58	1.75	4.75	2.24 cd	3.49 d	34.15 a	35.50 a
20 June	2.18	1.78	1.86	1.96	1.32	2.66	1.78 d	2.16 d	34.30 a	35.12 a
10 July	5.42	2.68	3.46	3.75	2.44	5.66	3.77 cd	4.03 d	28.80 ab	27.70 b
L.S.D.(0.05)							**2.86**	**2.61**	**7.82**	**6.62**

Data also showed that the highest yield amounts were found in the plots planted on 20th June, 5th June (34.30 and 34.15 kg/plot in the 1994 season and 35.12 and 35.5 kg/plot in the 1995 season, respectively), whereas the lowest ones were recorded in the plantings sown on 20th April (19.36 kg/plot in the 1994 season and 18.25 kg/plot in the 1995 season).

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AN OVERVIEW AND EVALUATION OF TRANSGENIC Bt CORN FOR EUROPEAN CORN BORER CONTROL IN THE USA

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ABSTRACT

Genetically engineered Bt hybrids providing season long protection against attack by the European Corn Borer, *Ostrinia nubilalis* (Hübner), were on the market in the USA in 1996. These genetically modified plants contain a gene that produces a toxic protein like that of the naturally occurring bacterium, *Bacillus thuringiensis*. Ciba Seeds and Mycogen hybrids which both use the insertion event 176 where trademarked as Knock*Out*™ by Ciba (sold as Maximizer™) and NatureGard™ by Mycogen in the USA. Northrup King, Asgrow, Beck's, Cargill, DeKalb, Golden Harvest, Pioneer Hi-Bred, and others use Monsanto's YieldGard™ technology which is derived from insertion events Bt-11 (Northrup King) and MON-810 (the others). The toxic protein is like the delta endotoxin produced by one or more of the Bt varieties. The toxin acts by breaking down the gut wall of the insect resulting in its death. This gene can be expressed throughout the plant or in specific plant structures or parts. In YieldGard™ Bt hybrids, the gene is expressed throughout most of the plant. While in Knock*Out*™ and NatureGard™ hybrids, the gene is expressed in the green tissue and pollen. In university trials in the USA, the data show that approximately 99% control of first and second generation corn borers can be expected when the YieldGard™ technology is used. Where the toxic protein is expressed only in the green tissue and pollen, as is the case for Knock*Out*™ and NatureGard™, 99% control of first generation and 50 to 75% of the second generation can be expected. An issue related to extensive use of Bt corn is the potential problem with resistance developing in the corn borer population. If this technology is widely adopted by farmers, this could limit the genetic diversity of the corn borer population thus resulting in the widespread development of resistance. To reduce the probability of this happening, corn without the Bt-like gene should also be grown in Bt corn areas. If problems with corn borers develop in these non-Bt fields, the population can be controlled with non-Bt insecticides. As one might expect, farmers are excited about this new technology. However, they should use these Bt hybrids intelligently to preserve this corn borer management tool.

Key words: Maize pests, Bt corn, *Ostrinia nubilalis.*

INTRODUCTION

Hybrids that have been genetically engineered to provide season long protection against attack by the European corn borer, *Ostrinia nubilalis* (Hübner), and which are referred to as "Bt corn," were on the market for the first time in the USA in 1996, albeit in limited quantities. These genetically modified corn hybrids contain a gene derived from a naturally occurring bacterium, *Bacillus thuringiensis*, which produces a protein that is toxic to corn borers. In several states in the midwestern USA, Ciba's Maximizer™ and Mycogen's NatureGard™ hybrids were marketed in 1996. Both use the insertion event 176 which is trademarked as Knock*Out* by Ciba and NatureGard by Mycogen. Northrup King's YieldGard™ (genetic material provided by Monsanto) received USEPA approval in August 1996. In 1997, Asgrow, Beck's, Cargill, DeKalb, Golden Harvest, Pioneer Hi-Bred, and others entered the market using YieldGard™ technologies. YieldGard™ is derived from insertion events Bt-11 (Northrup King) and MON-810 (the others). By 1998, more USA companies should have Bt corn on the market using YieldGard™ and seed supplies should be plentiful.

As noted above, transgenic corn for corn borer control is produced by genetically altering the corn plant so that its DNA includes a gene which produces a toxic protein. The toxic protein is like the delta endotoxin produced by one or more of the varieties of the Bt bacterium. The toxin acts by breaking down the gut wall of the insect resulting in its death. Although death is not immediate like with many insecticides, the insect does stop feeding and little movement occurs. This gene can be expressed throughout the plant or in specific structures or plant parts depending on the part of the plant needing protection, on regulations as to its location within the plant, or on the technique (event) used by the seed company to place the gene in the plant. In YieldGard™ Bt hybrids, the gene is expressed throughout most of the above ground portions of the plant.

While in Knock*Out*™ and NatureGard™ hybrids, the gene is expressed in the green tissue and pollen.

This new technology allows farmers who regularly experience problems with corn borers to use this as a tool to effectively manage this insect. It reduces the need for laborious scouting for this pest, although farmers should spot check areas within Bt corn fields to determine the effectiveness of this control technique. Obviously, scouting should not be eliminated as a result of Bt corn plantings. Other above and below ground insects may be present during the season and Bt corn provides little to no protection against these. Depending on the location within the USA, insects such as corn rootworm, *Diabrotica* spp.; black cutworm, *Agrotis ipsilon*

(Hufnagel); armyworm, *Pseudaletia unipuncta* (Haworth); white grubs, several species; corn flea beetle, *Chaetocnema pulicaria* Melsheimer; Japanese beetles, *Popillia japonica* Newman; corn leaf aphid, *Rhopalosiphum maidis* (Fitch), etc. can cause economic damage in corn. It is also important to note that although season long protection against the corn borer occurs, the level of control varies depending on the location of the toxic protein in the plant. With YieldGard™, where the toxic protein is expressed in most of the above ground plant, approximately 99% control of first and second generation corn borers can be expected. Where the toxic protein is expressed only in the green tissue and pollen, as is the case with Knock*Out*™ and NatureGard™, 99% control of first generation and 50 to 75% control of second generation are likely.

An issue related to wide spread use of Bt corn is the potential problem with resistance developing in the corn borer population. Initially, this will not be an issue since limited seed supplies will mean that most fields will not have Bt corn in them. This will allow the diverse gene pool in the corn borer population to be spread among the borer population within an area. However, if this technology is widely adopted over time and few if any non-Bt corn fields can be found, this will limit the gene pool and could result in the widespread development of resistance in the corn borer population. To reduce the probability of this happening, corn without the Bt-like gene should also be grown in Bt corn areas. This non-Bt corn will act as a refuge for some of the corn borers, thus preserving the genetic diversity that is now in the corn borer population. If corn borers reach economic levels in non-Bt fields, conventional insecticides can be used to manage populations (Bt-based insecticides should not be used where Bt hybrids are grown). If refugia are not provided, the Bt technology may be short lived or seed companies will constantly have to introduce new genes, if available, or stack genes to combat this situation.

Farmers are paying a premium for this new technology. Figures of USA \$9 to \$24 over the cost of a "normal" unit of corn is within the range of what is being charged (one unit plants approximately 3 acres (1.22 hectares). During early pricing discussions, some companies were talking about an additional USA \$20 to \$40 per unit. However, these figures have dropped considerably and additional competition are likely to bring the prices down even more. Certainly the potential for losses to occur from the presence of borers in a field, the high level of corn borer control achieved by planting Bt hybrids, and the ease of using this new technology are reasons enough for many farmers to pay a premium. With research that shows that losses from corn borer can range from approximately 2 to 6.6% depending on plant stage attacked (figure for one borer per plant, multiple borers cause greater damage), this causes farmers additional concern. Farmers use these, as well as many other factors when making management decisions (see Table 1 below, where US$ return from growing Bt corn is charted based on various yields, corn prices, and numbers of corn borers per plant). Another consideration is the level of probability that corn borer will be present in a field and cause economic damage. These data are not readily available, but based on many years of experience with this insect the likelihood of economic damage occurring in most fields is probably no greater than 2 years in 10. This tempers farmer enthusiasm somewhat, but the positives for controlling corn borer with Bt hybrids seem to outweigh the negatives in many farmers' minds.

Table 1 - Projected US$ return per acre (per hectare) with Bt corn

Yield*	Corn Price**	Corn borers per plant				
		0	0.5	1	1.5	2
150	$2.50	-10.00	-0.63	8.75	18.13	27.50
(9,415)	**($99.97)**	**(-24.70)**	**(-1.56)**	**(21.61)**	**(44.78)**	**(67.93)**
	$3.00	-10.00	1.25	12.50	23.75	35.00
	($120.09)	**(-24.70)**	**(3.09)**	**(30.88)**	**(58.66)**	**(86.45)**
175	$2.50	-10.00	0.94	11.88	22.82	33.75
(10,985)	**($99.97)**	**(-24.70)**	**(2.32)**	**(29.34)**	**(56.37)**	**(83.36)**
	$3.00	-10.00	3.13	16.25	29.38	42.50
	($120.09)	**(-24.70)**	**(7.73)**	**(40.14)**	**(72.57)**	**(104.98)**

* bushels per acre (Kg/ha)
** price per bushel (US$/tonne, where 1 tonne= 1,016 kg)

Assumptions: Bt corn costs $10 per acre ($24.70/ha), each corn borer reduces yields by 5 percent, and Bt corn provides 100 percent control.
Source: Iowa State University Extension, 1997: modified by Purdue University, 1997.

Farmers are excited about this new technology, as well they should be. However, they should use these transgenic hybrids intelligently. They should be used where corn borers have historically caused problems and farmers should plant non-Bt corn nearby to reduce the likelihood that resistance will develop in the corn borer population. Also, farmers should regularly check all of their corn fields for level of corn borer control, comparing Bt fields with non-Bt fields. Farmers should not stop scouting any of their corn fields, whether it be Bt or non-Bt fields. There are many insects that can attack and cause economic damage to corn!

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ALTERNATIVE METHODS TO ASSESS EFFECTIVENESS OF *BACILLUS THURINGIENSIS* AGAINST *OSTRINIA NUBILALIS* (*LEPIDOPTERA*: *PYRALIDAE)* IN CORN

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ABSTRACT

Bacillus thuringiensis Berliner (Dipel® ES, potency of 16.8 X 10^9 International Units (IU)/l) was applied to corn plants at 6.6 and 8.7 X 10^9 IU/ha. Plants were infested with *O. nubilalis* larvae on the same day as *B. thuringiensis* application and others 1, 2, 4, 8, and 12 d after application. Evaluations were made by counting the number of live larvae in the whorl of the plant, the number of *B. thuringiensis* spores in the whorl of the plant, the larval mortality from *B. thuringiensis* crystals taken from the whorl of the plant, and the cm of tunneling in the stalk 40 d after *O. nubilalis* larval infestation. *B. thuringiensis* reduced the number of live larvae per whorl by 63% and the cm of tunneling by 62%. The r values (regression analysis) were 0.65, 0.49, -0.47, and -0.45, for tunneling vs. live larvae in the whorl, spores in the whorl vs. larval mortality, live larvae in the whorl vs. larval mortality, and tunneling vs. larval mortality, respectively. The r values for the relationship between larval numbers and tunneling when *B. thuringiensis* was applied as a granule, were 0.97, 0.93, 0.97, 0.95, and 0.93, respectively, for 5, 7, 9, 11 and 18 days after larval infestation. Counting live *O. nubilalis* larvae in the whorl of the plant 4-10 d after larval application is an accurate alternative to stalk splitting to determine effectiveness of *B. thuringiensis*.

Key words : *Ostrinia nubilalis*, European corn borer, *Bacillus thuringiensis*

INTRODUCTION

For many years chemical insecticides have been applied to corn (*Zea mays* L.) to evaluate their effectiveness in killing larvae of European Corn Borer, *Ostrinia nubilalis* (Hübner). Effectiveness of these products is generally measured by splitting the plant from tassel to base approximately 40 d after application (Berry *et al.* 1974). The same technique has been used to evaluate products formulated with the bacterium *Bacillus thuringiensis* Berliner (Lynch *et al.* 1977a,b, McGuire *et al.* 1990, 1994). This technique is tedious, labor intensive, and results are highly variable between persons doing the evaluation, furthermore, data are not available until harvest time, and all ears must be removed to prevent volunteer corn in next year's crop. Evaluation for *O. nubilalis* tunneling for whorl and pollen-shedding-stage corn is made at the same time because of labor requirements. Occasionally, a secondary fungal infection in the tunnels makes those made by 1st-generation larvae indistinguishable from those made by 2nd-generation larvae.

There are unique differences between chemical insecticides and *B. thuringiensis*. Chemical insecticides generally kill insects immediately by contact or ingestion. They are also relatively toxic to nontarget organisms including humans. In contrast, *B. thuringiensis* causes a cessation of larval feeding but the larvae do not die for 3-4 days (Lewis 1985). *B. thuringiensis* is not as toxic to nontarget invertebrates and is nontoxic to vertebrates including humans (Laird *et al.* 1989). Because of this, a field can be entered immediately after application. Therefore, many parameters such as *B. thuringiensis* spores remaining on the plant, live larvae on the plant, and insecticidal activity of the crystal toxin can be measured over time. However, when *B. thuringiensis* is applied to corn as a spray or granular formulation, environmental factors such as ultraviolet light may destroy much of the insecticidal activity of the bacterium (Dunkle and Shasha 1988). Longevity of *B. thuringiensis* has been measured when applied with over the row equipment (Lynch *et al.* 1980), but not when placed directly into the whorl of the plant.

In this manuscript, we present data from experiments designed to develop methods other than stalk splitting for evaluating the fate and effectiveness of *B. thuringiensis*.

MATERIALS AND METHODS

Pioneer Brand 3541® hybrid corn, which is susceptible to leaf feeding by *O. nubilalis* larvae, was planted in rows at 55,800 seeds/ha, 0.75 m apart. The experiment was designed as a randomized complete block with a split-plot arrangement of treatments and six replications. Whole plots (2 40 m long rows) were treated with Dipel ES® (0, 6.6 x 10^9, 8.7 x 10^9 International Units (IU)/ha (Abbott Laboratories, North Chicago, IL 60064). Subplots were the days after treatment (0, 1, 2, 4, 8, 12) when the plants were infested with *O. nubilalis* larvae. Day 0 corresponds to the day *B. thuringiensis* was applied.

B. thuringiensis was applied with a compressed air sprayer to assure uniform application to each plant and to protect the maximum amount of material from exposure to ultraviolet light. Approximately 50 neonate *O. nubilalis* larvae were applied to each plant within a subplot (Mihm 1983). Five d later, whorls were pulled from 15 plants/subplot, carefully unrolled, and the number of live larvae counted. At this time, whorls from an additional five plants/subplot were randomly selected to be analyzed for the presence of B. *thuringiensis* spores. For each plant, a section of the whorl, 7.6 cm in length, was cut beginning at the point where the leaves were tightly furled and extending upward. Whorls were immediately sealed in a labeled plastic bag and frozen until they could be analyzed. Forty d after larval infestation, 25 plants/subplot were split from tassel to base and the cumulative cm of tunneling were recorded.

Presence of *B. thuringiensis* spores was analyzed on a per-plant basis (Lynch *et al.* 1980). Each sample was homogenized in a blender for 2 min. with 100 ml sterile distilled H_2O. The homogenate was serially diluted and plated in triplicate on nutrient agar plates by spreading 0.1 ml of the diluent across the surface with a sterile glass spreader. The nutrient agar plates were amended with filter-sterilized polymixin B sulfate at 8 ppm (Saleh *et al.* 1969) and neomycin sulfate at 1 ppm. Colony counts were made after the plates had incubated for 24-30 h at 30°C.

Virulence of *B. thuringiensis* (crystal protein) was determined against neonate *O. nubilalis* larvae using a droplet assay (Hughes & Wood 1986). Thirty neonate *O. nubilalis* larvae were allowed to imbibe from droplets of the homogenate from each ground plant. Larvae were transferred individually to laboratory diet minus aureomycin (Guthrie *et al.* 1985) and incubated for 7 d at 27°C, 50% RH, and under constant light, after which mortality was recorded.

A second experiment was conducted to determine if the time interval between infestation with larvae and pulling of whorls influenced the relationship between live larvae and tunneling. Choice of corn and planting procedures were the same as in the first experiment. The experimental design was a randomized complete block with a split plot arrangement of treatments. Whole plots included hand application of Dipel® 10G (1.4×10^9 IU/ha) and an untreated plot. Subplots were the days following larval infestation when whorls were pulled and the number of live larvae counted.

Larval infestation was made 1 d before application of *B. thuringiensis*. At 5, 7, 9, 11, and 18 d after larval infestation whorls were pulled from 50 plants/subplot, and the number of live larvae were recorded. Forty d after infestation 25 plants/subplot were split from tassel to base and the cumulative cm of tunneling were recorded.

Data (live larvae in the whorl and cm of tunneling) from both experiments were analyzed by using the analysis of variance for a split-plot experimental design (SAS 1985). Means were separated by using Duncan's multiple range test (Duncan 1955). Relationships between all measured parameters were determined by correlation and regression analysis (SAS 1985).

RESULTS

The number of live larvae per whorl (whorl counts) in control subplots increased gradually, (68 to 78 larvae/15 plants) during the first three infestation days and then decreased (50 to 21), during the last three infestation days (Figure 1).

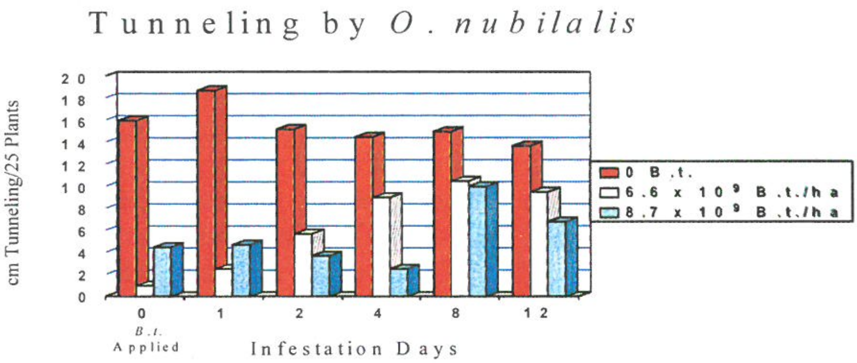

Figure 1 - Numbers of live *O. nubilalis* larvae in whorls of corn plants. Infestation days refer to days plants were infested with *O. nubilalis* larvae. Day 0 corresponds to the day *B. thuringiensis* was applied to plants.

The average number of live larvae in the corn in the subplots treated with *B. thuringiensis* increased (averaged over both *B. thuringiensis* levels) from 8 to 30 larvae/15 plants during this same period. Whorl counts for the 0 level were significantly different from counts for the 6.6 x 10^9 and 8.7 x 10^9 IU/ha levels at all samplings except infestation d 12. At this time there were no differences in larvae/plant between any of the treatments ($F = 12.51$, $df = 10, 75$, $P < 0.001$).

Cumulative cm of tunneling in control plants increased from 16 to 19 cm/25 plants between infestation d 0 and 1 and decreased from 15 to 14 between d 2 and 12 (Figure 2). There was a corresponding increase in cm of tunneling in treated plants during this time. These data ranged from a low of 1 cm of tunneling/25 plants at d 0 (6.6 x 10^9 IU level) to a high of 11 at d 8 (6.6 x 10^9 IU level). Tunneling in untreated plants was significantly greater than from treated plants at infestation d 0, 1, and 2. At infestation d 4 there were significantly fewer cavities in plants treated with the high level of *B. thuringiensis* than in those treated with the low or 0 levels of *B. thuringiensis*. On d 8 and 12 there were no differences in cm of tunneling between levels of *B. thuringiensis* ($F = 2.73$, $df = 10, 75$, $P < 0.006$).

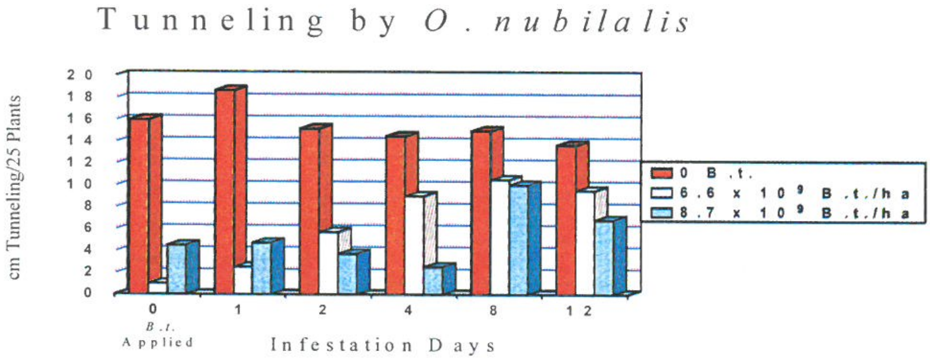

Figure 2 - Cumulative tunneling by *O. nubilalis* larvae in corn 40 days after larval infestation. Infestation days refer to days plants were infested with *O. nubilalis* larvae. Day 0 corresponds to the day *B. thuringiensis* was applied to plants.

The number of *B. thuringiensis* spores/plant decreased rapidly over time (Figure 3), with only 10% of the original spores remaining 2 d after application. A similar trend was recorded for larval mortality (Figure 4), although the decrease was not as great with the high level of *B. thuringiensis* as with the low level.

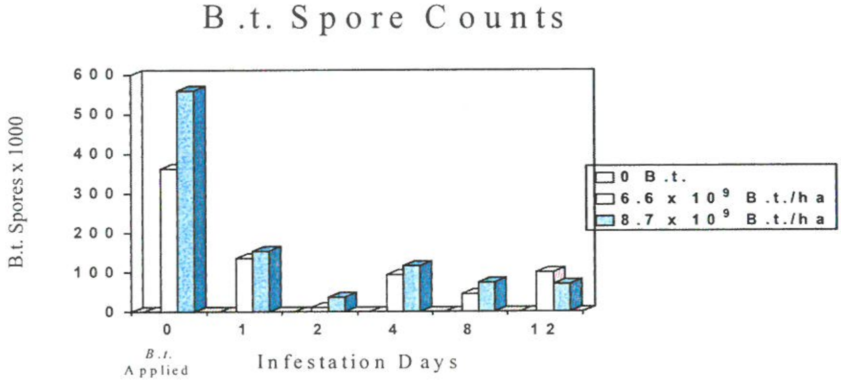

Figure 3 - Number of *Bacillus thuringiensis* spores remaining in the whorl of corn plants at intervals after application. Infestation days refer to days plants were infested with *O. nubilalis* larvae. Day 0 corresponds to the day *B. thuringiensis* was applied to plants.

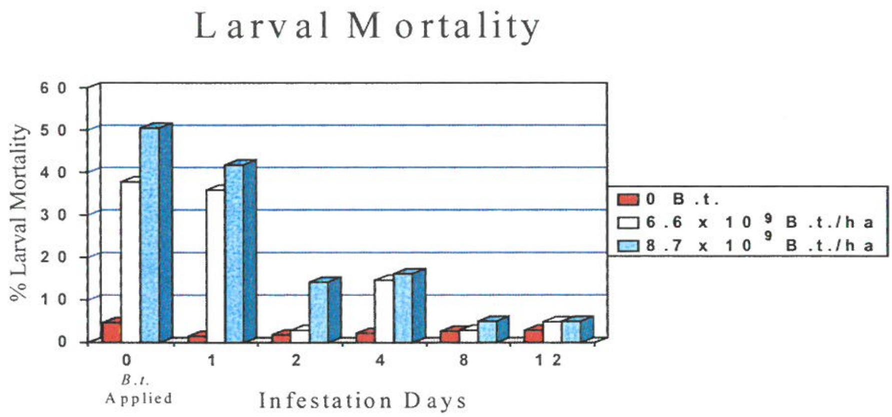

Figure 4 - Insecticidal activity of *Bacillus thuringiensis* remaining in whorl of corn plants as indicated as % of larval mortality. Values were determined in laboratory assays. Infestation days refer to days plants were infested with *O. nubilalis* larvae. Day 0 corresponds to the day *B. thuringiensis* was applied to plants.

Regression coefficients were calculated for all possible relationships between the measured parameters. The r values, for tunneling vs. whorl count (larvae), spores vs. larval mortality, whorl count vs. larval mortality, and tunneling vs. larval mortality were 0.65, 0.49, -0.47, and -0.45, respectively.

For the second experiment, a regression analysis was used to determine relationships between counts of live larvae in the whorl and cumulative tunneling in the corn stalks. The r values were 0.97, 0.93, 0.97, 0.95, and 0.93, for 5, 7, 9, 11, and 18 d after infestation with larvae, respectively. When all data for whorl counts vs. cumulative tunneling were combined, the r value was 0.83.

DISCUSSION

This is the first report of the numbers of live *O. nubilalis* larvae remaining in the whorl of a corn plant being used as an indicator of effectiveness for a crop protection product. The number of larvae in the whorl of plants treated with *B. thuringiensis* remained significantly lower than the number in untreated check plants for up to 8 d after infestation (Figure 1). An interesting observation was the decline in live larvae/15 plants in the 4 and 12 d subplots within the untreated whole plot. This decline in numbers of larvae may be explained by migration of larvae from the whorl to the sheath collar area and by natural mortality of larvae which includes predation, disease, and enviromental factors. For infestation d 12, there were equal numbers of larvae in all subplots within all whole plots. A corresponding slight increase in larvae/whorl in the *B. thuringiensis*-treated subplots (Figure 1) was most likely caused by the substantial loss of toxicant (measured as *B. thuriniensis* spores) within the whorl (Figure 3) and larval mortality (Figure 4). The cm of tunneling by *O. nubilalis* larvae in all subplot treatments (Figure 2) followed the same general pattern as for live larvae (Figure 1). An exception occured for plants treated with the high level of *B. thuringiensis* which were infested on d 4 after application of toxicant in those plants. This slight decrease in cm of tunneling corresponded to an increase in spore counts and larval mortality (Figures 3 and 4).

When data from the two *B. thuringiensis* whole plots were combined, there was a reduction of 94% in tunneling at day 0 compared to untreated whole plots. In other studies with a liquid formulation of *B. thuringiensis*, researchers reported reductions in tunneling that ranged from 43 to 68% by applying *B. thuringiensis* over the row with a high clearance machine at levels of 11.8 to 35.3 X 10^9 IU/ha (Lynch *et al.* 1977b). In another study, tunneling by *O. nubilalis* larvae was reduced by 8 to 20% when a liquid formulation of *B. thuringiensis* was applied at levels of 4.5 to 9.0 X 10^9 IU/ha (Lynch *et al.* 1980). Our levels of application were substantially lower (6.6 and 8.7 X 10^9 IU/ha), but the reduction in tunneling was greater than or equal to that previously reported by other researchers. This indicates that only a small percentage of the *B. thuringiensis* was reaching the whorl when applied by machine.

The number of *B. thuringiensis* spores/plant had a half life of less than 1 d when placed directly in the whorl. When a high clearance machine was used to apply *B. thuringiensis*, the half life of the spores was less than 2 days (Lynch *et al.* 1980). The half life of the spores in our research is similar to those reported when *B. thuringiensis* spores were applied to soybean leaves (Ignoffo *et al.* 1974) and to redbud trees (Pinnock *et al.* 1971). The corresponding loss of insecticidal activity as indicated by larval mortality (Figure 4) demonstrated that *B. thuringiensis* had a half life of less than 2 d. Thus the insecticidal activity of *B. thuringiensis* crystals remained in the plant longer than did the spores. Spores are indicative of the presence of *B. thuringiensis* not of insecticidal activity. The crystal must be present to kill a substantial number of *O. nubilalis* larvae (Mohd-Salleh and Lewis 1982).

This report is also the first assaying the *B. thuringiensis* crystal within the whorl of a corn plant for insecticidal activity. Mortality of larvae in the laboratory assay declined very rapidly over time indicating a 50% loss in insecticidal activity in less than 2 d (Figure 4). Beyond this point in time there was only a slight additional loss of insecticidal activity. The highest mortality obtained in a laboratory assay was 32% at the day of toxicant application. In plant whorls in the field, 63% of the larvae were killed when averaged over all days. At d 0 69% of the larvae were killed or were no longer in the whorl. The technique used in preparing the material for mortality determination required the addition of 100 ml of H_2O thus a substantial dilution that may account for the relatively low mortality percentage in the laboratory assay.

Regression analysis showed a highly significant relationship between many parameters measured. The strongest correlation was between the whorl count (live larvae) and the cm of tunneling ($r = 0.65$). Heretofore, the only reported data on regression analysis of the fate of *B. thuringiensis* after application for control of *O. nubilalis* in corn was Lynch *et al.* (1980). For 2 separate years they reported r values of -0.52 and -0.48 ($\underline{P} > 0.001$) between spores in the whorl and tunneling. However, they averaged their spore data over application techniques that included granules and foam in addition to a liquid. All applications were made over the row with a high clearance machine. In this study, the r value of spore count vs. tunneling was -0.29 ($\underline{P} < 0.0027$). The average percentage reduction in tunneling was 62% (78% 2 days after treatment) whereas Lynch *et al.* (1980) reported a mean reduction in tunneling of only 20%. Even though there is a significant correlation between these two parameters the relationship between live larvae in the whorl and tunneling has more practical application.

In the second study, we showed an even stronger correlation between whorl pulling and tunneling. This confirms the correlation for these parameters shown in the first study. A researcher, however, should be cognizant of the changes in the slope of the r values between 11 d and 18 d after larval infestation (0.32 to 1.02). This change can be explained as the time when the *O. nubilalis* larvae begin migrating from the whorl area and begin feeding behind the leaf-sheath area or entering the stalk. Thus, if one was to use the whorl-pulling technique, data should be gathered between day 5 and 11 following larval infestation.

From these data, we concluded that even though *B. thuringiensis* is placed directly into the whorl of corn, the numbers of spores and larval mortality decreased very rapidly. This indicates that the whorl of the plant acts as a funnel to concentrate the toxicant not as a screen to ultraviolet light. The requirement for maximum kill of *O. nubilalis* larvae is that the toxicant be available to the insect. This confirms the work of McGuire *et al.* (1990). The latter research demonstrates that equal control can be obtained with a 4-fold reduction in IU of *B. thuringiensis* if COAX® is incorporated into the formulation. COAX acts as a phagostimulant (Bartlet *et al.* 1990), and if placed with *B. thuringiensis* in proximity to insects, and it stimulates them to consume the bacterium, excellent control can be obtained.

In conclusion, the number of live *O. nubilalis* larvae remaining in the whorl of the corn plant is strongly correlated with tunneling in the stalk. We propose that the whorl technique is an effective alternative to stalk splitting for evaluating the effectiveness of *B. thuringiensis* formulated products. It is more objective than a leaf-rating system such as the one used by Guthrie *et al.* (1960) because an exact number of live larvae are counted. Furthermore, in efficacy evaluations of *B. thuringiensis*, shot-hole feeding does occur, but, subsequently, most of these larvae may die. One additional assumption made in the whorl pulling technique is that no additional *O. nubilalis* infest the plants after the counts are made.

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Footnotes

Names are necessary to report factually on available data; however, neither the USDA nor Iowa State University guarantees or warrants the standard of the product, and the use of the name implies no approval of the product to the exclusion of others that may be suitable.

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RESISTANCE OR TOLERANCE ? PHILOSOPHY, MAY BE THE ANSWER

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ABSTRACT

The rapidly increasing world population and its demand for new outputs in food production, together with the recent advances in biotechnology application to plant breeding, brings to light the ultimate decision about the right philosophy to deal with this XXI century paramount issue.

It is a matter of fact that the Green Revolution, that so successfully stopped the Malthusian apocalyptic previsions, was based on a USA born productivist philosophy rooted upon fossil energy, high mechanisation levels, monocropping technology and a breeding approach that favoured yield (quantity) as the first priority trait. It is also known that after some decades of its application, some negative consequences came along such as genetic erosion and high pollution levels.

Maize, as one of the three big crops, has been and it surely will be, at the centre of this storm among the most serious candidates to challenge the new food demands. This is expected to be done both through the yield increases (quantity and/or quality) and also through a new improved equilibrium with its natural enemies (resistance and/or tolerance). Adding to this the new generalised concern for environmental protection, safety and sustainability, we are forced to figure out what should be the most adequate philosophic approach for the new century agriculture.

Portugal, as a small country recently incorporated (1986) in the European Union (EU) that adopted and fiercely imposed the American productivist philosophy through its Common Agricultural Policy (CAP) is at the edge of a big transformation in its fragile agriculture. This is especially evident at its traditional maize area located at the mountainous Northwest, where the polycrop self-sufficient systems were predominant. In this social/anthropological/environmental turmoil that is leading to the rapid disappearing of these quality-oriented sustainable systems, and in order to test their real potentialities, a pilot-project was initiated in 1984/85. This project that has been running at the Portuguese Sousa Valley (VASO), was designed to adopt a new philosophical approach that roots on the antipodes of the American model, either at its basic paradigms or at its breeding methodologies that lead to different final products.

The first results of this on-farm maize breeding project show a 17% gain in yield after the first cycle (C_1) of S_2 lines recurrent selection, maintaining the quality priority imposed by the local small farmers and respecting the polycropping system. Diversity, as the basis for a tolerance over resistance pathological approach, has been favoured, since the final product is intended to be an improved open-pollinated seed.

A comparison is made between the farmer's breeding methodology (phenotypic recurrent selection) and the breeder' methodology (S_2 lines recurrent selection), with a clear advantage to this last one.

Two contrasting philosophies – **productivist** *vs* **integrant** – are defined and compared through their respective paradigms and consequences, as well as their implications in plant breeding. Our project VASO, supported by its first results, presents some evidence suggesting that the integrant philosophy, favouring tolerance and sustainability, may be an alternative to the productivist model.

Key words: Plant breeding philosophy, tolerance, population improvement, S_2 lines, *Zea mays* L.

1 - INTRODUCTION

Portugal is a small country at the western border of the European Union (EU) with a fragile agricultural structure in which maize has played an important role as the responsible plant for the "agricultural revolution" that took place in the Portuguese Northwest during the XVII and XVIII centuries. As a result of that "revolution" a typical "agricultural system" was developed with a consequent selection criterion based on three common grounds:

- Polycropping. Plants that were able to grow in togetherness with other crops (in the same plot at the same time), that is to say, in a more or less complex "system".
- Quality priority. For both human and animal consumption and with a plant ideotype that could fit the system.
- Genetic diversity. For different types of soils, altitudes, pests, water and stress tolerances.

The general criteria that were assumed by traditional farming led to a family based self-sufficient farming, where a low input agriculture was looked upon as a sustainable way of life.

More recently, however, after the Portuguese integration in the EU (1986) and as a result of the Common Agriculture Policy (CAP), the Portuguese agricultural environment has been suffering a three fold negative impact.

First, an area reduction since only a small part of its arable land is fitted for the so-called modern intensive agriculture. The original area devoted to agriculture, which included the mountain slopes, has been reduced to the coastal plains and some river valleys where the competitive agriculture – the only one favoured by the CAP economic benefits - can survive. That reduction led to the actual 250.000 ha devoted to maize from the 350.000 ha in late 70´s.

Second, human erosion. The majority of farmers - small farmers of the Northwest mountainous regions - left alone without any economic compensation and without competitive yields, went to bankruptcy and were forced to abandon their home lands and emigrate to the coastal border looking for new jobs around the crowded cities.

Third, genetic resources erosion. With this internal migration, the rural world that embraces a larger spectrum than the agricultural concept itself, was deeply affected in its environmental equilibrium and an inevitable genetic erosion took place. In fact, in spite of a previous national germplasm collection realised during the late seventies, this big transformation during the early eighties led to a considerable genetic erosion, having in account that these self-sufficient models always carry with them a set of diversified crops and animals.

Altogether, these three consequences gave rise to such a social and environmental transformation that largely exceeds the normal spectrum of the "competitive agriculture" narrow-minded conception. In fact, Portugal can be considered today as a privileged study field for agricultural transformation and its implication with the rural world environmental and anthropological impacts. This can be envisioned through two analytical approaches:

1 – Within the last 10-15 years, the farmers' population was reduced from 20-30% to 5-10%. This fast and severe reduction originated a migration from the mountainous interior to the coast, with a consequent pressure on housing, health and education facilities, as well as all kinds of social adaptation problems.

2 – The former farmer who migrated from the hill slopes to the coastal border, used to run a four-fold agricultural system, which included, in fact, such different activities as:

Food production. Mostly for self-consumption, part for selling.
Genetic resources conservation. Due to his polycropping self-sufficient agricultural system, he was the real curator of a large diversity of plant and animal genetic resources.
Environmental sustainability. He was the best manager of soil and water resources, taking care of their quality and control, and the administrator of the plant and animal equilibrium within a sustainability maintenance concept.
Forest protection. Besides cleaning and administrating the forest, he was also the best fireman.

However, in spite of these four activities with a direct impact on the society in general, this farmer was only paid for the first activity (food production), exactly the one that, by itself and under its limited natural conditions, could not be competitive in normal market terms.

As a result of this policy - which the CAP should be responsible for - a heavy social problem and an environmental disaster came along upon the Portuguese rural world. As an evidence of this "disaster" it will enough to face this new reality: our country has been building up a new "army" that is growing year after year (34.000 in 1996 and still being implemented), with all the inherent paraphernalia of trucks, helicopters and aeroplanes – the forest firemen structure.

Today, like yesterday, following along this national transformation from an "agriculture as a way of life" to an "agriculture as a business", either on the hills or on the plains, always the same witness – maize! Will it be that it has something to tell us about tolerance or resistance behaviour?

2 – RESISTANCE OR TOLERANCE: HOW TO CHOOSE FROM?

Due to its weight in the global economy, maize has been a target for continuous improvement both at the technological and breeding levels. Among the different approaches to implement the yield mean, the battle against its natural pest enemies - the IWGO scope - certainly constitutes a pursued goal to achieve. Therefore, based on the understanding of the pest/plant interaction given by entomology, both technology (agronomic and chemical) and genetics (breeding) should play partners, side by side with farmers, in a race that has been targeted to steadily diminish the yield losses.

At the breeding level, this has been attempted and partially accomplished either through resistance or through tolerance genetic mechanisms. Assuming that, in a broad sense:

- **Resistance** is mostly connected with single gene expression (qualitative traits) that directly counteracts the plant enemy.
- **Tolerance** is more related with polygenic expression (quantitative traits) responsible for a common action that buffers the pest and/or disease even without killing it.

We are now in position to better understand that the breeder's decision in choosing between one and the other, implies an option between "high pest control efficiency /plant uniformity" and "lower pest control efficiency /plant diversity".

In fact, the different ways breeders have dealt with this issue, can fit within the historical framework of genetics itself: firstly, with a Mendelian qualitative genetics approach, secondly, with a quantitative genetics methodology and more recently with the new "biotec" molecular genetics solutions. In other words, this historical picture can be characterised by an alternate sequence of emphasis on resistance (Mendelian genetics), tolerance (quantitative genetics) and again resistance (molecular genetics).

So, in a time when the new transgenic maize seeds rapidly spread into the market place, how to choose between tolerance and resistance mechanisms? Does maize itself have a word to say about this?

To answer these questions, let us go back to the foundations of any breeding programme: genetic resources. From the slopes of Portuguese Northwest Mountains to the river valleys, maize gave rise to the "agricultural revolution" of the XVII and XVIII centuries. This means that the new plant introduced in Europe by Columbus, underwent such a selection pressure of adaptation that conducted it to a panoply of different land races, each one adapted to a micro-ecology among a diversity of soil/climate conditions. This can be easily understood by a parallel reading of the diversity of the Portuguese wine types that still are produced in the same spots where maize used to grow.

This richness of germplasm has been collected since the early 70's in a narrow collaborative effort with IBPGR/IPGRI and constitutes a more than 3000 accessions collection stored at the Portuguese Plant Germplasm Bank

(BPGV) at Braga. From such a diversity of local open-pollinated populations grown at so different edapho-climatic conditions, some common features, either related with their genetic background or the adopted technology, can bring, in our opinion, some light upon the resistance / tolerance issue.

In what concerns the genetic diversity background, only a few scientifically based studies have been carried out besides preliminary evaluation at the botanical level. Nevertheless, they allow foretelling that a promisable genetic diversity was really collected with quantitative and qualitative potentials that are suggested through some evidences such as:

- Some accessions collected in some northern mountainous slopes have shown to possess abnormally high nitrogen efficiency.
- The general selection criteria carried out by farmers during the last centuries all over the traditional Portuguese maize area did favour the kernel flint types, mainly white coloured, but also some yellow/orange flints that fit the best standards for corn-flakes and grits utilisation.
- Some early maturity populations have shown a good tolerance to water and aluminium toxicity stresses.
- Grain quality was the first criterion for selection, mostly for food, but also for feed purposes, followed by the plant ability to fit the polycropping agricultural system within a limited biological cycle around maturity FAO 300. Quantity, as represented by the yielding potential, was then the second priority in selection.
- Selection for plant (stalk) quality favoured the digestibility over the stalk lodging resistance, in an incompatible like manner, due to two main reasons:
    - The traditional technology used to practice the detasseling after flowering, for forage complement, so buffering the wind effect and masking the lodging rate.
    - The stalks used to be stored in open air vertical meadows to be gradually used as feed during Winter and early Spring, so the preferred high digestible plants were also the poorest for lodging quality.
- Selection for both pest and disease control was effectively poor and essentially based on tolerance. Two main pests with some economic

relevance - *Sesamia nonagrioides* (Hub.) and *Ostrinia nubilalis* (Lef.) - have been present in the Portuguese maize fields. As it can be deducted from the former item, the best germplasm to select from was the one located close to the straw meadows, since those maize populations were the first to be heavily infested by the closer inoculation sources at Spring time pest eruption. And, as it is known, these conditions are always the best for selection.

So, in our attempt to read the message about tolerance/resistance coming out from the Portuguese maize germplasm, we can arrive to these conclusions:

- Diversity was the basis pursued by farmers to adapt the new crop to a large variety of edaphic/climatic conditions of the traditional maize area - the Portuguese Northwest.
- Population improvement, mainly as mass selection with quality priority criterion, was the pursued empirical methodology.
- In their battle for adaptation to the local conditions and agricultural system technology, as well as a direct consequence of the pursued selection methodology, farmers followed up a quantitative genetics approach.
- In the sequence of this natural framework that proved to pursue sustainability, the direct impact upon the pest control was necessarily favouring tolerance and neglecting resistance.

On the other hand, and more recently after our affiliation in the EU, the hybrids literally invaded our fields putting aside the local land races as they were forgotten in the same way as their creators (the small farmers) were erased out of the rural world.

This recent big transformation that included both an enormous genetic erosion and the building up of the new age of uniformity in our landscape, brings with it a different agricultural approach that is imposed by the competitiveness of market economy and affects altogether farmers, breeders and policymakers. Moreover, because within this philosophic framework the power of decision always belongs to the politicians, and since the CAP definitively has favoured this last approach, we came across a situation that can be defined under the following parameters:

- Diversity is no more the favoured option. Instead, uniformity - well exemplified by the intensive hybrid maize production - is the key for competitive farming.
- Qualitative genetics based breeding, taking care of the heterosis effect and single crosses methodology, replaced the population improvement.
- The new scientific tools coming out from the recent advances in molecular genetics, namely biotechnological transgeny, are favouring the monogenic approach and even stressing the uniformity factor.
- This new scientific environment in modern agriculture, once confronted with the question about tolerance or resistance, easily favours the option for resistance.

If there is a clear message coming out from the maize plant when faced with these two contrasting situations - traditional *vs* modern maize cropping - that message is this: tolerance means diversity, and resistance means uniformity. Consequently, then the initial proposition between tolerance and resistance belongs to a much wider issue requiring an option between diversity and uniformity. And this again reaches the deep grounds of philosophy.

In fact, considering this big transformation in the Portuguese agriculture within such a short period of time, we realise that it has been adopting the American philosophy, translated into a model of intensive agriculture. This model requires from medium to big parcels of land, high levels of mechanisation, plenty of water and chemical fertilisers, chemical weed control, monocropping systems, uniform plants, that is to say, single cross maize hybrids.

While diversity is more related with population improvement, uniformity is the final result of breeding methodologies that have been supposed to be the unique economic alternative for food and feed production at the high standards of modernity. This has been, in fact, the "American recipe", so successful in the USA and exported to Europe and other continents after World War II. Nevertheless, in spite of its enormous success in yield enhancement (quantity), more recently it has shown some of its weak points like that one of chemical pollution (water and soil) which it has been accounted for after some decades of exultant exploitation.

Furthermore, when the environmental science is steadily raising in the horizon, showing up all the consequent troubles this agricultural approach has been causing, as well as the danger it represents in compromising the future, the neglected dilemma between uniformity and diversity comes again into place.

However, a historical contradiction should be noticed: at a time when the "genetic resources" issue was high in our scientific concerns and "diversity" was looked upon as the answer for tomorrow's agriculture, suddenly the scientific community became fascinated by the new advances in molecular genetics that, again, favour "uniformity". Consequently, the food industry all over the world was shaken and we could assist at its moves like the generalised buying of seed enterprises by the big chemical giants, looking for the easiest way to implement food production and their own profits. This has been made by using the existing uniform plants (inbreds) and inserting in them the "magic genes" that will booster production, either under a form that combats the natural enemies, or allow to support the polluting chemicals used for weed control.

In fact, the new genetic tools, like transgeny - which maize so well represents under the commercial successful form of Bt (*Bacillus thuringiensis*) plants resistant to European corn borer (ECB) - have been so tangibly welcomed by both breeders and farmers, that it seems everybody is under an euphoric wave! Nevertheless, it should not be forgotten that all this new stuff brings even more uniformity to our cornfields. In fact, this new technology is taking the best economic advantage when realised upon the best inbred lines, those already uniform genetic materials that breeders selected and tested for a stabilised environmental adaptation.

After all, this is a common situation with the big enterprises that look for already uniform adapted seeds in which they foresee an economic added value anytime a new "magic gene" is introduced. So, in the name of the food production or under the "feeding the world" slogan, diversity is being again neglected and uniformity implemented, at such levels that we wonder about future risks always connected with the genetic "bottle neck".

Adding to this and even worst as it may be, we have to face the generalised erroneous common idea that besides uniformity there is no other competitive alternative for food production.

In such a scenery, is there any place to bring back the virtues of diversity with its credits of sustainability, low level of chemical inputs, a controlled balance with the natural enemies of our crops, but with yield means that apparently don't fit the food needs?

Stepping forward, how to respond then to this dilemma: uniformity/resistance or diversity/tolerance?

In a time when everybody is expectedly looking to biotechnology as a tool to solve the food needs for the increasing population of the XXI century, how to sustain the diversity/tolerance thesis?

To answer this question it must be emphasised that, as revolutionary as it may be, biotechnology is only a scientific tool. And as a tool, it can be used to favour either uniformity or diversity.

3 - PHILOSOPHY: THE BREEDER'S BASIC DILEMMA

What are we supposed to choose for a sustainable agriculture?

Under a breeder's point of view, this is not a simple question. In fact, an array of other questions immediately arises at the breeder's mind: what to favour? Diversity or uniformity? Hybrids or improved open-pollinated populations? Resistance or tolerance? What are the real needs when confronted with determinant agents? What are the farmer's demands? What are the enterprise or governmental indications? What is the agricultural policy and what really determines the policymakers' decisions: market or ethic concerns?

It is our opinion that all big decisions are built upon some kind of philosophy. Based on it, a decision about agricultural policy will be taken in order to favour, at its extremes, either a market-oriented economy, or a sustainable self-sufficient type, with all the intermediate positions in between.

3.1 - PRODUCTIVIST PHILOSOPHY

In order to better expressing this contrasting idea, let us try to characterise these extreme philosophical approaches. We will start with the successful American model whose philosophy we call "productivist" since it definitely favours productivity (quantity - tones/ha) as the main parameter both at selection and farming levels.

This model is based on a fine scientific approach. In fact, USA should be credited for "planting universities" through all the Corn Belt and there, in the real plant environmental conditions, scientists have been exploiting the maximum potential yields of each crop and, at the same time, developing a technological package that fits their genetic capability. In short and according to this philosophical approach, it can be said that scientists are the ones who take all the essential decisions, working out a productive package that includes: an improved "seed" and an adequate technological "recipe".

The "recipe", represents the equilibrium coming out from pursued studies about environmental influences (soil, climate) and their interaction with the plant genotypes, and is based on replicated yield trials from which a generalised set of data was recorded. These data, constituting a set of instructions or recommendations that also contemplate machinery equipment and its management, as well as types and amounts of other inputs, are aimed to optimise the genetic yielding potential of that "seed".

The "seed", as the final product of the breeding chain, is designed to be competitive in an open market economy which, in the case of an allogamous species like maize, is also intended to fit the commercial system that requires the seed renewing (hybrid) in each cropping season. So, at the breeder's level, besides taking good care of the non-additive gene action to booster heterosis, selection criteria usually follow this sequence in a decreasing order of priorities: yield, stalk and root quality, pathology and plant ideotype.

Yield

The yielding ability, being considered the most important selection factor, has been mostly associated with quantity (tones/ha). The favoured gene action that better fits the pursed heterosis for hybrid production, is one of the non-additive type, mainly dominance. This leads to uniform plants that are supposed to be grown in monocropping and that are able to fit high levels of mechanisation and chemical inputs.

Stalk and root quality

Since both are related with the plant structure that absorbs and transports the mineral nutrients and supports the ear, they have given the important second place in selection criteria due to their two-fold and inter-linked functions:

- To avoid lodging, required by the high mechanisation, mainly at harvest.
- To counter influence pests and diseases that, besides lodging, directly contribute to lower the yielding.

Pathology

Because this philosophical model definitely favours the uniform hybrid plants used in the intensive modern agriculture, selection for healthier plants - the pathology factor - tends to prefer resistance to tolerance. In fact, this preference has been more recently emphasised through the new generation of hybrids coming out from transgeny or other biotechnological applications. This is the case of ***Bt*** (*Bacillus thuringiensis*) hybrids with incorporated pest resistance, and others that tolerate some herbicides.

However, in what the ***Bt*** maize is concerned, attention should be paid to the possible consequences of this option for resistance, especially if considered in a medium/long term perspective. In fact, mother Nature always reacts to pressures and a generalisation of ***Bt*** hybrids is expected to contribute for the selection of resistant insects that eventually can arise and so overcome this biotechnological barrier.

Being aware of this expected mechanism and in order to maintain its feasibility, breeders and seed companies are already recommending the parallel planting of a border plot of normal susceptible plants, in order to maintain a susceptible insect population and so avoiding the appearance of a resistant generation. Basically, the practical recommendation is based on:

- The insect resistance, being recessive, only functions in the homozygous condition.
- If all the maize plants were resistant (***Bt***), only the resistant insects would survive.
- The mating among resistant insects would lead to resistant insect populations for the following cropping / infection.
- By having a plot of normal maize plants (insect susceptible) this would favour the maintenance of a susceptible insect population which, once mating with either resistant or susceptible partners, would breed

heterozygous susceptible offspring.

- This methodology, in spite of the inconvenience of requiring the planting of border plots of normal plants, would thus prevent the jeopardy of a new technology that promises to be somehow a breakthrough in maize production enhancement.

It should be stressed, however, that this new technology always gives preference, essentially by economic reasons, to uniform already adapted genetic materials, like elite inbred lines which are stepped forward into still more uniform hybrids at the field. In other words, if at the farm level we could observe an already risky uniformity among hybrids, from now on we risk having all of them with *Bt* resistant genes.

All this reasoning leads us to the conclusion that this new scientific advance, that favours resistance and leads to even more uniformity, is somehow being achieved at the expenses of diversity.

Plant ideotype

Last, but not the least, this is also an important factor since any increase in plant density (number of plants/ha), turns to be an indirect way of increasing yields. In fact, the canopy that better takes advantage of the disposable soil conditions and better explores the sunlight, is always a matter of selection preference. So, the plant ideotype that better fits the productivist philosophy can be defined as a plant with:

- Up-right leaves (low leaf angle)
- Little ramified panicles
- Low ear placement
- High plant population capability
- Good ear size
- Stalk and root quality

3.2 - INTEGRANT PHILOSOPHY

On the other extreme in our analysis, we have what we call "integrant philosophy". We call it "integrant" because it contains the idea of integration, that is, a demand to integrate a set of pieces together, in such a way that all of them can work harmoniously in a system.

Contrasting with the "productivist philosophy" in which each plant/crop is considered isolated "per se", the "integrant philosophy" embodies the reasoning that each plant/crop must be considered a piece of a system, in which it has to fit (integrate) in order to maintain and still improve that same system.

So, while the productivist model brought about, as consequence, the monocropping, the integrant approach is intended to serve the polycropping and intercropping. While in the first model the plant / crop is looked upon as the only species in the field plot and herbicides are required to eliminate the competitors, in the second what counts is the system, composed by several crops that share the same plot at the same time.

Another distinctive character between the two models is that while in the "productivist" the decision making belongs to the scientist (breeder), in the "integrant" the decision maker is the farmer himself. This is due to another differential requirement: while in the first case (productivist) scientists work out all the technological package at lab/station conditions, in the second case (integrant) almost all the scientific work is carried out at farmer's place and conditions.

So, because the scientist (breeder) is working at the "farmer's home", it must be allowed to the farmer the decision making power. The breeder is expected to study, understand and catch the farmer's intentions and priorities, and translate them into a scientific language and programme. Moreover, the breeder is supposed not to disrupt a sustainable system that, in most of the situations, is the result of a long way of farmer's investment and empirical knowledge concentrated into a system that reflects, in its diversity, the surviving diversity itself from which it came from.

Once this system had been understood and each of its "pieces" or components identified, the breeder is "invited" or "challenged" to implement, according to the farmer's priorities, each one of those "pieces" in order to harmoniously improve the system itself.

As a matter of fact, any time we approach these generally complex systems, we realise that the selection criteria are different. In fact, from a breeder's point of view and stepping into the former descriptive analysis, the integrant philosophy usually faces the following farmer's decreasing priorities: quality, system integrity, yield and pathology.

Quality

This first priority selection factor embraces not only grain but also stalk quality, that is to say, the quality of the entire plant.

Grain quality, as being considered the decisive factor, is intended to fit both food and feed requirements, since it will be essentially needed as a complement (sometimes a staple food) for human consumption and also for animals.

In maize, the grain quality is more related with the flint type, mainly white coloured (preferred for food) and also the yellow flints, more adequate for poultry. Finally, the grain storage conservation efficiency completes the meaning of quality.

Plant quality, is here meant in a sense that the entire plant but roots, is utilised for feed. First, as a forage supplement as the plants used to be detasseled after flowering. Second, because the straws are harvested and stored in vertical meadows to be used during winter as a feed supplement for cattle. So, the digestibility is also within the quality meaning, since farmers used to give selection preference to those stalks that cattle could eat completely. This means that the stiff stalks that favour a good lodging and pest tolerance are, in this case, a bit neglected.

System integrity

The agricultural system, more or less complex, is the result of a set of conditions or limitations faced by small farmers around the world where two seemingly antagonist factors coincide: high demography and shortness of arable land. This is, indeed, the real situation characteristic of many developing nations in parts of the world like Central Latin America, Africa and Asia, where a limited arable land is disputed by a crowded demography.

It is a matter of fact that, in a situation like this, farmers have always come up with the same solution: intercropping systems that occupy the soil twelve months a year, in small parcels of land from which they must collect all the possible food. It is also a matter of fact that, contrarily to what has been diffused, the global productivity of these polycropping systems is usually high, and that the generalised poorness so associated with these systems should be allowed more to the area and inputs limitations, than to the systems themselves.

For most of the small farmers in the world the maintenance and improvement of this kind of agriculture represents everything which they are dependent from. They are aware that the disruption of this self-sufficient system can jeopardise their food chain, and due to their financial limitations they cannot afford but little risks, like those resulting from an unbalanced control of the natural enemies of their crops.

Consequently, the way breeders are supposed to aboard a situation like this should be one that firstly respects the system, and secondly tries to implement each one of its components. In fact, the breeder's challenge will be to improve (breed) each crop plant that is a "piece" of the system, having in mind that all different "pieces" (plants) have to behave harmoniously, without disruptions, in a common sustainable togetherness. In other words, he needs to "breed the system".

Yield

In this context, the yield component - quantity - comes in third place priority, since this "yield" is only a part of the whole "system yield" that includes all the other crop components. Nevertheless, it is also a stressed goal, that generally is pursued by selection through two different angles: first, through the ear size and total kernel weight; second, by the plant ability to take advantage of low plant densities and transform that extra-light into increased yields.

In fact, as a consequence of the poly/intercropping systems, high plant densities are not allowed, in order to avoid too much shadow for the other crops. Consequently, plants that show a better ability to take care of more disposable sunlight - like prolific plants - are natural selection targets.

However, it should be noticed that selection for those better yielding plants, normally linked with longer vegetative cycles, is not allowed, because they could disrupt the system.

Pathology

Because this factor only comes in fourth priority order, no wonder that Portuguese maize open pollinated populations characteristic of these agricultural systems, usually show low levels of pest and disease tolerance. Consequently, this is a point to be looked upon as it represents not only a yield limitation, but also a potential for breeding implementation, since the starting point is generally so low.

These generalised low levels of pest and disease tolerance that are commonly found in the maize open pollinated populations, can be better understood having in account that:

- Because mechanisation has been a limitation in intercropping and small farmers are used to harvest their field plots by hand, all the plants, even the lodged ones, are collected. So, the lodging effect, in its direct contribution to lowering the yield is somehow overcome and the selection for it neglected.
- The lodging effect tends also to be masked by the generalised detasselling practice, since the shortened plants can better tolerate the wind effect and so mask some stalk weakness due to pest/disease damages.
- Because the stalk quality is primarily intended for a better animal appetency and digestibility, this selection criterion turned to be one that favoured the pests.
- Due to the fact that traditional selection was a limited mass selection only carried upon the ears, after harvesting, at the store facilities, this practice didn't allow farmers to select for stalk quality since, at selection time, the ears were already isolated from the stalks.

This explains why selection for pest tolerance was always poor.

Finally, considering these selection criteria, the maize plant ideotype that turns to better fit the integrant philosophy can be defined by these five preferences.

- Plant size: medium/high
  Two main reasons justify this preference for tall plants. First, because at a low level of the canopy there is a need to leave room and sunlight for the other crops of the system, like beans and forages. Second, because what is intended to harvest is the whole plant: grain for food and stalk for feed.
- Ear placement medium/high
  Contrarily of the low ear placement ideotype pursued by the productivist philosophy, in this case the medium/high ear placement is justified by two main reasons. First, because the negative correlation over the

lodging effect is somehow counterbalanced by the detasseling practice, which shortens the plant. Second, because after detasseling - always performed above the upper ear – a sufficient number of left over leaves is needed to complete the kernel filling until the harvest.

- A canopy that favours neither a low leaf-angle nor up-right leaves
  By the contrary, due to the low plant densities, the plant is allowed to expand its leaves in generous leaf angles.
- Prolificacy
  In order to take advantage of the low plant densities, prolific plants are always preferred in selection.
- Flint kernel types
  We assume that this generalised preference for the flint kernel type by small farmers, all over the world, engaged in these self-sufficient systems covered up by the integrant philosophy, represent a real quality preference based on solid empirical grounds.

Furthermore, since these systems belong to a sustainable type of agriculture, the plant must be able to grow under low input requirements. This requisite is needed not only because the local economy doesn't allow good amounts of fertilisers, but also because some extra nitrogen would have a negative interference over some other components of the system, like the leguminosae.

3.3 - THE BASIC DILEMMA

The two models - productivist *vs* integrant - in spite of being so widely differentiated in their conceptions and consequences, can be better visualised if we compare them through an inter-dependent "like-orbital model", similar to that of "sun-earth gravitational system" (Figures 1 and 2). For this characterisation we will use three differentiated parameters:

Profession of faith
This means the basic assumption, the main accepted truth or "scientific dogma", upon which everything else is built on and that really conditions all further essential decisions.

Decision centre

This concept tries to identify who is allowed to take the real decision making power, in the production process. In other words, this parameter will show where the philosophy is focused on, the relative positioning of science and farmers in the food chain, or the identification of the main actor in the agriculture theatrical representation.

Dynamic action

Within the understanding of an "orbital model", this parameter is supposed to make a contrasting comparison between centripetal and centrifuge forces and their consequences as potential multiplying effects. So, while centripetal forces are more related with influences coming from outside and imposed into the system, centrifuge forces are those that emanate from inside the system and normally expand to the outside. What really matters with this parameter is the answer to these questions:

- What kinds of forces are responsible for the multiplying effects?
- What is the basis of the extension: "pulling" or "pushing" forces?

So, following up this "orbital model", we will have this characterisation:

Productivist philosophy

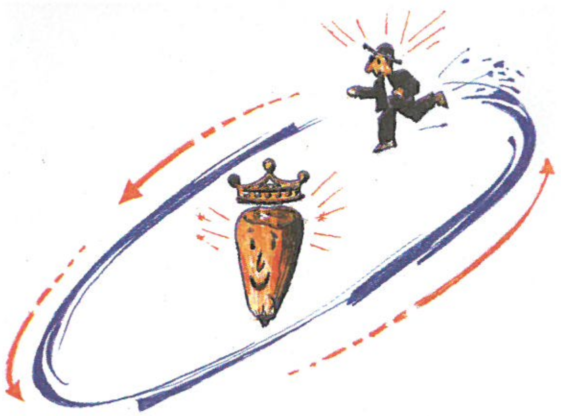

Figure 1 - Schematic representation, in an orbital like manner, of the forces and agents that characterise the productivist philosophy.

Profession of faith

Yield is not only the most important trait in selection, but also strong enough to push forward the whole production chain.

Decision centre

The decision power belongs to the scientist (breeder) who explores the genetic potential of the "seed" and builds up a technological package presented to the farmer in a "recipe" like manner.

Dynamic action

The dynamic forces are essentially of centripetal type, since the "technological package", that includes the improved seed, is "imposed" to the farmer from outside. In fact, that "package" is supposed to "push" the production system forward into the modernity.

Integrant philosophy

Figure 2 - Schematic representation, in an orbital like manner, of the forces and agents that characterise the integrant philosophy

Profession of faith

The farmer (the real available farmer), whenever integrated in an ecological system, behaves in a rational manner and takes rational decisions. So, instead of arguing about his low educational level or perhaps judging stupid some of his decisions, it will be advisable to credit him with rationality and try to understand his own reasoning.

Decision centre

The power of decision belongs to the farmer. The breeder is supposed to fit the farmer's criteria, propose improving alternatives, perform demonstrative comparisons and submit them to the farmer's decision.

Dynamic action

The dynamic forces are mostly of the centrifuge type. Consequently, the extension potential of this philosophical approach is supposed to be somehow automatic, since the farmer himself will be the most involved disseminating factor, in his quality of "decision maker" and "author" of any success.

In order to elaborate upon this contrasting philosophical pathway, it will be advisable to root our reasoning in some clear solid grounds, such as:

- Any production system, whatever it will be, always follows this threefold pathway: **energy**, **row materials** and **science.**

  - There are two main kinds of **energy** from which we have to choose: fossil and renewable.

  - For food and feed production, available genetic resources represent the **row materials**. Germplasm collection, conservation, characterisation, evaluation and enhancement are dramatic issues to an increasing world population. Moreover, the adaptation process performed by generations of world-wide farmers, either to their particular edapho-climatic conditions, or to the most common pests and diseases, should be considered a must that no plant breeder can neglect. Two main blocks of row materials to choose from are generally available: local adapted or exotic germplasm.

  - **Science** is always at the basis of any technological advancement. So, the choice of the most adequate breeding methodologies constitutes a key point for any philosophical approach. The adopted philosophy will determine not only the row materials (local *vs* exotic) to start from, but also the type of gene action (additive *vs* nonadditive) to favour. Ultimately, this combination of factors will end up in the option to be taken between tolerance and resistance.

Based on these common grounds we can now consider a more enlarged contrasting scenario between the two philosophical models, as it is given in Table 1.

Table 1 - Contrasting issues and/or consequences between the two philosophical models: **productivist** vs **integrant**

Contrasting Factors	**Philosophical model**	
	Productivist	**Integrant**
1. Profession of faith **2. Decisive centre** **3. Dynamic action**	Yield is the determinant factor The seed (breeder) Centripetal	Farmer's decisions are rational The farmer Centrifuge
4. Energy **5. Row materials** **6. Science** 6.1 - Gene action 6.2 - Breeding methods 6.3 - Pathology 6.4 - Technology	Fossil Exotic, inbreeds Non-additive (heterosis) Genealogical selection, (+) biotechnology Resistance (+) Mechanisation, (+) agrochemical, (-) manpower and monocropping	Renewable Local adapted, populations Mainly additive Recurrent selection, (-) biotechnology Tolerance (-) Mechanisation, (-) agrochemical, (+) manpower and polycropping (system)
7. Type of seed **8. Final output**	Hybrid, uniformity High yielding, quantity	Open-pollinated, diversity Moderate yielding, quality
9. Environmental effects 9.1 Protection level 9.2 Genetic resources 9.3 Farming continuity	 Soil, water and air pollution Erosion Leading to exhaustion	 Soil, water and air cleanness Conservation Sustainability

4 - UNIFORMITY or DIVERSITY ?

As it has been previously shown and summarised in Table 1, the option for the type of philosophy will determine a set of positive and negative consequences, which we have to face and balance. Nature is a living organism composed by ecological systems in which man plays a decisive role, either through a positive understanding of its rules and reactions, or through a negative action by disturbing the Nature equilibrium. Agriculture, as the most important linkage between man and Nature, is responsible for 95% of the food production for a rapidly increasing demographic population. This explains why it must be considered as a global sustainable system without which mankind will not survive. Moreover, this sustainability needs to have in account that the agriculture natural enemies have their own survival systems that we need to fight back without jeopardising our own survival.

After decades of worldwide generalised adoption of the productivist model, more than ever, it is necessary to stop and reason if this is the recommended model to pursue though the coming millennium. So far, it is possible to conclude that:

1. The productivist philosophy has been the predominant food-producing model, so successfully created and perfectioned by the American scientists that it was exported to both the developed and developing world as a "miracle recipe".

2. The integrant philosophy is a remnant survival model common in all places of the world where a high demography needs to share a limited piece of arable land. Essentially linked to severe social problems and generally associated with poorness, this philosophy has been neglected by any kind of science applications that could endeavour its own potentialities. Nevertheless, it still maintains some of its virtues: cleanness, sustainability and environmental friendliness.

3. The productivist model, being entirely dependent on the fossil energy, turned to be highly pollutant and makes poor countries, those without an easy access to oil, the most food vulnerable.

4. Because fossil energy, in the medium/short term, is limited, there is a need for an alternate energy in the food chain production. Renewable energy, which is at the basis of the integrant model, must be studied and implemented to serve the next generations of farmers.

5. Productivist philosophy, as it favours the use of narrow based genetic resources, has been contributing to a generalised genetic erosion and to uniform crops, grown in monocropping systems that always require big extensions of arable land.

6. By favouring the uniformity at the expenses of diversity, the productivist model is still implementing the uniformity through the new biotechnological procedures, which have an easier application on those already uniform materials.

7. Considering that credits should be due to the productivist philosophical approach for its success in the " Green Revolution ", it should also be pointed out some of its weaknesses, like: genetic erosion, soil, air and water pollution, as well as its complete dependency on fossil energy.

8. In what pests and diseases control is concerned, while the productivist model favours resistance, the integrant model favours tolerance.

9. Recent advancements in science have brought a new era in genetic resources evaluation and their potential use for an array of products that range from food production to medicine. These new scientific achievements that have shown up DNA as a common basis for all types of life, brought also into action the potential of transgeny as the most promisable tool to take advantage of diversity. In so being, diversity, both at the cultivated and at the wild germplasmic levels, has won extra credits that enable it a decisive factor for the future of humankind.

10. Having in account that both philosophical models agree on the emphasis to be given to diversity as the basis for further genetic improvements, it is clear, however, that it is the integrant approach that better favours and takes advantage of diversity.

However, one big question still remains: even accepting that the integrant philosophical approach is the one that better favours diversity, what about its performance and yield potential for food production? Does it have any possibility to compete with the productivist model in the food production chain that, besides quality, also desperately needs quantity?

In fact, there has been a generalised belief that there is no alternative for the productivist model, since the poor agriculture, more connected with the integrant model, has exhausted its yielding potential and must be considered obsolete for an increasing world-wide demand for food. In order to question that assertion and to test the potential of the integrant approach, we started in 1985 a pilot project of "on-farm maize breeding" at the Portuguese Sousa Valley (VASO) region.

5 – *VASO* - A TESTED MODEL for DIVERSITY and TOLERANCE

We are among those who foresee that the modern productivist model, even considering its past and present successful career, is somehow condemned to fail, at least partially, in the decades to come. In fact, its environmental aggressiveness, either directly through the pollution (soil, air and water) due to the heavy utilisation of agrochemical, or indirectly through its food toxicity, altogether with its fossil energy dependency, will slowly shorten its life span in the long run.

Consequently, an alternative model that could substitute the productivist philosophy is needed. The integrant philosophy model, as it aims the sustainability with a friendly environment, may constitute, at least partially, a valid alternative. And, in between the two opposite philosophical approaches, a large spectrum of "hybrid" or complementary solutions should be required for this transition into the new century agriculture, supposed to sustainable feed an increasing world population.

In order to test this alternative, we moved out to the headquarters of the local farmers' organisation Centro de Gestão Agrícola do Vale do Sousa (CGAVS) at Lousada. Since then, we have been testing the integrant model, side-by-side with some local farmers and conducting the breeding practices in their own field plots.

It should be said, that this initiative came along as a result of a joint multidisciplinary small team of scientists sharing a set of common basic ideas favouring a change in philosophic approach for agriculture. Our personal engagement in this project, was motivated by our professional experience with the Portuguese traditional intercropping and also as a FAO consultant for Africa and Asia, where the same common ground was detected: the failure of the developed world to understand and deal with the developing countries agriculture.

This explains why from the very beginning the VASO project was aimed at three main goals: it should be simple, cheap and exportable, having in mind its potential application to the Third World Agriculture, namely the Portuguese speaking African countries (PALOPs).

MATERIALS AND METHODS

The option for the integrant philosophical approach and the practical consequences of this decision can be better understood going back to the contrasting comparisons laid down at Table 1. From a breeder's point of view, three main decisions were required as a basic starting point: (1) the best location to represent the region, (2) the germplasm to start from and (3) the farmer to work with.

Location

The Sousa Valley region was chosen, having in account the following factors:

- Located within our traditional maize area – the Portuguese Northwest – characterised by polycropping systems, where maize still plays an important role.
- Among the Northwest areas, Sousa Valley was one among the richest, with fertile flat soils and tender slopes going up to medium altitude hills covered by a forest of pine trees. These good agricultural conditions explain why, at the time (1985), 20-25% of its soils were planted with hybrids, while the national average was only 15%. This means that in a region where the national maize production champion (18 tones/ha with a single-cross hybrid) was located, still 75-80% of the maize area was grown with regional open pollinated varieties.
- The availability of a basic amount of agro/sociologic/economics data, previously collected by some members of the original multidisciplinary

team, allowing the breeder with a like "X rays" of the region.
- The support of a local elite farmers' association (CGAVS) which accepted to be a part of the project.
- The possibility (challenge!) to test the efficiency of an alternative project supposed to improve the local germplasm in order to be competitive, at least in certain specific circumstances, side by side with the local hybrids production.

Germplasm

As a pre-requisite of the "integrant" option, as it can be seen in Table 1, the raw genetic material was local adapted germplasm. This was made to respect the farmers' selection pursued along the last four centuries and also assure the already obtained environmental adaptation either for the soil/climate and quality preferences.

In order to fulfil this requirement, the Summer time of 1984 was spent in a survey, jumping from one plot to the other along the Sousa Valley region, looking for the best open pollinated varieties in the field. This survey allowed us to make a reasonable choice of the germplasm to start from.

Two land races, one an early maturity (FAO 200) yellow flint adapted to stress conditions (Al toxicity and water limitations) and other a medium maturity (FAO 300) white flint with a strong fasciation expression, were finally chosen.

Farmer

Choosing the right people to work with is also a major decision in a project like this, which is supposed to be worked out side by side with the farmer himself, to whom the decision power will be allowed. Here again the initial personal contacts and the information collected by the scientific team were decisive for the choice.

Two farmers, each of them representing one of the two chosen land races, became engaged. Their initial acceptance and enthusiasm to join the project turned to be the best guaranty of success along this project.

So, with a careful respect for the local traditional agriculture, a kind of a deal was made with the involved farmers: while ourselves would proceed according to our chosen breeding methodologies, they would continue a parallel programme with their own mass selection criteria.

Out of this agreement between breeder and farmer, three consequences became clear:

- Respecting the "system" would imply to accept its low input and intercropping characteristics, as well as accepting and respecting the local farmer as the decision maker himself.
- With two simultaneous breeding programmes – the farmer's and the breeder's – the farmer would have a permanent possibility to compare the effectiveness of both. This would allow the farmer to base his decisions on solid grounds.
- By choosing the farmer's local adapted germplasm as the genetic raw material to start from, the breeding approach turned to favour an option for diversity and the first priority trait – quality – was automatically safeguarded.

Tolerance *vs* resistance

In what pests and diseases are concerned, since a clear option for diversity was taken, it comes out that a parallel option for tolerance over resistance is implicit.

Two main pests are common in the Sousa Valley region: *Sesamia nonagrioides* (Hub.) and *Ostrinia nubilalis* (Lef.). Besides these two pests which can be considered of economic importance, some damage can also occur, in certain years, due to *Agrotis segetum* during the early stages of crop development.

In what *Agrotis segetum* is concerned, a curious biological pest control methodology was found in the region, coming out from the farmers' empirical knowledge.

Due to the pest biology, the larvae, which are located and hidden in the first layer of the soil, take advantage of the night moisture to reach the surface and feed on the young corn plants. With such nightish habits, when the sun rises and starts drying the soil surface, the larvae go back again to the soil, hiding themselves under the superficial layer.

The pest control methodology consists in irrigating the cornfield immediately after the first young plants are found cut down. With this practice, the soil superficial layer becomes moistful even during the day, so inviting the larvae to stay longer at the surface, exposing them to sunlight and so allowing birds to execute an efficient cleaning of the field!

Breeding methodologies

The breeding approach to cover up both the yield component and the two main pests – *Sesamia and Ostrinia* – has been conducted in quantitative genetics way through population improvement selection, combining three main recurrent selection methodologies: phenotypic, S_1 and S_2 lines

Phenotypic recurrent selection

This methodology – a two parents control mass selection - has been the breeding tool used by the farmer, who has been advised to carry it out under a three steps sequence (A-B-C). The first two steps (A and B) in the field and the third one (C) at the store. The sequence follows this pattern:

A - Immediately before the pollen shedding, the selection is performed over the male parent by detasseling all the undesirable plants. These, would be not only the weakest and all that do not fit the desired ideotype, but essentially all the pest and disease susceptible looking plants, like those that show up evident symptoms of *Sesamia* and *Ostrinia* attacks.

B - Some days before the harvest, besides selecting for the best ear size, the plants are foot kicked at their base (first visible internodes) to evaluate both their root and stalk quality. And, as an indirect measurement, the pest and disease tolerance can also be evaluated. In practical terms, if the plant doesn't resist to the impact and breaks down, it is eliminated. So, since the *Ostrinia* and *Sesamia* attacks cause a weakness in stalk and ear quality, this practical selection methodology allows a progressive reduction in the population lodging mean, so leading to the improvement of its tolerance level. Moreover, a special preference in selection is given to prolific plants.

C - At the store, after harvest. Here again, selection always includes, besides ear length and kernel row number, the elimination of all damaged ears.

Therefore, the same pursued goal of improving the population mean of healthy plants is attempted.

S_1 and S_2 lines recurrent selections

These methodologies that we have been using in this project – S_1 lines for the AMIUDO and S_2 lines for the PIGARRO land races – both contemplate the **pathology** trait following up the same indirect pattern that leads to favouring tolerant genotypes. First, when selecting the plants to be selfed. Second, at harvest time, when these selfed plants are foot kicked before collecting the ears. Third, at the yield trial season, when the counting of root and stalk lodged plants are recorded for statistical analysis.

For the **yield** polygenic trait, the S_2 lines recurrent selection was initially preferred due to its good taking care of the additive component of the genetic variance ($3/2\ \sigma_a^2$). However, the S_1 lines methodology was applied to the land race AMIUDO because of its heavy inbreeding depression revealed after the first selfing generations. On the other hand, an entirely different situation was found to occur with PIGARRO which showed up, along the selfing generations, a surprisingly low inbreeding depression, so allowing a better fit for S_2 lines methodology application.

In this last case – S_2 lines – a variable number of plants, close to 1000 S_1 lines and around 500-600 S_2 lines, were obtained. Out of these, the best 200 S_2's were selected for yield testing, where a 15-20% selection pressure was applied and a final set of 30-35 elite S_2's were selected for the recombination season in order to form the first cycle seed (C_1).

FIRST RESULTS

While the VASO project was initiated in 1985 and still continues nowadays with the same original land races, we will present here only some data referring to the first cycle (C_1) through S_2 lines recurrent selection of the white flint land race - PIGARRO. The reason for that is our attempt to present some evidence suggesting that it is possible, to question that generalised idea which says that population improvement methodologies that favour diversity and tolerance are not able to fit the yield competitive requirements imposed by the hybrid seed industry.

In order to frame a joint analysis of the yield and pathology traits and how they have been balanced, some basic assumptions upon which this philosophical approach has been based should be pointed out:

- Among the four most important selection factors required by the integrant approach – quality, system integrity, yield and pathology – the first two were automatically safeguarded by our options: local adapted germplasm (quality) and all the breeding procedures realised at farmer's place and conditions (system integrity). So, our next concern was to manage the last two factors in such a way that to the quantitative trait yield was always given priority over pathology.

- PIGARRO is a curious open pollinated land race in what it presents, besides a high plant size and high ear placement, a high level of ear fasciation. These characteristics turned to be somehow contradictory with the pathology evaluation, because any gains in ear size had a tendency to increase the lodging effect and so buffering any real gain in pest tolerance. For the first increases in yield, an apparent decrease in pest tolerance should be expected, due to a heavier ear that could jeopardise a precarious equilibrium between the plant gravity centre and its stalk and root stability. Consequently, the pathological trait, as expressed by its tolerance levels, was considered a medium/long goal to be pursued and achieved along the breeding process.

- Since the detasseling after flowering was a farmer's common practice, this technology turned to moderate the lodging effect at harvest and mask the pest tolerance. However, while at the selfing stages (S_1 and S_2 lines) all the plants suffered the detasseling, at the yield testing stage (S_2 lines in yield trial tests) the plants were not detasseled. Consequently, the stalk and root lodging counting is referred to the entire plant, so allowing a more rigorous and unbiased reading of pest tolerance levels.

Having in account the above-mentioned assumptions, we are now in position to analyse our first data in which an attempt to compare the first C_0's from phenotypic recurrent selection with the first cycle (C_1) from S_2 lines recurrent selection, is made.

S_2 lines

To test for gain in **yield** after the first cycle (C_1) of S_2 lines methodology, a Latin Square yield trial was carried out. We used 70 plants per ten square meters plots (70.000 pl/ha), an organic matter fertilisation of 8 tones/ha and no agrochemical other than 70 nitrogen units/ha. This trial was implanted at the farmer's normal conditions, in an intercropping with beans (*Phaseolus vulgaris* along the same row of maize) and forage (*Lolium multiflorum* L.) sown at the maize plant 10 leaves stage.

Four entries were included: the initial population ($C_0$84), two sets of improved seed through phenotypic recurrent selection ($C_0$86 and $C_0$90) and our first cycle (C_1) from S_2 lines recurrent selection. With this choice it was intended to compare not only the progress being made through the breeder's methodology (C_1 *vs* $C_0$84), but also the farmer's methodology ($C_0$86 and $C_0$90 *vs* $C_0$84).

Our data show (Table 2) that a significant difference in yield (5%) was obtained among the populations.

Table 2 – Analysis of variance from a yield trial carried out at the Portuguese Sousa Valley, comparing the first cycle (C_1) of S_2 lines recurrent selection with $C_0$84, $C_0$86 and $C_0$90 of the local land race PIGARRO.

S.V.	D.F.	M.S.	F
Populations	3	1193195.9	6.977*
Rows	3	588415.0	3.440
Columns	3	1546732.6	9.044*
Error	6	171007.1	
Total	15	734071.6	
Coefficient of variation (CV)			5.6%
Least Significative Difference (LSD)			716.3 Kg/ha
* Significance at the 5% level			

As it can be seen in Figure 3, it was found that a substantial increase (1.2 t/ha) was obtained between $C_0$84 (7.0 t/ha) and C_1 (8.2 t/ha), corresponding to a 17% gain in yield. Moreover, significant differences were also obtained between C_1 and both $C_0$86 and $C_0$90 (LSD=0.7 t/ha), but no significant differences among the different C_0's.

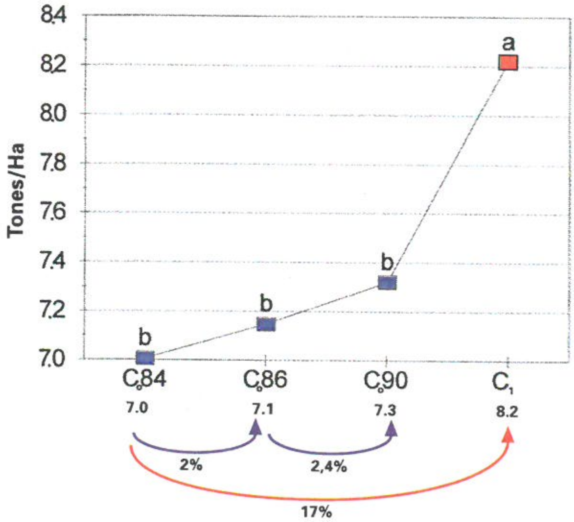

Figure 3 – Comparative gains in yield after the first cycle (C_1) of S_2 lines recurrent selection, compared with the outputs of phenotypic recurrent selection, simultaneously applied to the same land race PIGARRO.

When comparing the **pathology** trait performance shown in Figure 4, and having in account all that was already discussed about the pursued balanced criteria between yield and pathology, our data suggest:

Stalk quality, as the stalk lodging counting represents it, was not apparently affected by the S_2 lines methodology (C_1 similar to $C_0$84). Furthermore, since the pest tolerance against our two main enemies

(*Sesamia an*d *Ostrinia*) is mostly materialised in the stalk lodging effect, this suggests that our described methodology for pest tolerance selection seems to respond with a reasonable performance.

Root quality, as the root lodging counting represents it, revealed a tendency to decrease from $C_0$84 to C_1. However, this apparent negative results should be more allowed to the ear size increase, connected with the yield implementation, rather than to a real decrease in the root quality itself.

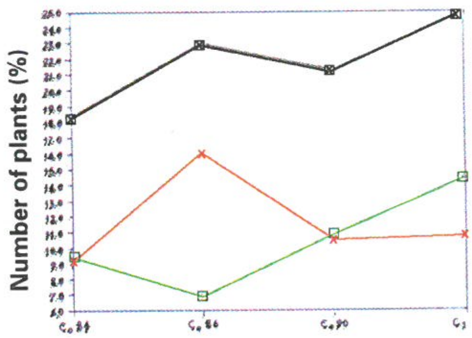

Figure 4 – Compared evolution of the pest tolerance levels, partially estimated through the lodging effects, after the first cycle (C_1) of S_2 lines and six cycles ($C_0$84-$C_0$90) of phenotypic recurrent selection of the land race PIGARRO.

Root lodging ☐
Stalk lodging ×
Total root and stalk lodging ☒

Summing up both stalk and root lodged plants of C_1; we arrive to a situation where we face a challenge to better combine further yield implementations with root and stalk qualities of this population. This means that a special attention should be paid to the root quality trait if we want our plants to support an heavier ear that is likely supposed to be achieved through the yield trait improvement.

Phenotypic recurrent

In what the parallel phenotypic recurrent methodology is concerned (farmer's approach), the selection evolution from 1985 to 1990 seems to suggest:

In **yield** improvement (Figure 3), as it was already mentioned, no significant differences were obtained. Nevertheless, it seems that a slow, but positive, tendency in on the way (2% between $C_0$86-$C_0$84 and 2.4% between $C_0$90-$C_0$86). As a curious anthropological appointment about the farmer's reaction to the contrasting results obtained by the two methodologies, he only showed a moderate optimism, still maintaining his own reserves about the breeder's capabilities and attributing the S_2 lines advantage to some imponderable climatic factors. His reaction was one that he was expecting to reach the same results with his mass selection approach!

In **plant quality and pest tolerance control** (Figure 4) the farmer has got a kind of contradictory results with root and stalk lodgings, between the first (84-86) and the second (86-90) periods.

This circumstance brings to light a real situation of communication and acceptance between farmer and breeder. In fact, what happened was that, in the beginning of our relationship, the farmer was not completely open minded to accept a simple recommendation like foot kicking the plants for a direct root and stalk quality and an indirect pest tolerance selections. Only after seeing by him the bad results ($C_0$86) in stalk lodging, he accepted to change his mind and follow the breeder's suggestion.

The performance of $C_0$90 already shows the effect of that change and turns to be a good example about mutual conquering of confidence, a goal that breeders need to accomplish all along a progressive relationship with the farmer himself.

This same situation also shows who really is the decision-making agent!

6 - DISCUSSION AND CONCLUSIONS

Contrasting with the polycropping, the EU monocropping productivist model that was imposed through its CAP, didn't fit the Portuguese traditional maize area located at the Northwest mountainous regions, where family based small sustainable farming used to be predominant.

Such a transformation that was responsible for a reduction in the agriculture population from 20-30% to less than 10% within the last twenty years, brought about both a human and a genetic resources erosion with an inherent environmental disruption and all kinds of social and anthropological consequences.

On the edge of this recent transformation, a pilot project of on-farm maize breeding (VASO) was installed aiming to test the social and economic viability of a different and alternative philosophy for the productivist model.

Assuming that any agricultural policy roots upon a basic philosophy and that plant breeding also needs to be led by a consequent philosophical approach, the VASO project suggests that the "integrant model" can be considered an alternative within a quality based sustainable agriculture conception.

Our data show that, besides safeguarding the quality priority and the system integrity, the yield component was not neglected. In fact, our results support the validity of population improvement, providing evidence for a 17% gain in yield after the first cycle (C_1) of S_2 lines recurrent selection.

It should be noticed that the yield mean was obtained with only 70 nitrogen units/ha of chemical fertilisation, after a pre-ploughing organic matter application of 8 tones/ha. Moreover, that yield was achieved in intercropping with beans and forage, whose values should be added at the maize yield mean itself. In summary, data show that a reasonable mean yield (8.2 t/ha) was attained for a FAO 300 maturity open pollinated population, grown in polycropping.

Data also support our good choice of the local adapted land race PIGARRO as the basic germplasm. Its revealed low inbreeding depression, together with its high fasciation levels, turned to be positive, if not determinant, factors for yield enhancement. In fact, fasciation expression was a useful potential for kernel row implementation at which the yield itself shouldn't be strange.

Diversity, as the foundation for population improvement, constituted the basis for a pest tolerance breeding approach. Our results suggest that the pest tolerance levels, indirectly measured by stalk and root lodging counting, were not improved, but also not significantly lowered, after this first breeding period (C_1). Results suggest that some confounding effects coming out from an ear size implementation, should be responsible for a small increase in root lodgings. Nevertheless, even being considered a secondary priority after yield, pest tolerance should be a trait to deserve further attention in the coming cycles.

The S_2 lines methodology, when compared with the phenotypic recurrent selection, proved to be superior, responding with significant outputs regarding the yield trait. On the other hand, phenotypic recurrent methodology, in spite of showing some apparent positive tendencies, showed no significant improvements in yield when the different C_0's were compared.

Our data suggest that the polycropping agricultural systems, in spite of having been neglected and, in a certain manner, even combated by the CAP, still maintain their potentialities, which adequate breeding methodologies can take advantage of.

Our first results also support the idea that the integrant philosophy can be looked upon as an alternative to the productivist model, especially fitted for small farming predominant in crowded areas of the globe. Furthermore, as it appeals to the farmer's involvement and favours diversity, tolerance and sustainability, it seems to better defend and preserve the environmental equilibrium.

Finally, it should be stressed that no scientific fundamentalism is defended by the authors' approach. In fact, it is our conviction that both philosophies have their specific niches of application for many years to come, and that some "hybrid" philosophic adaptations will be preferred in certain situations. Nevertheless, it should also be clear that we have the feeling that, as the integrant approach should be raised, the productivist approach should be lowered, in a progressive balanced manner that in the long run, sustainability, quality and environmental protection can be guaranteed.

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