EVALUATION OF SOME SUNFLOWER GENOTYPES CONCERNING THE REACTION TO *ALTERNARIA* SPP. PATHOGEN

Steluþa RARANCIUC, Maria JOIÞA PĂCUREANU *)

ABSTRACT

***Alternaria* spp. is the pathogen which produces the brown spot of sunflower leaves, stems and calathidium. The paper presents the *Alternaria* species identified on sunflower in different vegetation stages. The results regarding the behaviour of an assortment of sunflower hybrids and lines to artificial inoculation with *Alternaria* spp. in three vegetation stages, are also emphasized. The tested genotypes had a different behaviour to *Alternaria* spp. attack depending on the phenological stage in which the parasite/host plant impact, took place. A high plant sensitivity was registered under artificial inoculation in cotyledons stage, after that, this sensitivity has diminished at the same time with the leaves appearance, followed by a new increasing when the artificial inoculation was performed during postflowering. The most resisting genotypes proved to be: LC-985, Performer, Select, LC-1029, LC-1093, during postflowering, the most important economical stage of disease.**

Key words: *Alternaria* spp., brown spot, sunflower genotypes.

INTRODUCTION

In Romania, the *Alternaria* brown spot on sunflower is produced by three species: *Alternaria helianthi (*Hansf.), *Alternaria zinniae (*Pape) and *Alternaria alternata (*Fr.). The *Alternaria* species can affect the sunflower plants during the emergence, leaves, stems and calathidium stages (Ciurea et al., 1983). The *Alternaria helianthi* and *Alternaria zinniae* attack the leaves, stems and calathidium in the shape of dark brown spots, surrounded by an yellow halo. The spots produced by *Alternaria zinniae* are smaller and evolve into black lesions. On stem, *Alternaria zinniae* also produces spots in the shape of brown to black stripes, which form large necrotic areas. *Alternaria alternata* is frequent in sunflower crops, being generally saprophyte on vegetable residues (Lamarque, 1987).

The first paper about the reaction of sunflower genotypes to *Alternaria* spp. attack was made by Islam et al. (1976), when a great variability of genotypes as regards the resistance or sensitivity trait, was emphasized.

In accordance with the research performed by Block (1992) and Skoric (1992), an absence of genetical resistance vs. *Alternaria* spp. in the cultivated sunflower was reported. Variations of tolerance or sensitivity were also noticed by Agrawat et al. (1979), Morris et al. (1983), Islam and Maric (1983), Lipps and Herr (1986). A high resistance level was reported by Mirza and Hoes (1996) at Suncross 25–3 hybrid.

Kong et al. (1977) studied the components of sunflower quantitative resistance to *Alternaria helianthi*. The authors suggest that the lesions size determined after 7–9 days from the plants inoculation can be used in selection for resistance to *Alternaria helianthi* under greenhouse conditions.

A relationship between the reactions to natural inoculation with *Phomopsis helianthi* and *Alternaria* spp. was observed by Iliescu et al. (1985) who showed the good behaviour of Fundulea 59, Felix, Select and Super hybrids to the attack of both pathogens.

In 1988, Skoric reported the fact that the NS-H-43, NS-H-44 and NS-H-45 hybrids, very resistant to *Phomopsis helianthi*, were resistant to *Alternaria helianthi* too, fact that could indicate that the resistance to these pathogens is controlled by linked genes.

Based on the estimation of some sunflower hybrids reaction to artificial inoculation with *A. zinniae*, Iliescu et al. (1990) underlined that these hybrids have a different behaviour, depending on the genetical nature and phenological stage in which the parasite/host plant impact takes place. The authors consider that the different sunflower genotype reaction, supposes the existance of some resistance genes and subsequently the possibility of some breeding works in sunflower resistance to *Alternaria* brown spot.

MATERIALS AND METHODS

*) Agricultural Research and Development Institute (A.R.D.I.), 915200 Fundulea, CalarasiCounty, Romania

In order to identify the *Alternaria* species involved in sunflower brown spot complex, samples of infected leaves, stems, capitula in different vegetation stages were collected and subsequently microscopically analysed and then, the pathogen fungus was isolated.

In order to estimate the resistance or sensitivity degree to the attack of this pathogen, 14 sunflower hybrids and 12 parental lines, 6 female and 6 male, were tested.

The testing of sunflower resistance to *Alternaria* spp. in field was performed by the inoculation with suspension of conidia cultivated on GCA (Masirevic, 1995). The mycelium and conidia suspension proceeded from the fungus cultures on GCA medium, 7–9 days aged, was filtered for removing the medium particles. The tested plants were sprinkled three seconds with conidia suspension, at 5–7 cm distance from the leaf area. The plants were covered with plastic sheet for 48 hours.

The calculation of the attack degree for each variant was done after the estimation of the attack intensity on leaves and calathidium of each plant. For scoring up the attack on leaves and calathidium, the noting scale 0–9 was used, taking into account that an attack percentage of the leaf and calathidium area coresponds to each note.

The artificial inoculation was performed in three different vegetation stages: cotyledons, 4–6 pairs of leaves and the end of flowering.

The *Phomopsis helianthi* attack was also noted under natural infection conditions.

RESULTS AND DISCUSSION

After the microscopic examination, the isolation and cultivation of collected samples, the parasitic species: *Alternaria helianthi* and *Alternaria zinniae* were found in different proportions, as well as the saprophyte species *Alternaria alternata*. The results presented in table 1 show that the *Alternaria helianthi* species is dominant beginning with the 4–6 pairs of leaves stage (identified on leaves) till maturation stage (identified on all sunflower organs). The *Alternaria zinniae* species appears beginning with flowering stage, having a greater incidence during the maturation, on leaves and stems.

The *Alternaria alternata* species was identified on all plant organs, as saprophyte, in whole maturation stage, being also present in the existing mycoflora on sunflower seeds.

In order to evaluate the reaction of different genotypes (hybrids and their parental forms) under both natural and artificial inoculation conditions, in different vegetation stages and during two years, 14 hybrids and 12 sunflower lines were studied. Figure 1 presents a high plant sensitivity to the pathogen attack under artificial inoculation conditions, in cotyledons stage. The attack frequency ranged between 40.6 (Justin) and 90.3 (Super). Generally, the parental forms of hybrids presented a higher attack level in comparison with hybrids, in this stage of plant development. Among the female lines, the highest attack level was registered in the LC-1004A and LC-1019A lines, while, among the most utilized as male lines in commercial hybrids, the highest attack level was registered in LC-1095C, LC-1103C and LC-1085C lines.

When the artificial inoculation has been performed in the stage of 4–6 pairs of leaves, the plants manifested attack symptoms only on basal leaves, the attack degree on plant ranging between 5.8% (LC-985) and 35.1% (Minunea).

In the case of artificial inoculation performed at the end of flowering stage, the genotypes have been significantly differentiated with attack degree values between 5.9% (LC-1093A) - 80.1% (Romina).

The best behaviour to the pathogen attack

Table 1. Proportion of *Alternaria helianthi* and *Alternaria zinniae* species on sunflower vegetative organs in different vegetation stages, 2000

Attacked vegetative organ	4–6 pairs of leaves		Flowering		Maturation	
	A. helianthi (%)	*A. zinniae* (%)	*A. helianthi* (%)	*A. zinniae* (%)	*A. helianthi* (%)	*A. zinniae* (%)
Leaves	100	0	60	40	12	88
Stems	-	-	46	54	20	80
Calathidia	-	-	92	8	73	27

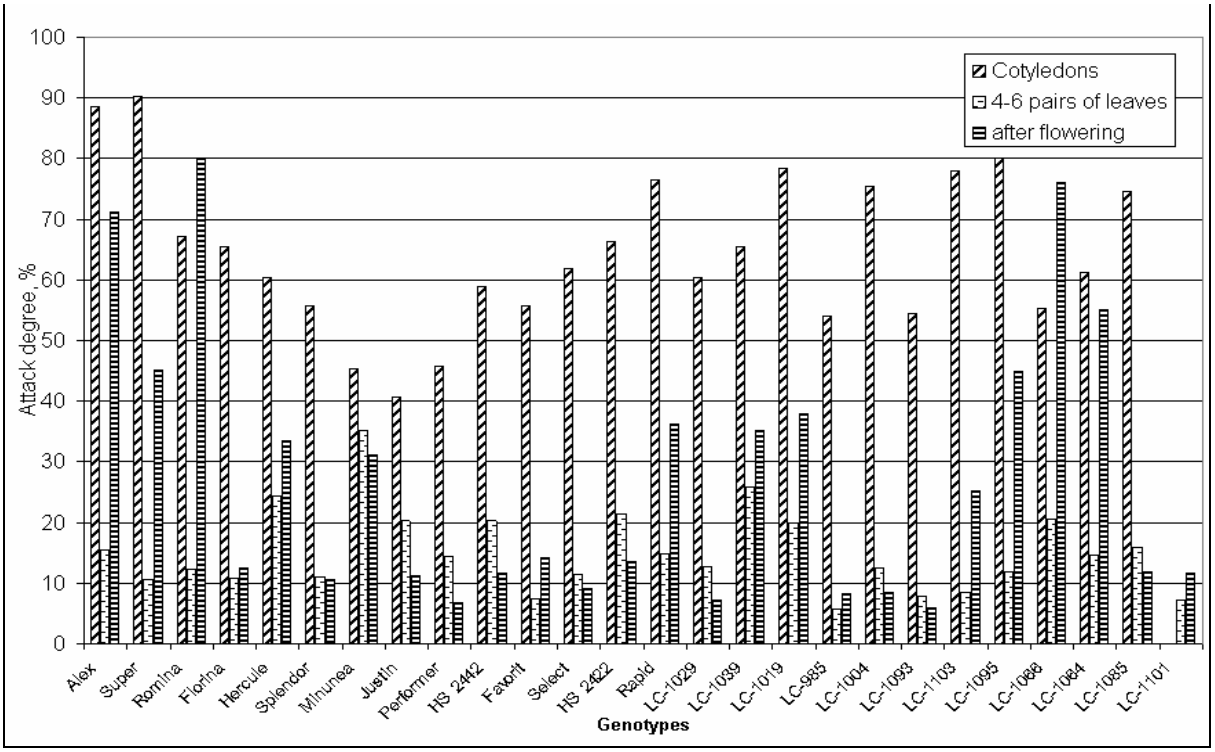

Figure 1. Attack of *Alternaria* spp. pathogen under artificial inoculation conditions in the three different sunflower vegetation stages

(attack degree below 10%) was registered by Performer, Select, LC-1029, LC-1004, LC-985, LC-1093 genotypes, the rest of them being medium resistant to susceptible (Figure 1). The results of inoculations in the three sunflower vegetation stages reveal a high plant sensitivity to inoculation in cotyledons stage, then this sensitivity has diminished at the same time with the appearance of true leaves, followed by an increase, when the artificial inoculation was performed during postflower stage.

The obtained results emphasized the fact that the appearance of the first symptoms of brown spot by artificial inoculation is positively correlated with the infection produced on natural way (Figure 2), at the investigated genotypes. The value of correlation coefficient is very significant: 0.82.

Observing the attack incidence of *Alternaria* spp. and *Phomopsis helianthi* under natural infection, on the investigated hybrids (Figure 3), a different reaction of them to the inoculation with these two pathogens, during postflowering, was noticed, moment in which the attack has a significant negative influence from the economical viewpoint on sunflower productivity elements (Allen et al., 1985; Chattopadhyay, 1999). Florina, Performer, HS 2442, Select, Favorit hybrids and LC-985, LC-1093, LC-1085 and LC-1109 lines had the best behaviour to the attack of these two pathogens.

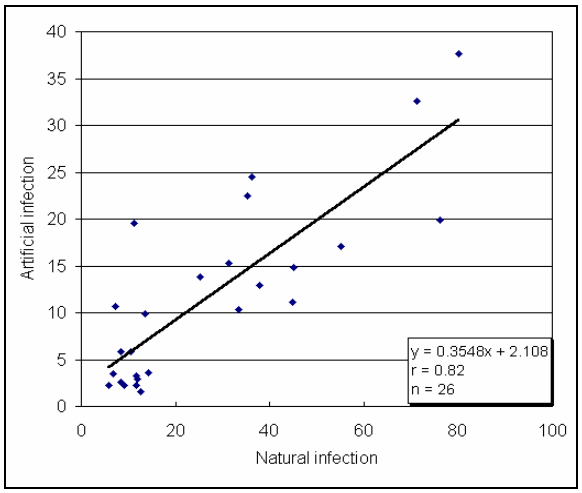

Figure 2. Linear regression for infection degree with *Alternaria* spp. under natural infection conditions and infection degree under artificial inoculation conditions, sunflower genotypes

A positive correlation between the reaction of genotypes to the attack of *Alternaria* spp. and *Phomopsis helianthi* under natural infection con-

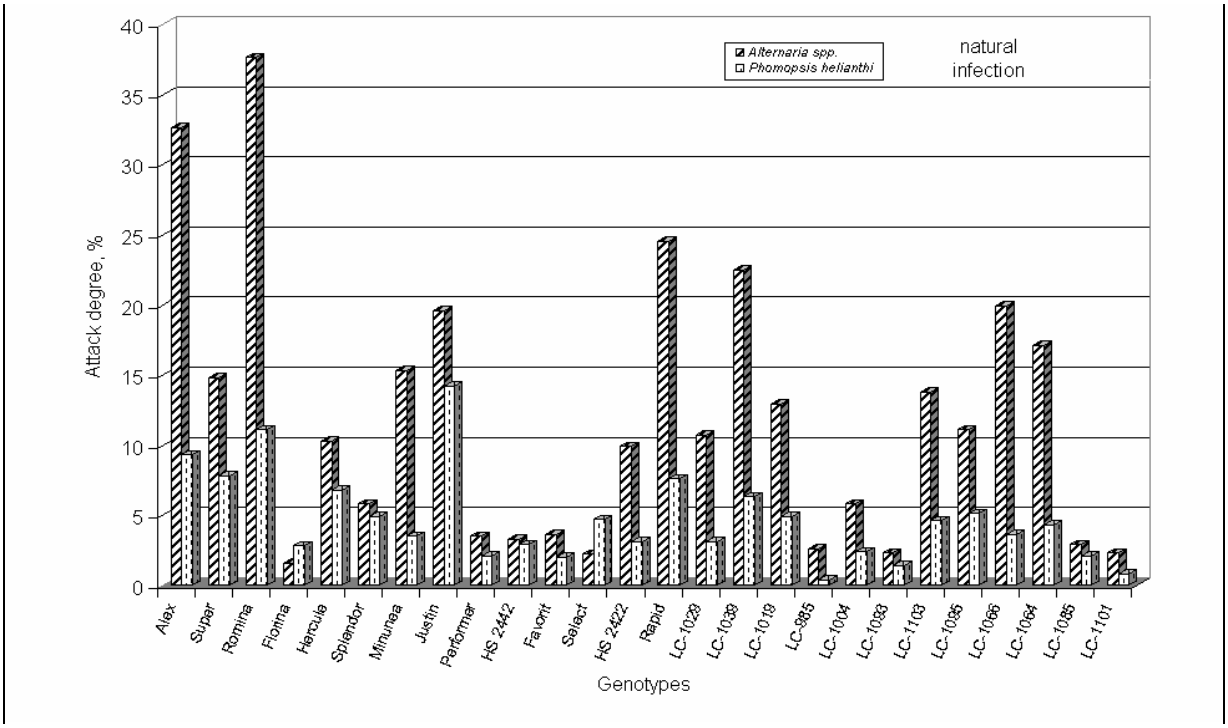

Figure 3 . Attack of *Alternaria* spp. and *Phomopsis helianti* on the investigated sunflower genotypes

ditions has been noticed (Figure 4). The hypothesis from the special literature regarding a possible linkage of genes which control the resistance to these two diseases, was confirmed.

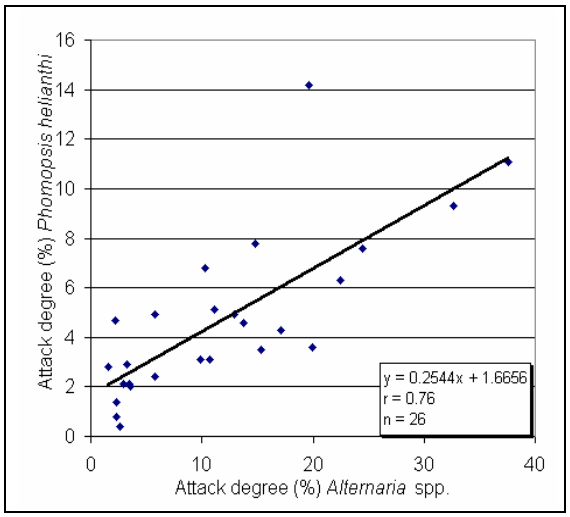

Figure 4. Linear regression for the attack degree whith *Alternaria* spp. and *Phomopsis helianthi* under natural infection conditions at different sunflower genotypes

CONCLUSIONS

In Romania, at sunflower crop, three species of *Alternaria: Alternaria helianthi, Alternaria zinniae* and *Alternaria alternata* were isolated. Among them, *Alternaria helianthi* is the dominant species in all sunflower vegetation stages.

The tested genotypes had a different behaviour vs. *Alternaria* spp. attack, depending on the phenological stage in which the parasite/host plant impact took place. A high plant sensitivity was registered under artificial inoculation in cotyledons stage, after that, this sensitivity has diminished at the same time with the leaves appearance, followed then by a new growth when the artificial inoculation was performed during postflowering.

The most resistant genotypes proved to be: LC-985, Performer, Select, LC-1029, LC-1093, during postflowering stage of sunflower.

The results demonstrated a tight correlation between the manifestation of *Alternaria* spp. attack under both natural and artificial infection conditions.

There is a positive correlation between the reaction of genotypes to *Alternaria* spp. and *Phomopsis helianthi* attack under natural infection.

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Table 2. Testing of restoration ability of *cms*-C type male sterility at some inbred lines at A.R.D.S Turda, during 1995-2001.

	Male-sterility maintenors (*rfrf*)		Fertility trstores(*Rf*)	
Check lines *cms*	Group I	Group a II-a A	Group a V-a	Group a IV-a
W633 *cms*-C	K2051, K2057B,		TC109A, TC221, TB364,	
TC208 *cms*-C	TC184, TE235, Lv92, Lv94, Lv113, Lv1700, TC317, TC371, TB368, TB363, TC316, A665, TC333, TC382		TD235, TD218, TB369, TC322, TC314, TC335, TC331, TC327, TC328, K1077, K1042, K1080, P131	
TA 367cms-C	TD106, TD221, TE233, TD269, TC344, TD359, TA417	PT9704 ,TD301, TD344	TD232, TD236, TD237, TD241, TE208, TE220, TB346, TC330, TC331, TD330, TD336, TD350, TD351, TD352, TD355, TD356, TD357, TD358, TA436, K3159B,	TD238, A439, TA435
TC243cms-C	TD268, TE201, TE210, TC318, TC365, TC383, TC384, TC385, TC391, TC395, TC399, TD341, TD347, TA425, TA432, TA438, F426, MV463, P951, K18018, K2148C, K2443B, K3056A, NF1042, NF1109, NF2028,	TD298, TE223, TD320, TA419, TA431, K1801B, NF1032	TD287, TD263, TD279, TD289, TD296, TD297, TD299, TE203, TE206, TE213, TE221, TE226, TE227, TE228, TC339, TC357, TC360, TC361, TC362, TC364, TC368, TC373, TC379, TD329, TD335, TD342, TD345, TD353, TD354, TA430, TA431, TA433, TA437, TA438, TA441, F1218, F1188/89, F858/89, K1653, K1795, K1796, K1800B, K1802A, K1805C, K1511, K2188, K2227, K3041A, K3046B, NF1005, NF1041, NF1090, NF1091, NF1092, NF1093, NF1098, NF1111,	TD278, TE202, TD369, NF1103, TD320
TC184 *cms*-C	TD283, TD284, TC396, TC397, TC398, TD302, TD303, TD304, TD305, TD346, TA426, TA427,	TD301, TD344, TD348, TA424 PT9704	TD290, TE217, TC386, TD336, TD346, TD359, TA435, F40/88, PT9707, PT9708, PT9716, PT9717, K3043, K3044, NF1014	

	F43/93, F768/82, F1870/86, PT9712, PT9714, K2174			
Proportion	**34%** (67 / 198)	**7**%(15/193)	**55**% (108 / 198)	**4**%(8/198)

Table 3. Testing of restoration ability of *cms*-ES type male sterility at some inbred lines at A.R.D.S Turda, during 1995-2001.

Check lines *cms*	Male-sterility maintenors (*rfrf*)		Fertility trstores(*Rf*)	
	Group I	Group a II-a A	Group I	Group a II-a A
TC208 *cms*-ES	TC184, T248B, TC243, TD242, TB367, TB368, TC316, TC317, TC371		TC218, T241, TB369, TC314, TC322, TC328, TC335, K1042, K1077, K1080, P131, W401	TC 327
TC243 *cms*-ES	TE201, TC383, TC384, TC385, NF 2028	TD298, NF1032, NF1109	TD278, TD296, TD297, TD298, TD299, TE202, TE203, TE206, TC330, TC378, TD361, NF1005, NF1041, NF1090, NF1091, NF1092, NF1093, NF1098, NF1111	TD369, NF1103
TA367 *cms*-ES	TD106, TE223, TE222, TE225, TE 235,TD341, TD346, TD 348, TA432, TA438, WN7, F426/88, F1153/89, F43/93, Lv 86, Lv 92, Lv 94, Lv 113, Lv 1700	TD320, TD348, TA439, F768/82, PT9714,	TC241, TD263, TE208, TE217, TE220, TE221, TE226, TE227, TD329, TD330, TD354, TA431, TA431, F1218, F1188, F40/88, F858/89	TE202, PT9716
Proportion	**35%** (33/94)	**9%** (8/84)	**51 %** (48/94)	**5 %** (5/94)

Table 4. Testing of restoration ability of *cms*-M type male sterility at some inbred lines at A.R.D.S Turda, during 1995-2001

Check lines *cms*	Male-sterility maintenors (*rfrf*)			Fertility trstores(*Rf*)	
	Group I	Group a II-a A	Group a II-a B	Group a V-a	Group a IV-a
MKP33 *cms*-M	TC245, TE207, TB329, TB371, K1075	T248, T291, TC245, TB362, TB366, TB371, P17,W153R,W 633, A665, ND245	TC209, TC243, TA367, TA404, K1079, W117, CM105	Precoce46, CM25, CO216, CO255, A661, K2006	T169a, TC208, TC114

TC 208 *cms*-M	TC109B, TC114, TC184, T243, T248, TD221, TB362, TB366, TC321, TC328, TC331, TC335, TC384, TC385, TA419, K1075, P17, PT9716, ND245	T291, TC243, TC245, TD241, TB364, TB371, TC331, TA404, TA432, K3048A, CO216	A661, TC209, K1080, PT9707	T248B, TC242, TD233, TD283, TE210, TB363, TB367, TB369, TC386, TC391, TC395, TC396, TC397, TD303, TA424, TA428, TA436, PT9708	TD106, TC287, TD233, TD284, TD290, TE208, TA367, TC314, TC344, TC379, TC399, TD301, TD302, TD359, TA425, TA426, TA427, TA429, TA433, TA435, K1042, K1077, K1801B, K2051, K3046B, PT9704, PT3905, PT9710, PT9711, PT9712, PT9713, PT9714, PT9715, PT9717, A665, CM25, CO255
Proportion	**20 %** (24/121)	**18 %** (22/121)	**9 %** (11/121)	**20 %** (24/121)	**33 %** (40/121)

Distribution of inbreds in groups on the basis of fertility restoration, performed by Josephson et al. (1978).
Group I - does not release anthers.
Group II A - release less than 1/2 from anthers, but they are small, dried and hard, without pollen.
Group II B - release many anthers, but they are small, dried and hard, without pollen.
Group III - release partially fertile anthers with a little pollen. The proportion of released anthers was variable.
Group IV - release about 75-100% a little abnormal anthers.

Table 5. Testing of restoration ability of cms-T type male sterility at some inbred lines at A.R.D.S Turda, during 1995-2001.

Check lines *cms*	Male-sterility maintenors (*rfrf*)		Fertility trstores(*Rf*)	
	Group I	Group a II-a A	Group a V-a	Group a IV-a
W633 *cms*-T	TC184, TC221, K2051	K2057B	TC109A,	
TC208 *cms*-T	TC182, TC246, TD232, TD233, TD235, TD236, TD242, TB365, TB368, TB369, TC313, TC314, TC322, TC327, TC328, TC331, TC333, TC335, TC344, TA416, K1042, K1077, K1080, K2131, P22, P131, CO120, W401, A661, A665		TC317, TB331, TC316, TC360, TC364	TC371
TC243 *cms*-T		**TD345,** TC394, TD359, TD335		
TA367 *cms*-T	TD268, TD276, TD278, TD296, TD297, TD298, TD299, TE201, TE203, TE206, TE233, TC337, TC351, TC361, TC362, TC365, TC368, TC378, TC380, TC381, TC383, TC384, TC385, TD336, TD343, TD344, **TD345,** TD350, TD351, TD352, TD353, TD355, TD356, TD357, TD358, TD359, TD360, TD361, TA419, TA425, TA430, TA433, TA441, P951, MV463, PT9711, PT9712, PT9713, PT9714, PT9715, PT9716, K1093, K1511, K1795, K1796, K1800B, K1802A, K1805C, K1806, K2148C, K2274, K2308, K2443B, K3041, K3161B, NF1005, NF1014, NF1032, NF1041, NF1042, NF1065, NF1090, NF1091, NF1092, NF1098, NF1103, NF1111, NF2028, NF2188	TD279, TE202, TE212, K2274, TC384	TC326, TD359, TE210, TC395, TC391, TC399, TD287, TD289, K1653	
TC 208 *cms*-T	TC287, TD284, TE208, TE223, TC379, TC386, TD301, TA426, TA428, TA429, TA431, TA432, TA435, PT9701, PT9703, PT9704, PT9705, PT9707, PT9708, PT9710, K3043, K3044, K3046A		TC391, TC397, TC399, TD302, TD303, NF2028	
TC335 *cms*-T	TC241, TE217, TE220, TE222, TE225, TE226, TE227, TD320, TD329, TD330, TD341, **TD345,** TD346, TD347, TD348, TD354, WN7, F426/88, F1970/86, F1188/89,	TE202, TE223, TE221, TE226, TE227, F1870/86,	TD263, TD287, TD289, TC360, F1218, TD290, TD287, TD289,	

	F858/89, F1153/89, F768/82, K2174	F1970/86		
TC184 *cms*-T	TD342, K1801B, K3056A, K3159B, PT9707, PT9717,	TD221, TE212, TE227, TE230, TE231, PT9706, PT9711	TD335, TE229	TE228,
Proportion	**74 %** (165 / 223)	**9%**(21/223)	**16 %** (35 / 223)	**1%** (2/223)

Table 6. Behaviour of some inbreds vs.different checks – *cms*

Check lines - *cms*	Male sterility maintenors (*rfrf*)	Polyfertility restorers (*Rf*)
S 42 *cms* T	-	T 169a
W 33 *cms* T	T 169a	-
T 153 *cms* T	T 169a	-
T 248 *cms* T	T 169a	-
A 218 *cms* T	-	T 169a
TA 367 *cms* T	TD 345	-
TC 335 *cms* T	TD 345	-
TC 243 *cms* T	-	TD 345

Table 1. Reproduction ability of the *E. integriceps* recent generations, as compared with multiannual average (1970-2000) and with the specific years: favourable (1986) and unfavourable (1989).

Natural gene ration of *E.*	Prolificacy (egg/female) under field condi tions	under controlled conditions	
integriceps		average	maxi-mum/fe male
1970-2000	40.2	57.9	311
1986	56.3	71.3	298
1989	18.8	27.1	87
1996	47.1	69.9	302
1997	46.6	68.6	197
1998	37.5	53.8	209
1999	38.8	54.5	219
2000	39.3	55.7	208

Table 2. Prolificacy level of some *E. integriceps* populations (fertile females), from generations with different fat body levels, collected from

the field, at the beginning of migration and studied under controlled conditions.

Fat body	Generation	Prolificacy (egg/female)	
		aver-age	maxi-mum
23.4	1989-1990	32.1	97
22.5	1972-1973	33.4	127
26.5	1971-1972	46.4	148
27.9	1977-1978	67.5	186
28.0	1984-1985	83.6	210
29.7	1985-1986	95.3	234
29.8	1994-1995	104.7	246

Table 3. Level and stages of fat body diminution at *E. integriceps* (multigeneration average).

Stages	Fat body level		Diminution	
	limits	average	limits	average
Diapause beginning	33.03-37.58	35.69	0	0
End of dia-pause	21.97-27.64	25.43	24.57-36.33	27.39
End of ovi-position	8.12-10.39	8.78	66.50-78.69	74.43

Table 4. Mortality registered at the *Eurygaster integriceps* populations, during diapause in different generations, from Romanian area

E. integriceps natural popula-tion	Mortality (%)	
	Limits in coun-ties	Total area (mean)
2000-2001	4.6-35.7	8.7
1995-1996	3.7-36.4	10.2
2001-2002	5.1-32.3	12.7
1985-1988	3.8-41.2	14.8
1999-2000	4.8-97.6	24.5
1973-1974	11.6-85.0	39.5
1988-1989	17.5-68.4	48.2

Table 5. Fat body value at *Eurygaster integriceps* populations, established on female groups, distributed in weight classes, at the beginning of diapause (multigeneration average).

Weight (mg)	% from the total of	Fat body (%)

	population limits	average	limits	average
below 0.110	3.7-7.7	5.6	26.2-26.6	26.4
0.111-0.118	7.6-23.1	13.3	26.5-28.8	28.7
0.119-0.126	15.9-24.7	19.7	32.8-33.5	33.6
0.127-0.134	32.5-34.8	33.7	34.9-36.4	35.4
over 0.145	22.4-30.8	28.6	35.7-39.8	38.7

Table 6. Fat body value at *Eurygaster integriceps* populations, established on male groups, distributed in weight classes, at the beginning of diapause (multigeneration average).

Weight (mg)	% from the total of population		Fat body (%)	
	limits	aver-age	limits	aver-age
below 0.105	7.0-19.7	12.3	25.3-26.7	26.2
0.106-0.113	16.8-19.9	17.3	27.2-28.5	27.7
0.114-0.121	20.3-29.5	23.7	29.4-33.8	31.5
0.122-0.129	19.2-32.7	28.5	31.2-35.5	32.6
over 0.130	15.5-23.9	19.4	31.4-36.6	33.8

Table 7. Mortality (%) registered at *Eurygaster integriceps* female populations, depending on the fat body (multigeneration average).

Fat body (%)	Mortality (%)			
	During August-October		During November-March	
	limits	average	limits	average
26.4	17-22	20.4	59-64	61.3
28.7	13-15	12.9	43-54	47.6
33.6	9-17	12.5	41-52	46.2
35.4	4-11	6.6	29-34	33.6
38.7	4-7	5.8	26-35	30.9

Table 8. Mortality (%) registered at *Eurygaster integriceps* male populations, depending on the fat body (multigeneration average).

Fat body (%)	Mortality (%)			
	During August-October		During November-March	
	limits	average	limits	average
26.2	22-31	22.6	62-71	67.1
27.7	11-24	20.4	53-62	57.4
31.5	12-19	14.3	39-47	44.0
32.6	9-18	12.7	30-44	37.6
33.8	5-14	9.1	24-45	32.3

Table 9. Sterility and prolificacy registered at the *Eurygaster integriceps* populations, depending on the fat body (multigeneration average).

Fat body (%)	Females sterility (%)		Mean prolificacy (egg/female)		
	limits	aver-age	limits	aver-age	maxi-mum
26.4	100	100	0	0	0

28.7	60-72	63.5	4.1-6.6	5.4	42
33.6	54-63	57.3	16.2-22.8	19.5	78
35.4	35-44	39.1	26.4-33.1	30.3	135
38.7	25-32	29.8	38.9-51.7	45.8	194

Table 10. Multiplication index at the *Eurygaster integriceps* populations, depending on the fat body (multigeneration average).

Fat body (%)	Multiplication (egg/female)	index
	limits	average
26.4	0	0
28.7	0.37–2.47	1.54
33.6	4.54–9.62	6.95
35.4	28.57–40.18	35.22
38.7	49.38–64.83	56.47