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PRELIMINARY RESULTS ON THE PREFERENCES OF *CALLOSOBRUCHUS MACULATUS* ON APULIAN GERMPLASM OF *CICER ARIETINUM* ([1])

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Panzarino O., Bari G., Vernile P., de Lillo E. – Preliminary results on the preferences of *Callosobruchus maculatus* on Apulian germplasm of *Cicer arietinum*.

The susceptibility of six genotypes of *Cicer arietinum* L. (Fabaceae) to *Callosobruchus maculatus* (Fabr.) (Coleoptera: Bruchidae) was evaluated through comparative laboratory bioassays. The egg-laying amount, pattern of adult emergence, median development time and pre-adult mortality were assessed under *free-choice* and *no-choice bioassays* on three local genotypes (Altamura, Grumo Appula White and Grumo Appula Black) and three commercial varieties (Barraco, Sultano and standard of large-scale retail trade as control). Trials were performed on a completely randomized experimental design under artificial conditions with the release of males and females (ratio 1:3), at the most 24-h old. Among the assayed genotypes, the local one provided with a black coat (Grumo Appula Black) was significantly ($P<0.05$) the least susceptible to the cowpea beetle owing to the least number of laid eggs (2.0±1.8 under free-choice and 2.2±1.3 under no-choice bioassays), the delayed median developmental time (30.3±0.6 and 32.3±0.6, respectively), the lower adult emergence (45.6 and 46.2, respectively) and susceptibility index (7.7±2.04 and 7.8±0.51, respectively).

KEY WORDS: Bruchidae, Fabaceae, local genotypes, free-choice bioassay, no-choice bioassay.

INTRODUCTION

World production of chickpea seeds (*Cicer arietinum* L.) (Fabaceae) constitutes one of the main and cheapest sources of plant proteins for the human diet and it is second only to soya and bean. Several bruchids infest seeds of legumes in field and storage environments (SRINIVASAN *et al.*, 2008). The infested pulses become inedible also for animal consumption owing to the increased content of moisture (favoring fungal growth and mycotoxin hazard), uric acid, trypsin inhibitors, saponins and further anti-nutritional factors such as phytic acid (BOEKE *et al.,* 2001).

Cowpea weevil (CW), *Callosobruchus maculatus* (Fabr.) (Coleoptera: Bruchidae), is most common in tropics and sub-tropics. Seeds of *Vigna* spp., mainly *V. unguiculata* (L.) Walp, represent its elective food. But CW is largely polyphagous infesting heavily also chickpea, pea, lentil and other legume seeds and greatly adapts to storage conditions (TIMMS, 1998; OLAKOJO *et al.,* 2007). Seed losses due to CW infestations in field usually do not exceed 1-2% but, often, they extend up to 60-80% of the pulses stored for 6-8 months (SOUTHGATE *et al.,* 1979).

Several studies have assessed the genotype susceptibility to *Callosobruchus* species within the legume family (*cfr.* SRINIVASAN *et al.*, 2008; KAZEMI *et al.*, 2009; SWELLA & MUSHOBOZY, 2009), such as within some pulse species, *e.g. Vigna* spp. (*cfr.* ASANTE & MENSAH, 2007; SRINIVASAN & DURAIRAJ, 2008; BADOOR *et al.*, 2009; JHA *et al.*, 2011), and chickpea (*cfr.* JHA, 2002; KELLOUCHE *et al.*, 2004; ASLAM *et al.*, 2006; SHAHEEN *et al.*, 2006). The host suitability of chickpea cultivars to *C. maculatus* was investigated in some countries (*cfr.* AHMED *et al.*, 1989; JOHNSON *et al.*, 1990; PARAMESHWARAPPA *et al.*, 2007; ERLER, *et al.,* 2009) but knowledge on the Italian genotypes is scanty.

The detection of the variety susceptibility of seeds could allow the selection of natural resources for the management of these pests through the genetic improvement of resistance in pulses (TARVER *et al.*, 2006; OLAKOJO *et al.,* 2007). Following this overall objective, the present preliminary study was undertaken in order to screen the suitability of some commercial and local genotypes of chickpea to the CW.

MATERIALS AND METHODS

STOCK CULTURES OF *CALLOSOBRUCHUS MACULATUS*

Stock cultures of the weevil were maintained on standardized *kabuli* chickpea seeds always using the same trademark provided from the local mass retailers. Groups of about 50 seeds were placed in PVC boxes (n=4) (5 cm in diameter and 8 cm high) of 0.1 L, infested by *C. maculatus* and plugged with nonwoven fabrics mounted on the lid. The boxes were kept in dark, at 30 ± 1°C and 70 ± 5% RH (TALEKAR, 1988). Insects were reared for several generations in conditions favoring the distinct prevalence of normal morphs (UTIDA, 1972) before their use in the current trials.

SEEDS

The bioassays were performed on three local genotypes, two commercial varieties of *C. arietinum*, and the seeds used for the stock cultures as control (Tab. 1). One (Grumo Appula Black) out of six is a *desi*, while the other are *kabuli* type. Seeds were cold treated (-18°C for at least 48 h) in order to avoid any pre-storage infestation or egg-laying by any pest. These seeds were then conditioned to a room temperature before being used for the bioassays.

OVIPOSITION

Oviposition was assessed according to free-choice (modified by SHUKLA *et al.*, 2007) and no-choice bioassays. The free-choice bioassay was aimed at evaluating the

[1] Authors jointly planned and implemented the research.

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Tab. 1 – Germplams of *Cicer arietinum* L. tested in the current bioassay.

Germplasm	Seed type	Weight of 100 seeds (g)	Characteristics of the seeds	Proteins (%)
Control	*kabuli*	48-52	cream, irregular-wrinkled	–
Altamura (local ecotype)	*kabuli*	28-33	cream, spherical-smooth	24-26
Grumo Appula White (local ecotype)	*kabuli*	30-35	cream, spherical-smooth	23-25
Grumo Appula Black (local ecotype)	*desi*	20-25	black, irregular-rough	23-26
Sultano (commercial variety)	*kabuli*	25-30	cream, spherical-smooth	21-24
Barraco (commercial variety)	*kabuli*	35-40	cream, irregular-wrinkled	21-23

influence of the discriminating attractiveness of the contemporary seed genotype presence toward the weevil oviposition. The no-choice bioassay was carried out in order to assess the influence of each seed type on the oviposition without any interference by the other tested genotypes.

FREE-CHOICE BIOASSAY

The six-way device (fig. I, 1) applied in this bioassay consisted of the following PVC parts:

– a "common release arena", got from a cylindrical box (12 cm diameter, 7 cm depth for ~ 0.5 L) provided with a nonwoven fabric lid. Six circular holes (~ 1 cm diameter), equidistant among them, were made on the sidewalls of this box, at ~ 1 cm from its bottom;
– six "oviposition arenas", got from smaller cylindrical boxes (5 cm diameter, 8 cm depth for ~ 0.1 L) and their intact lid. A circular hole (~ 1 cm diameter) was made on the sidewall of each box at ~ 3.5 cm from its bottom;
– six "tubes" (pipes 6 cm long and 1 cm for the external diameter) connected the release with the oviposition arenas.

Each oviposition arena was filled up 30 seeds of only one type of tested chickpeas (one germplasm for each box, including the control seeds); seeds of all germplasms were contemporary tested. No seed was placed in the common arena. Fifteen females and five males of *C. maculatus* adults (all emerged within the last 24 hours) were collected from the maintained culture and released in the common arena. They could freely come back from each oviposition arena and visit the other ones. The device was kept at dark, 30 ± 1°C and 70 ± 5% RH (TALEKAR, 1988). The adults were removed just two days later, before first larval hatching, and the seeds of each arena were examined to count the eggs laid on their surfaces. The bioassay was thrice replicated with genotypes placed randomly into the device.

NO-CHOICE BIOASSAY

The test was performed using PVC cylindrical boxes (5 cm diameter, 8 cm depth for ~ 0.1 L), with nonwoven fabric lids. One box for each genotype, including control, was filled up 30 seeds (fig. I, 2). Three females and one male of *C. maculatus* adults (all emerged within the last 24 hours) were released in each box. Adults were collected from the maintained culture and three replicates were performed per genotype. The microcosms were kept in the dark at 30 ± 1°C and 70 ± 5% RH (TALEKAR, 1988) and the adults were removed just two days later, before first larval hatching, and eggs were counted on the seeds of each genotype.

COWPEA WEEVIL EMERGENCE

Only one egg was left on each seed removing the exceeded ones in order to avoid that more larvae, contemporarily growing into the same seed, could negatively interfere with the juvenile development (OFUYA & AGELE, 1990). Then, the seeds from each replicate and genotype were kept separately in tubes (BD Falcon™ Conical Tubes) under the previous rearing conditions. Since three weeks after the infestation, the seeds were checked daily to

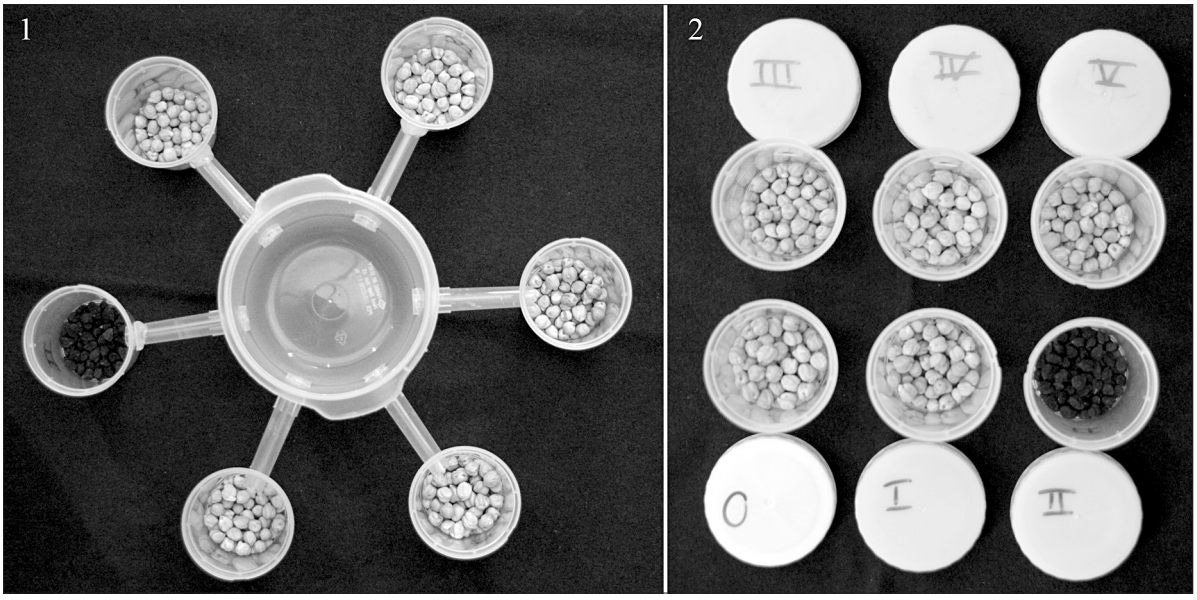

Fig. I – 1: six-way device applied in the free-choice bioassay; 2: six microcosmos with nonwoven fabric fenestrated lids applied in the no-choice bioassay.

detect the emergence of new adults that were recorded and removed.

The percentage of adult emergence, the median developmental period (MDP) (*i.e.*, number of days taken for 50% of the adults to emerge since the oviposition period) and the susceptibility index (SI) (*i.e.*, percentage of the natural logarithm of the total number of emerged adults on the MDP) were calculated (DASBAK *et al.*, 2009). No classification of the SI was followed to rank the susceptibility of the genotypes because of the variability of the classification available in literature on different pest-pulse combinations.

Just for the free-choice bioassay, seeds which did not give any emergence, even though an egg was left on them, were carefully dissected looking for the remnant stage of the beetle. The mortality of each stage (embryos, larvae, pupae/not emerged adults) was calculated as percentage of the selected eggs.

STATISTICAL ANALYSIS

Treatment means and MDP were compared by non-parametric U-Mann & Whitney test and performed on the elementary data applying Statistica 8.0 software. A significance level of $P<0.05$ was considered. Appropriate data transformations were done for the pre-adult mortality (arcsine) and for MDP (logarithmic).

RESULTS

In both bioassays, beetles laid eggs sparsely on seeds and box surfaces, but any particular preference or correlation was detected concerning egg-laying location. Obviously, more eggs were often counted on the same seed.

OVIPOSITION: FREE-CHOICE BIOASSAY

Adults and eggs were observed in all oviposition arenas of the six-way device. About two eggs per seed were counted on Grumo Appula Black and this oviposition rate was significantly lower than that assessed on Altamura, Grumo Appula White, Barraco and control seeds (Tab. 2). No difference was ascertained among Sultano, both Grumo Appula germplasm and control. On Barraco and Altamura, about four eggs per seed were counted and the oviposition rate was not distinctive to the control.

WEEVIL EMERGENCE: FREE-CHOICE BIOASSAY

Adult emergence started from the 26th day after the beginning of the bioassay (fig. II) on Altamura, Grumo Appula White, Barraco and control. One day later, beetles started to emerge from seeds of Sultano, and three days later from seeds of Grumo Appula Black. Emergence period ranged from seven days for Grumo Appula Black up to 14 days for Barraco seeds. Emergence peaks were achieved at 28th day for Altamura, Grumo Appula White and Barraco, at 30th day for Grumo Appula Black and control, and at 31st day for Sultano seeds.

The highest CW emergence was detected for Grumo Appula White seeds (85.0%); and no difference was pointed out among control, Altamura, Barraco and Sultano seeds (fig. III). The lowest emergence was observed for Grumo Appula Black seeds (45.6%) and it did not show any statistical difference with emergence from Sultano seeds.

No significant differences were observed in the embryos mortality within the assayed genotypes and the highest percentage of unhatched eggs (about 22%) was recorded on Sultano seeds. Larval mortality was highest for Grumo Appula Black and Sultano (about 10%) and significant differences ($P<0.05$) were observed between the first of them and control, Barraco and Grumo Appula White seeds; no mortality of larvae was observed for the last genotype. The highest mortality at pupa/not emerged adults was detected again for Grumo Appula Black seeds (about 35%) with significant differences ($P<0.05$) in respect to the all the other treatments.

MDP was significantly lower (28.7 ± 0.6) for Altamura, Grumo Appula White, Barraco and control, than that for Grumo Appula Black (30.3 ± 0.6) and Sultano (30.7 ± 0.6) (tab. 3). SI of Grumo Appula Black was significantly lower (7.7) than that for Altamura, Grumo Appula White, Barraco and control (ranging from 10.9 to 11.1); Sultano exhibited an intermediate SI (9.4).

OVIPOSITION: NO-CHOICE BIOASSAY

Adults laid on all germplasm seeds. A bit more than two eggs per seed were counted on Grumo Appula Black and it was the distinctively lowest oviposition rate among the assayed genotypes (Tab. 2). The highest oviposition rate was assessed on Barraco seeds (4.0 eggs per seed) which did not exhibit significantly differences with Sultano and control seeds (3.6 eggs per seed on both genotypes).

Tab. 2 – Callosobruchus maculatus: mean number of eggs laid per seed (± SD) under free- and no-choice bioassay on different genotypes of chickpea. Means in a column followed by the same letter do not differ significantly at $P<0.05$ level (U-Mann & Whitney test).

Germplasm	Free-choice bioassay eggs (means ± SD) per seed	No-choice bioassay eggs (means ± SD) per seed
Control	3.4 ± 2.3 ab	3.6 ± 1.3 ab
Altamura	4.0 ± 1.7 a	3.1 ± 1.5 bcd
Grumo Appula White	2.9 ± 1.9 b	2.8 ± 1.2 d
Grumo Appula Black	2.0 ± 1.8 c	2.2 ± 1.3 e
Sultano	2.6 ± 1.7 bc	3.6 ± 1.1 ac
Barraco	4.1 ± 2.3 a	4.0 ± 1.9 a

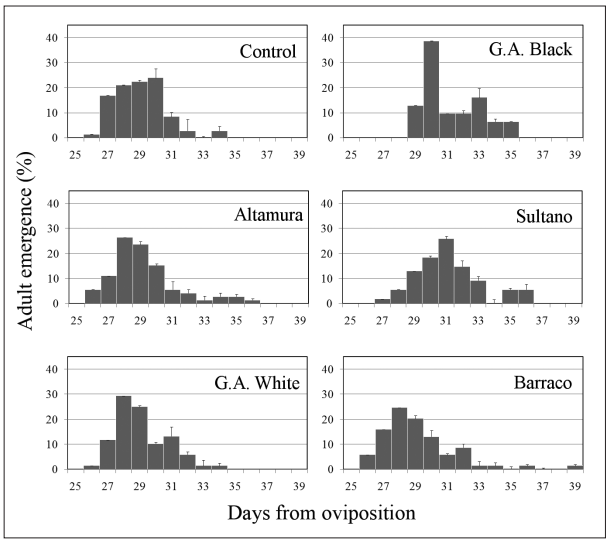

Fig. II – Emergence trend of *Callosobruchus maculatus* adults under free-choice bioassay on different genotypes of chickpea. The daily emergence is expressed as percentage of the total emerged adults occurred in all replicates for the same genotype.

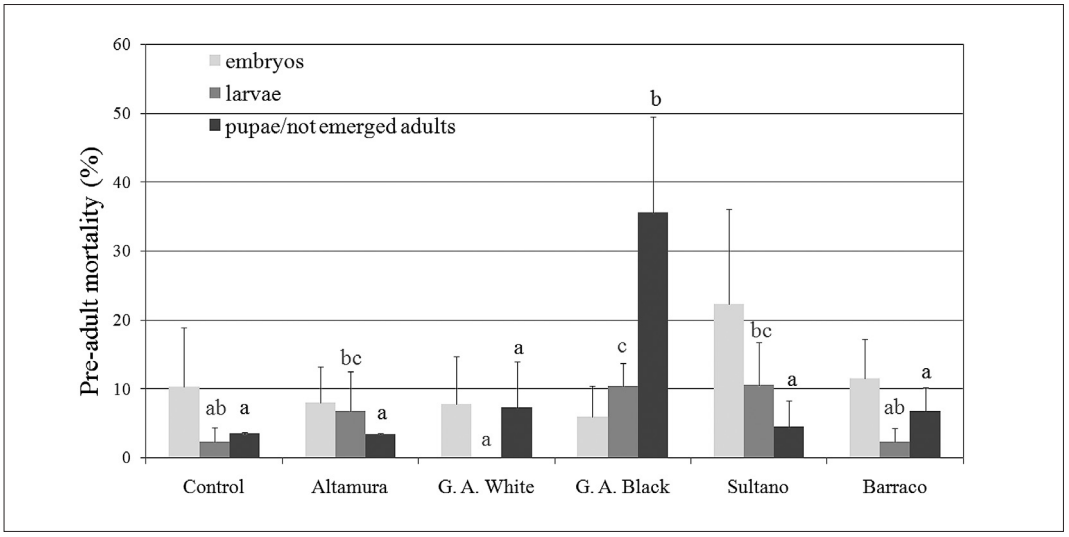

Fig. III – Mortality of the different development stages of *Callosobruchus maculatus* under free-choice bioassay on different genotypes of chickpea. Mean percentage of the replicates + SD is reported. Different gray letters indicate a significant difference among the larval mortality on each germplasm. Different black letters indicate a significant difference among the number of pupae/not emerged adults mortality on each germplasm ($P<0.05$ at U-Mann & Whitney test).

Tab. 3 – Callosobruchus maculatus: Median Development Period (MDP ± SD) and index of susceptibility (SI ± SD) under free- and no-choice bioassay on different genotypes of chickpea. Means in a column followed by the same letter do not differ significantly at $P< 0.05$ level (U-Mann & Whitney test).

Germplasm	Free-choice bioassay		No-choice bioassay	
	MDP ± SD	SI ± SD	MDP ± SD	SI ± SD
Control	28.7 ± 0.6 a	11.0 ± 0.32 b	29.7 ± 0.6 b	10.9 ± 0.32 b
Altamura	28.7 ± 0.6 a	11.1 ± 0.48 b	30.0 ± 0.0 b	10.8 ± 0.30 b
Grumo Appula White	28.7 ± 0.6 a	10.9 ± 0.96 b	29.7 ± 0.6 b	10.7 ± 0.87 b
Grumo Appula Black	30.3 ± 0.6 b	7.7 ± 2.04 a	32.3 ± 0.6 c	7.8 ± 0.51 a
Sultano	30.7 ± 0.6 b	9.4 ± 0.75 a	29.3 ± 0.6 b	11.0 ± 0.47 b
Barraco	28.7 ± 0.6 a	10.9 ± 0.74 b	29.0 ± 0.0 a	10.6 ± 1.05 b

WEEVIL EMERGENCE: NO-CHOICE BIOASSAY

Also in this bioassay, adult emergence started from the 26th day after the beginning of the bioassay (Fig. IV) on Barraco and control seeds. The day after, adults started to emerge also from Altamura, Grumo Appula White and Sultano. A certain delay was ascertained for Grumo Appula Black whose adults started to emerge four days later (30th day from the beginning of the bioassay). Emergence period ranged from just seven days for Grumo Appula Black up to a maximum of 12 days for Barraco and Altamura seeds. Emergence peaks were achieved at 32nd day for Grumo Appula Black seeds and at 29-30th day for seeds of the other genotypes.

Barraco showed the significantly shortest MDP (29.0 ± 0.0), followed by Sultano, Altamura, Grumo Appula White and control. Grumo Appula Black (32.3 ± 0.6) displayed the significantly longest MDP (tab. 3). Grumo Appula Black expressed significantly lower (7.8) SI than that of other genotypes which ranged from 10.6 to 11.0.

The lowest CW pre-adult mortality was detected for Grumo Appula White seeds (15.2%), and no differences were pointed out with control, Altamura, Barraco and Sultano seeds (fig. V). The highest mortality was observed for Grumo Appula Black seeds (54.0%).

DISCUSSION AND CONCLUSIONS

Results of the current investigation pointed out that the CW biology was largely unaffected by the applied bioassay (free-choice *versus* no-choice).

Egg-laying on seeds of control, Grumo Appula White and Black, and on Barraco exhibited very slight differences in a range of 0.1 to 0.2 eggs per seed between the two bioassays. Vice versa, higher differences were observed for Sultano (1 egg more per seed) and Altamura (0.9 eggs less per seed). The lowest oviposition rate for the black *desi* genotype was ascertained under both experimental bioassays. However, the fecundity of CW was quite similar to that recorded in literature at the same temperature by GIGA and SMITH (1987) on a chickpea variety of medium size (about 38 g per 100 seeds), and by other authors in different experimental conditions (KELLOUCHE *et al.*, 2004; PARAMESHWARAPPA *et al.*, 2007; BADOOR *et al.*, 2009; KAZAMI *et al.*, 2009).

Several and not completely understood factors related to the seeds seem to affect the CW fecundity (SOUTHGATE *et al.*, 1979; SALES *et al.*, 2005; ERLER *et al.*, 2009; SWELLA & MUSHOBOZY, 2009). Some physical characteristics, as seed size or color, hardness and type of seed coat of cowpea, were demonstrated to be ineffective in favoring resistance

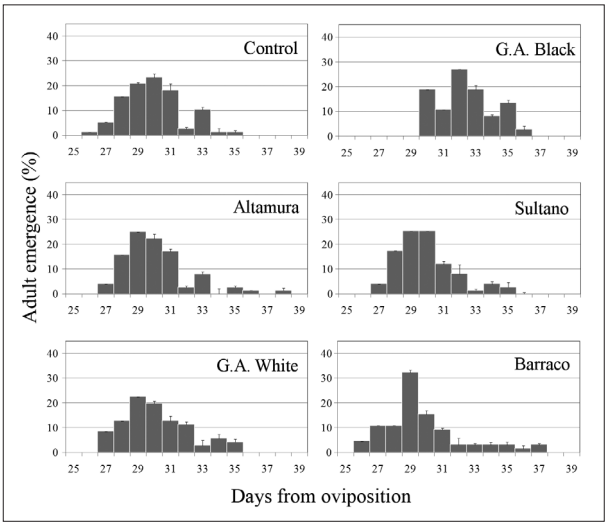

Fig. IV – Emergence trend of *Callosobruchus maculatus* adults under no-choice bioassay on different genotypes of chickpea. The daily emergence is expressed as percentage of the total emerged adults occurred in all replicates for the same genotype.

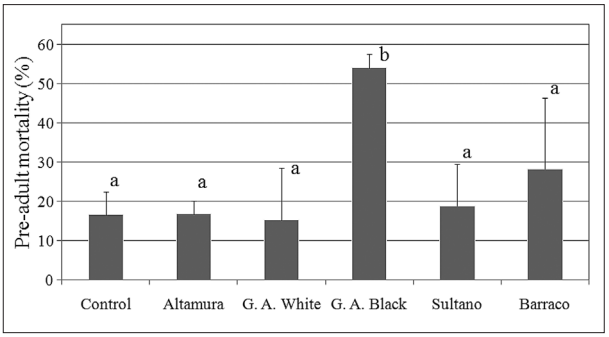

Fig. V – Mortality of *Callosobruchus maculatus* under no-choice bioassay on different genotypes of chickpea. Mean percentage of the replicates + SD is reported. Different letters indicate a significant difference among the pre-adult survival on each germplasm ($P<0.05$ at U-Mann & Whitney test).

to the egg-laying of *C. maculatus* (ILOBA, 1985; SINGH & SINGH, 1990; EDDE & AMATOBI, 2000 and 2003), even though seeds with smooth, soft and thin coats were more preferred than those rough, hard, wrinkled and quite spiny (AHMED *et al.*, 1989). Actually, CW females could perceive an oviposition attractant of the seed coat (EDDE & AMATOBI, 2003) and their oogenesis and oviposition could be stimulated by seed coat components (MONGE, 1983; CREDLAND & WRIGHT, 1988). Egg-laying should not be influenced by nutrients of the tested seeds because laying females are usually considered unfeeding on those seeds, even though DOMENICHINI (1951) observed adults fed on seeds or their fragments. However, DE LIMA *et al.* (2004) did not find distinctive differences in the fecundity of *C. maculatus* on susceptible cowpea genotypes by adults emerged from resistant ones as well on other genotype combinations. On the contrary, antibiosis effects were observed on the fecundity of F1 females of other bruchids like *Zabrotes subfasciatus* (Boheman) and *Acanthoscelides obtectus* (Say) emerged from resistant bean varieties (CARDONA *et al.*, 1989).

Emergence trend, peaks, duration and MDP were largely overlapping on the same tested genotype for both bioassays with very minor differences (of about one day). Weevils from seeds of Grumo Appula Black exhibited two-three days of delay for emergence beginning and peak. Similarly, emergence duration was the shortest for Grumo Appula Black (only seven days under both bioassays) and the longest for Barraco (12 days under no-choice and 14 days under free-choice bioassay). The MDP on chickpea genotypes currently assayed at 30 ± 1°C ranged from 28 to 32 days and it was a bit shorter than that assessed on mung seeds at 25°C by FOX *et al.* (2003). A slower larval development was also appreciated for the genotype Cassano Black (Tarasco, *pers. comm.*). The emergence trend in the current trials cannot be considered affected by paternal age (OFUYA & AGELE, 1990) and larval crowding (FOX *et al.*, 2003). Therefore, the retarded emergence pattern could be related to biochemical factors as known for other pulse species (APPLEBY & CREDLAND, 2003). Current data are quite similar to those in previous breedings on chickpea seeds (GIGA & SMITH, 1987) even though a few of them evidenced a longer CW emergence duration at the same environmental conditions (BADOOR *et al.*, 2009).

Apart cowpea beetles emerged from Sultano, SI was rather similar in both bioassays and the lowest value was obtained always for Grumo Appula Black.

The analysis of the pre-adult mortality data showed a similarity of the outcomes between the two types of bioassays. Grumo Appula Black affected the weevils more than the other genotypes such as previously observed for the Cassano Black genotype (Tarasco, *pers. comm.*). For the other genotypes, weevil mortality ranged around 20% with the exception of Sultano at the free-choice bioassay. Particularly relevant was the mortality of pupae and adults within the seeds of Grumo Appula Black assessed under the free-choice bioassay.

Actually, both bruchid development and pre-adult mortality may be due to the presence of multiple anti-nutritional factors (*e.g.*, enzyme inhibitors; vicilins; lectins; etc.) contained into the cotyledons such as into the seed coat (*e.g.*, tannins) which can be combined with additive or synergistic action in deterring, poisoning and starving the pulse larvae (DICK & CREDLAND, 1986; PIERGIOVANNI *et al.*, 1994; EDDE & AMATOBI, 2000; LATTANZIO *et al.*, 2005; SALES *et al.*, 2005; GUZMAN-PARTIDA *et al.*, 2007).

In conclusion, the outcomes of this preliminary study have shown a lower suitability of the local type Grumo Appula Black with dark coat confirming previous observations on another local genotype (Cassano Black - TARASCO, *pers. comm.*). This local type shows relatively smaller size than other tested varieties. However, recent experiences on Turkish *desi* chickpeas pointed out that very small green seeds (10-11 g per 100 seeds) were actually the most resistant to *C. maculatus*, but their genetic basis for resistance has not been characterized, yet (ERLER *et al.,* 2009).

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REFERENCES

AHMED K., KHALIQUE F., AFZAL M., TAHIR M., MALIK B.A., 1989 – *Variability in chickpea* (Cicer arietinum *L.) genotypes for resistance to* Callosobruchus maculatus *F. (Bruchidae).* - J. Stored Prod. Res., 25 (3): 97-99.

APPLEBY J.H., CREDLAND P.F., 2003 – *Variation in responses to susceptible and resistant cowpeas among West African populations of* Callosobruchus maculatus *(Coleoptera: Bruchidae).* - J. Econ. Entomol., 96 (2): 489-502.

ASANTE S.K., MENSAH G.W., 2007 – *Evaluation of susceptibility of some elite cowpea cultivars to attack by* Callosobruchus maculatus *F. (Coleoptera: Bruchidae).* - Ghana J. Agric. Sci., 40 (1): 73-80.

ASLAM M., SHAHEEN F.A., ABBAS M.A., SABA A., 2006 – *Management of* Callosobruchus chinensis *Linnaeus through use of resistance in stored chickpea varieties.* - World J. Agric. Sci., 2 (1): 82-84.

BADOOR I.M., EMAM A.K., SALAMA S.I., HANAFY H.E.M., 2009 – *Tendency of certain pulse seeds to* Callosobruchus maculatus *(F.) and* Callosobruchus chinensis *(L.) infestation*. - Arab. Univ. J. Agric. Sci., 17 (1): 193-197.

BOEKE S.J., VAN LOON I., VAN HURS A., KOSSOU D.K., DICKE M., 2001 – *The use of plant material to protect stored leguminous seeds against seed beetles: a review.* - Wageningen Agric. Univ. Papers, 3: 1-108.

CARDONA C., POSSO C.E., KORNEGAY J., VALOR J., SERRANO M., 1989 – *Antibiosis effects of wild dry bean accessions on the Mexican bean weevil (Coleoptera: Bruchidae).* - J. Econ. Entomol., 82: 310-315.

CREDLAND P.F., WRIGHT A.W., 1988 – *The effect of artificial substrates and host extracts on oviposition by* Callosobruchus maculatus *F. (Coleoptera: Bruchidae).* - J. Stored Prod. Res, 24 (3): 157-164.

DASBAK M.A., ECHEZONA B.C., ASIEGBU J.E., 2009 – *Post-harvest bruchid richness and residual activity of pirimifos-methyl on* Callosobruchus maculatus *F. infested pigeon pea (*Cajanus cajan *L. Millsp.) in storage.* - Afr. J. Biotechnol., 8 (2): 311-315.

DE LIMA M.P.L., DE OLIVEIRA J.V., BARROS R., TORRES J.B., 2004 – *Alternation of cowpea genotypes affects the biology of* Callosobruchus maculatus *(Fabr.) (Coleoptera: Bruchidae).* - Scientia Agricola, 61 (1): 27-31.

DICK K.M., CREDLAND P.F., 1986 – *Changes in the response of* Callosobruchus maculatus *(Coleoptera: Bruchidae) to a resistant variety of cowpea.* - J. Stored Prod. Res., 22: 227-233.

DOMENICHINI G., 1951 – *Contributo alla conoscenza del* Callosobruchus maculatus *F. e dei suoi parassiti.* - Boll. Zool. Agr. Bachic., Milano, 17: 101-122.

EDDE A.P., AMATOBI C.I., 2000 – *Relative resistance of some cowpea varieties to* Callosobruchus maculatus *(F.) (Coleoptera: Bruchidae).* - J. Sustain. Agric., 17: 67–77.

EDDE A.P., AMATOBI C.I., 2003 – *Seed coat has no value in protecting cowpea seed against* Callosobruchus maculatus *(F.).* - J. Stored Prod. Res., 39: 1–10.

ERLER F., CEYLAN F., ERDEMIR T., TOKER C., 2009 – *Preliminary results on evaluation of chickpea,* Cicer arietinum, *genotypes for resistance to the pulse beetle,* Callosobruchus maculatus. - Insect Sci., 9: 1-7.

FOX C.W., BUSH M.L., WALLIN W.G., 2003 – *Maternal age affects offspring lifespan of the seed beetle,* Callosobruchus maculatus. - Funct. Ecol., 17(6): 811-820.

GIGA D.P., SMITH RH., 1987 – *Egg production and development of* Callosobruchus rhodesianus *and* C. maculatus *on several commodities at two different temperatures.* - J. Stored Prod. Res., 23: 915.

GUZMAN-PARTIDA A.M., JATOMEA-FINO O., ROBLES-BURGUEÑO M.R., ORTEGA-NIEBLAS M., VAZQUEZ-MORENO L., 2007 – *Characterization of alpha-amylase inhibitor from Palo Fierro seeds.* - Pl. Physiol. Biochem. / Soc. Fr. Physiol. Vég., 45(9): 711-715.

ILOBA B.N., 1985 – *The cowpea seed tests and its influence on oviposition and development in* Callosobruchus maculatus *Fabricus (Coleoptera: Bruchidae).* - Nigeria J Pl. Prot., 9: 11-22.

JHA AN., 2002 – *Response of chick pea cultivars to* Callosobruchus chinensis. - Ind. J. Entomol., 64 (4): 434-437.

JHA A.N., SRIVASTAVA C., CHANDRA N., 2011 – *Screening of greengram* (Vigna radiata) *cultivars to three species of pulse beetle (*Callosobruchus *sp.).* - Ind. J. Agric. Sci., 81 (3): 283-286.

JOHNSON Y.S., THANGARAJ E., GUNATHILAGARAJ K., 1990 – *Evaluation of chickpea genotypes for their resistance to the pulse beetle,* Callosobruchus maculatus *(F.) (Coleoptera: Bruchidae).* - Internat. Chickpea Newsletter, 23: 19-20.

KAZEMI F., TALEBI A.A., FATHIPOUR Y., FARAHANI S., 2009 – *A comparative study on the effect of four leguminous species on biological and population growth parameters of* Callosobruchus maculatus *(F.) (Col.: Bruchidae).* - Adv. Environ. Biol., 3 (3): 226-232.

KELLOUCHE A., SOLTANI N., HUIGUARD J., 2004 – *Activité de re production et capacité de dévelopment de la descendance de* Callosobruchus maculatus *(Fabricius) (Coleoptera: Bruchidae) dans des graines de différents cultivars de* Vigna unguiculata *(Walp) et de* Cicer arietinum. - Internat. J. Trop. Ins. Sci., 24 (4): 304-310.

LATTANZIO V., TERZANO R., CICCO N., CARDINALI A., DI VENERE D., LINSALATA V., 2005 – *Seed coat tannins and bruchid resistance in stored cowpea seeds.* - J. Sci. Food & Agric., 85 (5): 839–846.

MONGE J.P., 1983 – *Comportement de ponte de la bruche du haricot (*Acanthoscelides obtectus *Say) sur substrat artificial impregne d'extrait de la plante hote (*Phaseolus vulgaris *L.).* - Biol. Behaviour, 8: 205–214.

OFUYA T.I., AGELE S.O., 1990 – *Ability of ovipositing* Callosobruchus maculatus *(Fabricius) (Coleoptera: Bruchidae) females to discriminate between seeds with differing numbers of emergence holes.*- J. Stored Prod. Res., 26(2): 117-120.

OLAKOJO S.A., AYANWOLE J.A., OBASEMOLA V.I., 2007 – *Laboratory screening of seeds of some cowpea cultivars (*Vigna unguiculata*) for tolerance to cowpea beetles (*Callosobruchus maculatus*) in a hot humid environment*. - Am. Eurasian J. Agric. & Environ. Sci., 2 (5): 528-533.

PARAMESHWARAPPA S.G., DESHPANDE V.K., SALIMATH P.M., 2007 – *Studies on comparative response and seed quality attributes of certain chick pea varieties to pulse beetle (*Callosobruchus chinensis *L.) in storage.* - Karnataka J. Agric. Sci., 20 (3): 492-495.

PIERGIOVANNI A.R., DELLA GATTA C., PERRINO P., 1994 – *High antinutrient levels and bruchid resistance of cowpea (*Vigna unguiculata*) seeds*. - Euphytica, 80: 59–62.

SALES M.P., ANDRADE L.B.S., ARY M.B., MIRANDA M.R.A., TEIXEIRA F.M., OLIVEIRA A.S., FERNANDES K.V.S., XAVIER-FILHO J., 2005 – *Performance of bean bruchids* Callosobruchus maculatus *and* Zabrotes subfasciatus *(Coleoptera: Bruchidae) reared on resistant (IT81D-1045) and susceptible (Epace 10)* Vigna unguiculata *seeds: Relationship with trypsin inhibitor and vicilin excretion.* -

Comp. Biochem. & Physiol., Part A: Mol. & Integr. Physiol., 142 (4): 422-426.

SHAHEEN F.A., KHALIQ A., ASLAM M., 2006 – *Resistance of chickpea (*Cicer arietinum *L.) cultivars against pulse beetle.* - Pak. J. Bot., 38 (4): 1237-1244.

SHUKLA R., SRIVASTAVA B., KUMAR R., DUBEY N.K., 2007 – *Potential of some botanical powders in reducing infestation of chickpea by* Callosobruchus chinensis *L. (Coleoptera: Bruchidae)* . - J. Agric. Tech., 3 (1): 11-19.

SINGH B.B., SINGH S.R., 1990 – *Breeding for bruchid resistance in cowpea.* In: Fujii K., Gatehouse A.M.R., Johnson C.D., Mitchell R., Yoshida T. (Eds.), Bruchids and Legumes: Economics, Ecology and Coevolution. Kluwer Academics, Netherlands, pp. 219–228.

SOUTHGATE B.T., MITTLER T.E., RADOVSKY F.T., RESH V.B., 1979 – *Biology of the Bruchidae.* - Ann. Rev. Entomol., 24: 449-473.

SRINIVASAN T., DURAIRAJ C., 2008 - *Screening of certain green gram accessions to* Callosobruchus maculatus *Fab. (Bruchidae: Coleoptera).* - J. Pl. Prot. Environ., 5 (1): 22-25.

SRINIVASAN T., DURAIRAJ C., KUMAR B.V., 2008 – *Damage potential in different edible legumes and interspecific competition between two species of* Callosobruchus *spp. (Bruchidae: Coleoptera).* - Madras Agric. J., 95: 7-12.

SWELLA G.B, MUSHOBOZY D.M.K., 2009 – *Comparative susceptibility of different legume seeds to infestation by cowpea bruchid* Callosobruchus maculatus *(F.) (Coleoptera: Chrysomelidae).* - Pl. Prot. Sci., 45(1): 19-24.

TALEKAR N.S., 1988 – *Biology, damage and control of bruchid pests of mung bean*. In: Mung bean. Proc. II Internat. Symp. AVRDC, Taiwan: 329-342.

TARVER M.R., SHADE R.E., TARVER R.D., LIANG Y., KRISHNAMURTHI G., PITTENDRIGH B.R., MURDOCK L.L., 2006 – *Use of micro-CAT scans to understand cowpea seed resistance to* Callosobruchus maculatus. - Entomol. Exp. Appl., 118: 33-39.

TIMMS R., 1998 – *Size–independent effects of larval host on adult fitness in* Callosobruchus maculatus. - Ecol. Entomol., 23: 480-483.

UTIDA S., 1972 – *Density dependent polymorphism in the adult of* Callosobruchus maculatus *(Coleoptera, Bruchidae).* - J. Stored Prod. Res., 8: 111-126.