1 **Genetic and environmental factors reducing the incidence of**
2 **the storage pest *Sitotroga cerealella* in maize**

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Abstract

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), is found world-wide and infests maize grains in the field and during storage. Transgenic maize resistant to kernel attack by *S. cerealella* has been developed, but could pose a nutritional risk to humans and livestock. Therefore, alternative sources of resistance posing no threat to consumption should be identified. In this study, our main objectives were to assess genetic variability for kernel damage by *S. cerealella* under natural infestation and to determine genetic and environmental factors contributing to genotype, year, and genotype*year variability. Factorial regression was performed to obtain a biological explanation for the number of kernels damaged per ear. Seventy-seven Spanish maize landraces along with six hybrid checks were evaluated in 2004, 2005, and 2006. There was variability for kernel damage by natural infestation of *S. cerealella* among the landraces: popcorn landraces were among the least damaged, whereas hybrid checks were among the most susceptible genotypes. Plant characteristics associated with ear appearance could be the main stimulatory factors for oviposition and feeding of *S. cerealella* moths, followed by husk coverage of the ear. It was confirmed that temperature is the main environmental factor affecting *S. cerealella* development. Genotypes sensitive to infestation by *S. cerealella* were more negatively affected by higher temperatures than the least attractive genotypes, and lower temperatures reduced the number of kernels damaged on the most susceptible genotypes more than on the least susceptible.

Introduction

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae), is found world-wide and infests maize grains before and after harvest (Barney & Weston, 1996), causing considerable direct damage by larval feeding and indirect damage by making the kernel more susceptible to infestation by secondary insect pests and fungi (Misra et al., 1961; Weston et al., 1993), as well as reducing germination and seedling vigor, especially in popcorn (Everly et al., 1963; Santos et al., 1990). Surveys of insect pests in shelled maize stored on-farm detected an unexpected high abundance and widespread distribution of *S. cerealella* (Sedlacek et al., 1998). Eggs are deposited by the adult on or near the grain, and neonate larvae burrow into the kernel or enter through cracks in the pericarp (Arbogast & Mullen, 1987). Larval and pupal development take place within the kernel; moths leave the kernel through channels to

the outside cut by the larvae just before pupation and covered by weakly fastened flaps of pericarp (commonly called windows). In southwestern France, there are three generations of *S. cerealella* per year, the first two generations feeding on maize kernels of the previous year or on wild plant hosts, and the third generation infesting maize crops in the field (Stockel, 1971). Barney & Weston (1996) observed a similar situation with *S. cerealella* in Kentucky, USA, where the moth had 3-4 generations per year.

Many environmental factors may affect larval development and field infestation by *S. cerealella*. Under laboratory conditions, temperature was the main factor affecting egg incubation period, larval and pupal development time, and survivorship, the optimum conditions being 30 °C and high relative humidity (Hansen et al., 2004; Pérez-Mendoza et al., 2004). Earlier planting as well as leaving the maize in the field after it has dried to below a critical moisture content increases the risk of infestation by *S. cerealella* (Weston et al., 1993). Early maize crops with shorter ear shanks and flat-surface kernels have been described as being more attractive for oviposition and feeding by *S. cerealella* (Cartwright 1930; Villacís et al., 1972; Weston & Rattlingourd 1999). Kernel hardness and the proportion of amylose, sugar, protein, and oil in kernels have been proposed as factors affecting larval development in maize (Peters et al., 1972 a; b; Villacís et al., 1972). However, only few studies have focused on the genetics of traits that confer resistance to *S. cerealella* in maize. Genetically-engineered maize plants have been developed in order to control several storage pests, including *S. cerealella*. A transgene from chicken eggs that encodes avidin has been introduced into maize plants, and kernels containing more than 100 p.p.m. avidin have excellent resistance to storage insect pests (Kramer et al., 2000). However, only about half of the individual kernels from harvested lots of transgenic avidin maize possess insecticidal levels of recombinant avidin because transgenic plants are male sterile (Flinn et al., 2006). Moreover, avidin sequesters the vitamin biotin and may pose a health risk to humans and livestock consuming large quantities of transgenic grain (Bergvinson & García-Lara, 2004). Therefore, native insect resistance posing no threat to consumers should be identified. In this study, the main objective was to assess the genetic variability among maize genotypes and years for kernel damage by *S. cerealella* under natural infestation and to determine genetic and environmental factors contributing to the variability.

Materials and methods

In total, 77 Spanish maize landraces along with six hybrid checks (Clarica, Furio, Hórreo368, Magellán, Maverik, and Surtep) were used to assess genetic variability for kernel damage by *S. cerealella* and to determine genetic and environmental factors contributing to variability among genotypes (G), years (Y), and G*Y interactions. In each trial, genotypes were evaluated in a 9 × 9 triple lattice design under natural infestation by *S. cerealella*. Experiments were carried out in 2004, 2005, and 2006 at Pontevedra on the European Atlantic coast (42°24'N, 8°38'W, 20 m above sea level). Of all maize races used, only 75 landraces and four hybrid checks were evaluated in all years (Table 1).

Each two-row experimental plot consisted of 25 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 60 000 plants/ha. Damage by *S. cerealella* was recorded as the number of kernels with adult emergence holes and windows, at harvest and after 2 months of storage at room temperature. The genotypic covariates used included early vigor, recorded when plants were in the five-leaf stage by assigning visual ratings that ranged from 1 (weak) to 9 (vigorous), days to pollen shed (days from planting until 50% of plants shed pollen), days to silking (days from planting until 50% of plants showed silk), plant height (cm from the ground to the tassel top), ear height (cm from the ground to the ear node), plant appearance [on a visual scale from 1 (short, yellowish and weak plants) to 9 (tall, green and vigorous plants)], husk coverage [on a visual scale from 1 (bad coverage of the ear) to 9 (good coverage of the ear)], number of ears per plant, kernel moisture (%), ear appearance [on a visual scale from 1 (small ears with poor health and kernel filling) to 9 (big ears with excellent health and kernel filling)], ear length (cm), number of ear rows, kernel depth (from the ear cob to the exterior of the kernel, in cm), length (mean width of the ear row, in cm), width (length of the ear row occupied by a single kernel, in cm), weight (mass of 100 kernels, in g), and density (g/cm³). Data were recorded on 10 plants and 10 ears per plot, except for vigor, days to silking, days to pollen shed, and plant appearance, which were recorded on the whole plot, and kernel moisture which was measured in the grains from five ears. Environmental covariates considered were average daily temperature, mean of daily minimum and maximum temperatures, number of days with maximum temperature above 25 °C, number of days with average daily temperature above 15 °C, and annual rainfall.

Analyses of variance were performed for the number of kernels damaged at harvest and after storage. Genotype was the fixed factor and year and replication were random factors. Means were compared with Fisher's protected LSD. The general form for a factorial regression model with genotypic (k) and environmental (h) covariates is (Denis, 1980):

$$Y_{ij} = \mu + [\Sigma \rho_k \cdot G_{ik} + \alpha_i] + [\Sigma \delta_h \cdot E_{jh} + \beta_j] + [\Sigma G_{ik} \cdot \theta_{kh} \cdot E_{jh} + \Sigma \alpha'_{ih} \cdot E_{jh} + \Sigma \beta'_{jk} \cdot G_{ik} + \epsilon_{ij}],$$

where ρ_k and δ_h are the regression coefficients of genotypic (G_{ik}) and environmental (E_{jh}) covariates, respectively; α_i and β_j are the residuals of genotype and environmental main effects, respectively; θ_{kh} is the regression coefficient of the cross-product of covariates (G_{ik} and E_{jh}); and α'_{ih} and β'_{jk} are the genotype-specific (i) and environment-specific (j) regression coefficient of environmental covariate E_{jh} and genotypic covariate G_{ik} , respectively. ϵ_{ij} is the residual interaction effect. The covariates and their order in the factor regression model for yield data were obtained by performing a stepwise regression independently on genotype and environmental covariates, without considering the other set of covariates (Denis, 1988). After standardization of covariates, factorial regression analyses were performed with the computer package INTERA (Decoux & Denis, 1991). All terms were tested against the residual experimental error.

Results

The correlation coefficient between number of kernels damaged by *S. cerealella* after storage for 2 months and number of kernels damaged by *S. cerealella* at harvest was high ($r = 0.98$), suggesting that field infestation rather than barn infestation contributed to kernel damage when storage under non-controlled conditions is for a short period of time (2-3 months). Analyses are presented only for those kernels that were damaged by *S. cerealella* after storage.

The three popcorn landraces evaluated (Úbeda, Villanueva del Arzobispo, and Codoñera), along with the extra-early landrace Sajambre, were the least damaged (Table 1). However, about half of the genotypes did not differ from Codoñera for the number of kernels damaged. On the other hand, the hybrid checks showed a high incidence of attack by *S. cerealella*, especially Clarica, Maguellán, and Furio. These checks were

among the most damaged by *S. cerealella*, along with the landraces Acibeiro, Aranga1, and Cacheira. Most Spanish maize populations from the center, south, and east had low numbers of kernels damaged. In summary, the hybrid checks Clarica, Maguellán, and Furio, along with the landraces Aranga1, Acibeiro, Cacheira and Guernika, were the most susceptible maize varieties; meanwhile, 36 landraces having fewer than 23 kernels damaged did not significantly differ from the less-damaged variety, Codoñera (values are bold in Table 1). Popcorn genotypes have very distinctive plant, ear, and kernel characteristics, which advised the no inclusion of the three popcorn landraces in subsequent analysis.

Year main effects (Y) accounted for 41.3% of total variation for number of kernels damaged by *S. cerealella*, while genotypic main effects (G) accounted for 32.5%, and the genotype*year (GY) interaction accounted for 26.2%. The stepwise regression only detected one environmental covariate (number of days with average daily temperature above 15 °C) and six genotypic covariates (ear appearance, ear number, kernel density, kernel length, kernel weight and husk coverage) as significantly affecting the number of kernels damaged by *S. cerealella* (Table 2). The number of days with average daily temperature above 15 °C explained more than 99% of the variability in, and had a significant and positive effect ($\delta_T = 16.41$) on, the number of damaged kernels (Table 2). Ear appearance, husk coverage, kernel density, kernel length, and ear number explained approximately 55, 8, 3, 3, and 4%, respectively, of variation for G, with the residual being non-significant. The coefficient of regression of number of kernels damaged by *S. cerealella* on ear appearance (ρ_{EA}), kernel density (ρ_{KD}), and kernel length (ρ_{KL}) were positive, whereas those for husk coverage (ρ_{HC}) and ear number (ρ_{EN}) were negative. The direct relationship between ear number and number of kernels damaged by *S. cerealella* was not significant (data not shown), but became negative and significant after removing the effect of ear appearance. Similarly, the direct relationship between kernel density and number of kernels infested by *S. cerealella* was not significant, but became positive and significant after removing the effects of ear appearance and husk coverage.

The factorial regression model explained more than 75% of the GY interaction sums of squares for number of kernels damaged by *S. cerealella*; the residual GY was not significant (Table 2). The five genotype*environment covariate cross-products explained approximately 33% of GY variability, with three of them being significant

(Table 2). The regression parameters of number of kernels damaged by *S. cerealella* on number of days with average daily temperature above 15 °C*ear appearance (θ_{T-EA}) and on number of days with average daily temperature above 15 °C*kernel density (θ_{T-KD}) were positive, whereas the regression parameter on number of days with average daily temperature above 15 °C*husk coverage (θ_{T-HA}) was negative. A similar percentage of GY variability was explained by the interaction of the environmental covariate with the residual genotype variation (ca. 34%); GY variability explained by the interactions between genotype covariates and the residual environmental variation was trivial (ca. 7%).

Discussion

Some efforts have been made to date to assess maize genotypic variability for kernel damage by *S. cerealella*, but differences among maize genotypes for infestation and damage by *S. cerealella* have only been reported by Blanchard & Snelling (1942), Peters et al. (1960, 1972a, b), Villacís et al. (1972), and Weston et al. (1997). In the present study, the variability in kernel damage by natural infestations of *S. cerealella* of a representative collection of Spanish maize landraces has been explored, and genetic variability has been detected. Popcorn landraces (Codoñera, Úbeda, and Villanueva del Arzobispo) were among the least damaged, whereas hybrid checks (Clarica, Furio, Magellán, and Maverick) were among the most susceptible genotypes; however, among field maize landraces, populations as susceptible as the hybrid checks or as resistant as popcorn landraces were found. Peters et al. (1972a) pointed out that small size and weight of kernels from a popcorn variety appeared to reduce the size of female moths of *S. cerealella*; the same authors did not find differences in the number of emerging moths per kernel in a free-choice experiment including a popcorn variety and four non-popcorn varieties. Therefore, some distinctive characteristics of popcorn landrace plants could have a deterrent effect on oviposition by *S. cerealella* adults. Kernel moisture appears to be a likely feature to explain the diminished infestation by *S. cerealella* in popcorn landraces compared with other landraces. Weston et al. (1993) stated that maize kernels with a moisture content above 31% were virtually non-infested. The three popcorn landraces had mean kernel moisture contents between 27.8 and 31.1%.

Most populations from central, eastern, and southern Spain had few kernels damaged by *S. cerealella*. Previously, S₁ and S₂ populations derived from some populations collected in central, eastern, and southern Spain had low numbers of weevil

progeny (van Schoonhoven et al., 1975) and southeastern Spanish populations performed well under maize borer infestation (Malvar et al., 1993). As had been pointed out by Malvar et al. (2004), the comparative lower damage by insects on southern and eastern Spanish populations compared with other Spanish populations could be due to the origin (Central America) of maize brought to southern and eastern Spain (Revilla et al. 1998).

Between-year variation of number of kernels damaged by *S. cerealella* after storage was almost completely explained by changes in the number of days with average daily temperature above 15 °C, with higher damage in years with more days with average daily temperature above 15 °C. The Angoumois grain moth infests grain in the lower temperate and tropical areas (Dicke, 1977); therefore, in temperate areas with mild climatic conditions, such as the European Atlantic coast, warmer conditions are more favorable for *S. cerealella* development.

The proportion of the genotype effect explained by five genotypic covariates was 73%, but the first two covariates explained more than 60% of variation attributed to genotypes. Increased numbers of kernels damaged by *S. cerealella* were associated with better ear appearance, poorer husk coverage of the ear, and deeper kernels. The association of the number of kernels damaged with the other genotypic covariates, kernel density and number of ears per plant, became significant after removing the effects of ear appearance and husk coverage, showing the importance of performing factorial regression analyses for detecting genotypic or environmental factors with minor effects on the trait evaluated. Once the effects of the ear and husk coverages were removed, increased numbers of damaged kernels were associated to increased kernel density and reduced ear numbers. Factors related to ear appearance seem to have a great influence on field kernel infestation by *S. cerealella*. Visual stimuli are largely irrelevant for host finding by *S. cerealella* (Weston et al., 1997), but large and healthy ears normally produced by vigorous and healthy plants may emit higher levels of volatile compounds acting as attractant for *S. cerealella* adults, as suggested by Weston et al. (1997). Cartwright (1930) also found an association between better husk coverage and reduced infestation by *S. cerealella*, and Villacís et al. (1972) reported a significant correlation between kernel size and number of insects per kernel in a free-choice test with kernels from different maize varieties. The positive relationship found between kernel density and number of damaged kernels was also previously reported by Peters et al. (1972a, b) in a free-choice test in which higher numbers of moths emerged from the

1 harder kernels because kernel density and hardness are highly related. Finally, the
2 negative linear regression coefficient for kernel damage on number of ears per plant can
3 be explained as follows: if attraction of *S. cerealella* moths is more dependent on plant
4 than on ear characteristics, plots with high stands but low ear numbers would exhibit
5 increased kernel damage, because moths would be attracted to the plants but few ears
6 would be available for oviposition.

7 The three significant genotype*environment interactions (number of days with
8 average daily temperature above 15 °C (T)*ear appearance, T*kernel density, and
9 T*husk coverage) all have the same biological explanation: genotypes with favorable
10 characteristics for infestation by *S. cerealella* (good ear appearance, loose husks, and
11 high kernel density) are more unfavorably affected by higher temperatures than the least
12 attractive genotypes (with bad ear appearance, tight and long husks, and lower kernel
13 density). Lower temperatures reduced number of kernels damaged on the most attractive
14 genotypes compared with the least attractive. There were also significant environment-
15 specific responses to ear appearance, kernel density, and ear number that could not be
16 explained by differences in the number of days with average daily temperature above 15
17 °C; but the interactions between those genotypic covariates and the residual
18 environmental variation accounted for less than 8% of the sum of squares of the GY
19 interaction for number of kernels damaged by *S. cerealella*. Previous studies have
20 focused on detecting genetic mechanisms underlying differences for resistance to *S.*
21 *cerealella* (Peters et al., 1972 a, b; Villacís et al., 1972; Weston et al., 1993, 1997;
22 Weston & Rattlingourd, 1999) or environmental factors affecting the development and
23 survivorship of *S. cerealella*, but this study is the first to consider both kinds of factors
24 (genotypic and environmental) together, in order to explain the variation in number of
25 kernels per ear damaged by *S. cerealella* attributed to genotype, year, and their
26 interaction. In addition, the influence of each factor was quantified, indicating that plant
27 characteristics associated with ear appearance could be the main factor of attraction for
28 oviposition and feeding of *S. cerealella*, followed by husk coverage of the ear.
29 Similarly, it was confirmed that temperature is the main environmental factor affecting
30 *S. cerealella* development.

31 32 **Acknowledgments**

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Table 1. Means for genotype covariates across years and environmental covariates across genotypes and for number of kernels damaged by *Sitotroga cerealella* on 79 maize varieties evaluated during 3 years

Spanish geographic origin		Genotypic covariates					Number of kernels damaged ²
		Ear appearance (class 1-9) ¹	Husk	Kernel	Kernel	Ear	
			coverage (class 1-9) ¹	density (g/cm ³)	length (cm)	number	
Field corn landraces							
Acibeiro	Northwest	4.14	5.89	1.07	0.70	0.85	47.47
Almonte	Southwest	3.43	6.44	1.14	0.79	0.71	22.39
Amarillo de Marañón	North	2.90	5.44	1.72	0.62	0.40	21.02
Amarillo temprano de Aragón	Northeast	3.31	7.44	2.19	0.69	0.59	16.23
Andoain	North	4.09	6.00	1.18	0.79	0.73	40.27
Anero	North	3.60	7.33	1.11	0.68	0.74	17.29
Aranga1	Northwest	4.59	5.22	1.07	0.80	0.78	51.68
Arredondo	Northwest	3.06	6.22	1.14	0.68	0.64	16.27
Arzúa, Dombodan	Northwest	3.70	5.67	1.10	0.69	0.81	23.56
Ataún	North	3.37	5.89	1.08	0.67	0.56	22.54

Con formato: Inglés (Estados Unidos)

Ayala, Izoria	North	3.64	5.33	1.05	0.73	0.71	27.78
Azcoitia	North	3.17	6.44	1.09	0.67	0.65	22.04
Bande	Northwest	3.62	6.67	1.09	0.73	0.73	21.72
Basto	Center and south	3.57	5.89	1.16	0.72	0.73	13.84
BastoxBlanco	South	2.87	7.11	1.15	0.65	0.59	15.55
Berastegui	North	3.31	6.56	1.13	0.66	0.57	21.70
Betanzos	Northwest	3.77	6.33	1.12	0.78	0.86	26.81
Blanco	East and south	2.96	6.67	1.21	0.74	0.66	7.22
Cacheira	Northwest	4.06	5.89	1.10	0.73	0.80	45.82
Camariñas, Ponte do Porto	Northwest	3.59	5.78	1.11	0.74	0.71	30.58
Canicouva	Northwest	3.75	6.11	1.18	0.65	0.98	9.42
Castellote	East	3.40	6.89	1.04	0.66	0.60	9.72
Castro Urdiales	North	3.69	6.56	1.14	0.71	1.00	23.69
Coristanco	Northwest	3.60	6.22	1.12	0.74	0.81	24.92
Covelo, Piñeiro	Northwest	4.10	7.78	1.17	0.68	0.80	21.55
Dúdar	South	3.68	6.33	1.35	0.62	0.68	21.64
Dumbria, Castiñeiras	Northwest	3.69	5.89	1.10	0.73	0.68	24.20
Enano levantino x Hembrilla	East	3.79	6.44	1.07	0.80	0.86	20.46
Fica Gámiz	North	4.06	5.89	1.13	0.77	0.86	43.82
Fino	Center, north, and south	3.74	6.44	1.18	0.64	0.68	21.88

FinoxTremesino	South	2.87	6.56	1.12	0.69	0.50	10.97
Gallego x Hembrilla norteño	Northwest	3.47	5.11	1.06	0.70	0.71	30.05
Gomesende	Northwest	3.83	6.67	1.16	0.68	0.59	29.18
Guadix	Southeast	3.35	6.44	1.12	0.74	0.83	11.57
Guernika	North	3.83	5.89	1.11	0.72	0.70	44.60
Guetaria	North	2.83	6.11	1.41	0.67	0.46	29.11
Guillarey	Northwest	4.40	7.33	1.20	0.72	0.84	28.84
Hazas de Sobas	North	3.29	6.22	1.07	0.66	0.73	17.86
Hembrilla	All regions	3.47	5.22	1.18	0.73	0.66	23.41
Hembrilla norteño	Center, north, and northwest	3.77	6.22	1.13	0.69	0.66	37.33
Hembrilla x Queixalet	East	3.47	5.89	1.06	0.65	0.56	26.01
Lagarin	North	3.94	6.44	1.19	0.74	0.69	26.44
Lalín	Northwest	4.10	6.78	1.11	0.72	0.82	26.02
Lazcano	North	3.45	6.11	1.15	0.72	0.85	22.46
Lira	Northwest	4.15	6.22	1.07	0.80	0.93	43.35
Melide	Northwest	4.02	6.00	1.10	0.73	0.77	40.95
Moeche, Santa Cruz	Northwest	3.74	6.22	1.04	0.68	0.69	26.11
Mondariz	Northwest	3.94	7.33	1.12	0.74	0.69	14.46
Mondoñedo, Couboeira	Northwest	3.74	6.56	1.11	0.77	0.63	26.68
Moya	Center	3.30	7.89	1.07	0.72	0.68	20.22

Narcea	North	3.55	5.44	1.09	0.68	0.79	20.25
Negreira, Avite-Pesadoira	Northwest	3.91	5.56	1.11	0.78	0.81	31.80
Nieves del Caso	North	3.69	6.00	1.14	0.68	0.76	15.27
Norteño largo	East, north, and northwest	4.22	6.56	1.14	0.67	0.52	26.66
Oia, Sta María de Oia	Northwest	3.71	6.33	1.12	0.74	0.65	28.10
Padrón	Northwest	4.16	6.78	1.14	0.74	0.77	29.88
Puenteareas	Northwest	3.98	7.44	1.13	0.62	0.71	27.23
Puentedeume	Northwest	4.21	6.22	1.12	0.82	0.82	41.51
Rastrojero	Center, east, and south	3.23	6.11	1.09	0.73	0.52	21.49
Rebordanes	Northwest	4.22	5.44	1.11	0.71	0.84	42.89
Ribadumia	Northwest	3.65	7.22	1.18	0.78	0.65	24.40
Rojo temprano de Aragón	East	3.91	6.33	1.14	0.73	0.76	30.71
Sajambre	North	2.89	5.33	1.08	0.64	0.96	5.40
Salvatierra	Northwest	3.00	6.11	1.44	0.62	0.74	14.74
Santiago	Northwest	3.70	6.11	1.08	0.69	0.73	38.28
Sarreaus	Northwest	3.63	6.33	1.10	0.74	0.80	21.44
Sobrado, Grixalva, Ax	Northwest	3.89	6.22	1.09	0.70	0.82	15.31
Solares, Hermosa	North	3.93	6.67	1.14	0.67	0.76	27.76
Taboada, Gondulfe	Northwest	3.26	6.22	1.12	0.71	0.82	18.36
Tremesino	South	3.45	6.33	1.14	0.67	0.66	15.65

Tuy	Northwest	4.24	6.22	1.15	0.75	0.71	30.78
Viana	Northwest	3.57	6.44	1.10	0.75	0.87	17.18
Popcorn landraces							
Codoñera	East	-	-	-	-	-	3.10
Úbeda	South	-	-	-	-	-	6.27
Villanueva del Arzobispo	South	-	-	-	-	-	5.89
Hybrid checks							
Clarica		4.62	5.89	1.06	0.92	0.93	64.70
Furio		4.65	6.00	1.12	0.85	0.77	46.03
Maguellán		4.22	5.44	1.11	0.85	0.89	54.45
Maverik		4.68	5.61	1.10	0.84	0.88	43.87
LSD (5%)							19.83
Years		Environmental covariate³					
2004		150					
2005		175					
2006		205					

¹Subjective scale from 1 (bad) to 9 (excellent).

²Values for landraces non significantly different from the less-damaged variety, Codoñera, are bold.

³Number of days with mean temperature above 15 °C.

1 Table 2. Analysis of variance of the factorial regression model including one (out of six
2 recorded) environmental and five (out of 17 recorded) genotypic covariates for number
3 of kernels damaged by *Sitotroga cerealella*. Trials were conducted with 76 maize
4 varieties during 3 years

Source	d.f.	Sum of squares	Mean of squares	P ¹	% SS ²	Regression coefficient ³
Year (Y)	2	40933	20467	<0.0001		
Tmed15 ⁴ (T)	1	40929	40929	<0.0001	99.99	$\delta_T = 16.41$
Residual Y	1	4	4	-	0.01	
Genotype (G)	75	32201	429	<0.0001		
Ear appearance (EA)	1	17596	17596	<0.0001	54.64	$\rho_{EA} = 8.61$
Husk coverage (HA)	1	2597	2597	0.0001	8.06	$\rho_{HA} = -3.62$
Kernel density (KD)	1	1033	1033	0.0040	3.21	$\rho_{KD} = 1.88$
Kernel length (KL)	1	988	988	0.0048	3.07	$\rho_{KL} = 3.13$
Ear number (EN)	1	1248	1248	0.0018	3.88	$\rho_{EN} = -2.83$
Residual G	70	8739	125	0.4187	27.14	
G*Y	150	26020	173	0.0020		
T*EA	1	4679	4679	<0.0001	17.98	$\theta_{T-EA} = 6.13$
T*HA	1	588	588	0.0265	2.26	$\theta_{T-HA} = -2.56$
T*KD	1	2692	2692	<0.0001	10.35	$\theta_{T-KD} = 4.22$
T*KL	1	356	356	0.0832	1.37	
T*EN	1	384	384	0.0722	1.48	
T*G	70	8955	128	0.3666	34.42	
Y*EA	1	522	522	0.0361	2.01	
Y*HA	1	3	3	-	0.01	
Y*KD	1	729	729	0.0140	2.80	
Y*KL	1	177	177	0.2260	0.06	

Y*EN	1	574	574	0.0283	2.21
Residual GY	70	6362	91	0.7000	24.45
Error	423	51276	121		

1

2 ¹Probability level for which differences were significant.

3 ²% SS = percentage of sum of squares (SS) within the corresponding main or interaction
4 effect.

5 ³ δ = regression coefficient of environmental covariate, ρ = regression coefficient of
6 genotypic covariate, and θ = regression coefficient of the cross product of genotypic and
7 environmental covariates.

8 ⁴Number of days with mean temperature above 15 °C.