Running head: **Maize evaluation for pest resistance**

Searching for new sources of pink stem borer resistance in maize

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Abstract

The pink stem borer (*Sesamia nonagrioides* Lef.) is the main corn (*Zea mays mays* L.) pest in the Mediterranean area. Although, screening for resistance to this pest has been successful, the level of resistance shown by the most resistant varieties is not high. The objectives of the present work were: i) the evaluation for pink stem borer resistance of the non tested inbred lines, field and popcorn materials, from the collection maintained at the Misión Biológica de Galicia and ii) the study of the performance, under pink stem borer infestation, of inbreds selected for resistance to pink stem borer in hybrid combination. Forty four inbred lines non tested yet for resistant to pink stem borer along with five inbred lines previously reported as resistant to stem and ear attack, were evaluated in 2002 and 2003. In adjacent experiments, hybrids EP79 × W552, EP77 × B93, and A661 × EP42 were tested along with a resistant hybrid, MEB531-*Bt*, and a susceptible hybrid, INRA 260. New sources of resistance to pink stem borer have been detected among inbred lines improving the level of resistance presented by previously tested inbreds. Some of these lines were successfully developed by pedigree selection for resistance to pink stem borer.

Key words: Germplasm, Maize, Resistance, *Sesamia nonagrioides.*

In Europe, stalk damage by corn borers is the largest biotic constraint for corn crops. The pink stem borer (*Sesamia nonagrioides* Lef.) is the main threat for corn (*Zea mays mays* L.) growers in the Mediterranean area (Cordero et al. 1998). Corn varieties genetically modified by the introduction of *Bacillus thuringiensis* (*Bt*) toxin genes appeared as the solution to eliminate losses caused by many lepidopteran species. Several studies, however, have demonstrated that insect can adapt to *Bt* toxins and pyramiding of several resistance genes could delay insect adaptation (Tabashnik 1994, Huang et al. 1999, Andow et al. 2000). That could be done by combining the partial natural resistance present among corn varieties with the total resistance of *Bt*-toxin genes.

The search for resistant corn varieties to pink stem borer attack has been focused on inbred materials (Cartea et al. 1994, Butrón et al. 1999a, Velasco et al. 1999a) and populations (Malvar et al. 1993, Soengas et al., 2004. Velasco et al. 1999b; Malvar et al. 2004) coming mostly from United States and Europe. Although, the screening has successfully identified materials that performed significantly better than others under pink stem borer infestation, the level of resistance shown by the most resistant varieties is not high. The resistance level could be improved by recurrent selection in promising populations. In the long term, recurrent selection would probably render some progress, but new searches for resistance among inbred lines could supply parents for making more resistant hybrid than those available on the market.

As part of our breeding work, inbred lines are constantly generated, and materials from other germplasm banks are requested for attending particular demands of our breeding program. These materials could supply some favorable factors against pink stem borer attack, especially those inbred lines generated from materials previously categorized as resistant.

Evaluations for pink stem borer resistance of ‘new’ inbred lines should be done along with materials previously detected as resistant in order to know if the new accessions could improve the level of resistance already available. Malvar et al. (2004) found some European landraces that improve stem resistance to corn borers compared to that reported previously by Malvar et al. (1993).

Stem damage by the pink stem borer is more important than direct damage on the ear for field corn. However, for specialty corns intended for human consumption, ear damage might be the major constrain. When attacking popcorn varieties, corn borers affect agronomic traits as well as quality traits (Thomas et al. 1960, Jarvis et al. 1990). Popcorn materials have been evaluated for resistance to *Ostrinia nubilalis* (Wilson et al., 1991; 1993; Jarvis, 1988), the other important corn borer in temperate areas. Although resistance to both corn borers, *Sesamia nonagrioides* and *Ostrinia nubilalis*, is not totally independent (Velasco et al. 1999b), specific evaluations for resistance to the pink stem borer are necessary. Until now, only one popcorn population, ESP0070441, has been evaluated for resistance to stem and ear damage by the pink stem borer along with many field corn populations (Malvar et al. 2004). ESP0070441 was as resistant to stem and ear attack as the most resistant field corn populations.

Resistance to pink stem borer, measured as less tunnel length and/or damage on the ear, is mainly under additive gene control (Butrón et al. 1998, 1999b, Cartea et al.1999, 2001, Velasco et al. 2002). Nevertheless, yield under infestation conditions has been defined as the best trait for evaluating the level of defense against pink stem borer attack and general combining ability as well as specific combining ability effects are significant for this trait (Butrón et al. 1999b). Therefore, for evaluating the usefulness of an inbred line selected for resistance to pink stem borer, it should be interesting to test its performance in hybrid combination, under pink stem borer infestation

The objectives of the present work were: i) the evaluation for pink stem borer resistance of the non tested inbred lines, field and popcorn materials, from the collection maintained at the Misión Biológica de Galicia, and ii) the study of the performance under pink stem borer infestation of hybrids involving inbreds selected for resistance to pink stem borer.

Materials and methods

Inbred lines

Forty four inbred lines non tested yet for resistant to pink stem borer along with five inbred lines previously reported as resistant to stem and ear attack, namely A509, PB130, A635, EA2024, and Oh43 (Butrón et al. 1999a), were evaluated under pink stem borer infestation in the Northwest of Spain. Inbreds were classified in four germplasm groups: Reid, Lancaster, Corn Belt, and Other. The Corn Belt group included all the American germplasm that was neither Reid nor Lancaster (Table 1). Popcorn, European, and Latin-American germplasms made up the group "Other" because there were few inbred lines that come from each origin and we considered that it was better to cluster them. Trials were planted in Pontevedra (42°24'N, 8°38'W; 20 m above sea level) in 2002 and 2003. Inbred lines were sorted in two completely random blocks. Each experimental plot consisted of two rows with 11 two-plant hills. Rows were spaced 0.80 m and hills 0.21 m. Plots were overplanted and thinned to obtain a final density of approximately 60 000 plants ha^{-1}.

Number of days to silking (from sowing to 50% plants silking) and days to pollen shed (from sowing to 50% plants shedding pollen) were recorded. At silking stage, six plants from each plot were infested with a mass of ≈ 40 eggs of *Sesamia nonagrioides* per plant. Eggs, obtained as described by Eizaguirre (1989), were placed between the main ear and the stem (Butrón et al. 1998). At harvest, stems of five infested plants from each plot were measured (length in cm from the ground to the last node) and dissected for measuring tunnel length (cm) made by the pink stem borer and also for counting the number of larvae of *Sesamia*. The percentage of the stem damaged was computed dividing tunnel length by stem length. On the corresponding ears, general appearance of the ear, based on a 9-point scale (from 1 = wholly damaged ear, to 9 = ear without injury), was used as an estimate of damage produced by *Sesamia nonagrioides* larvae on ears.

Hybrids

Hybrids EP79 × W552, EP77 × B93, and A661 × EP42 were tested along with a resistant hybrid, MEB531-*Bt*, and a susceptible hybrid, INRA 260, to pink stem borer attack. Inbreds EP77 and EP79 were developed from crosses between resistant inbreds (Butrón et al. 1999a) by pedigree selection under infestation by pink stem borer. The cross A661 × EP42 was selected among 45 hybrids for its high yield under pink stem borer infestation (Butrón et al. 1999b).

Hybrid trials were adjacent to the inbred experiments. Hybrids were sorted following a randomized complete block design, with three replications. Each experimental plot consisted of four rows with 11 two-plant hills. Rows were spaced 0.80 m and hills 0.21 m. Plots were overplanted and thinned to obtain a final density of approximately 60 000 plants ha^{-1}. All plants of three or two rows per plot were infested as described above, in 2002 and 2003, respectively. Traits recorded were: days to silking, days to pollen shed, stem lodging (percentage of broken plants below the main ear), root lodging (percentage of plants leaning more than 45º from the vertical), tunnel length, percentage of the stem damaged, general appearance of the ear, yield under artificial infestation (Mg ha^{-1} at 140 g kg^{-1} moisture content), kernel moisture content (g hg^{-1}), number of larvae of *Sesamia nonagrioides*, and number of larvae of *Ostrinia nubilalis*.

For both kinds of trials, inbred and hybrid experiments, individual and combined analyses of variance over years were made for each trait using PROC GLM (SAS 2000). Mean comparison by Fisher's protected LSD for each trait and coefficients of phenotypic correlation among traits were also computed.

Results and discussion

Inbred lines

The combined across years analysis of variance showed significant differences among inbreds for all traits, except for tunnel length and number of larvae (data not shown). The year × inbred interactions were significant for tunnel length, and percentage of the stem damaged. Individual analyses of variance showed significant differences among inbreds for tunnel length in only one year. Nevertheless, as the year × inbred interactions were mostly due to magnitude differences rather than to rank differences, mean comparisons among inbreds was made across years.

Although there was a relatively good agreement between the inbred lines with the shortest tunnels and those with the lowest percentage of the stem damaged (Table 1), some inbreds lines, as BP2 and A670, showed large tunnel lengths and low percentages of stem damaged. Although the coefficient of correlation between tunnel length and percentage of the stem damaged was significant (Table 2), the degree to which both traits varied together was only 0.79 because variability for stem length among inbreds was high (data not shown). Percentage of the stem damaged takes into account the extension of the damage as well as the vigor of the genotype and gives a better estimation of inbred line performance under pink stem borer attack because taller plants normally are more productive; then, yield losses caused by the same extension of damage could be compensated by the higher yield potential (Butron et al. 1999b).

Removing those inbred lines that were missed in one of the trials (Oh43, A295, A678, A635, EP68, EP70, and CML349) or were excessively late (IML6, TD34, TD25, B52, W576, and CML240), 17 field corn and 3 popcorn inbreds did not differ significantly from W552, which presented the lowest percentage of the stem damaged. Nevertheless, when considering both kinds of damage, stem and ear damage, only one of the three popcorn inbreds, namely BP2, and nine of the 18 field corn inbreds, namely EP77, EP78, B97, B98, A671, LH51, TD16, W570, and W572, were among the least damaged. It could be possible to increase the stem and ear resistance of popcorn varieties without crossing to field corn varieties that could introduce unfavorable characteristics for kernel quality (Robbins and Ashman 1984).

Three of the inbred lines tested were obtained by a pedigree breeding program for developing inbred lines resistant to pink stem borer, i.e. EP77, EP78, and EP79. Two of them (EP77 and EP78) were derived from the same cross (EP31 × CM109) and stood up for presenting a low percentage of the stem damaged, although they showed large tunnel lengths (Table 1). Selection was made, on infested plants with pink stem borer eggs, against broken plants by choosing the most appealing ears. Tunnel length was not a selection trait, but selection favoring plant vigor and ear appearance under insect pressure could reduce the percentage of the stem damaged and ear damage. The success reached by this selection program is obvious when the relative better performance of EP77 and EP78 compared to the tester A509 is judged against the better performance of A509 compared to the parental inbred lines of EP77 and EP78 (EP31 and CM109) observed in a previous study (Butrón et al. 1999a). EP79 was obtained by pedigree selection from the cross EP39 × CM109. In that previous work (Butrón et al. 1999a), EP39 and CM109 did not differ from A509 for tunnel length and ear damage. Nevertheless, in the present evaluation EP79 showed significantly less damage on the ear than A509, although both inbreds did not differ for stem damage. The improvement of ear resistance observed in EP79 compared to their parental inbreds and the lack of improvement for stem resistance rely on the breeding methodology used, because selection was made two months after infestation discarding plants with weak stems and favoring plants with good ear appearance. Therefore, more attention was put on ear resistance than on stem resistance, because stem weakness depends on several factors other than stem damage.

Inbreds B97 and B98 were resistant to pink stem borer attack and were released as resistant to the first and second generations of *Ostrinia nubilalis* (Hallauer et al. 1994). Therefore, resistance to both corn borers, *Ostrinia nubilalis* and *Sesamia nonagrioides*, could be somehow related (Velasco et al. 1999b). Lines W570 and W572 have been registered (Coors and Mardones 1989) as resistant to stalk lodging and crushing and were derived from a cross involving the line A635, previously reported as resistant to the pink stem borer (Butrón et al. 1999a).

Among inbred testers (A509, PB130, A635, EA2024, and Oh43), A635 and Oh43 were missed in one year. PB130 was among the most resistant inbreds to stem attack, but was the least resistant to ear attack along with another inbred tester, A509 (Table 1). On the other hand, the inbred EA2024 was resistant to ear attack, but was among the most damaged inbreds on the stem. A509 was surpassed by most of the non previously tested inbreds either for stem resistance or ear resistance. These results support the importance of the evaluation of new materials because they can improve the level of resistance already achieved. Similarly, Malvar et al. (2004) found some corn landraces that improved stem resistance to corn borers compared to that reported by Malvar et al. (1993).

There was a significant and negative relationship between percentage of stem damaged and days to silking (Table 2), agreeing with Cartea et al (1994) that pointed out that late inbreds were, in general, less damaged by the pink stem borer than early inbreds. No relationship was found between resistance to pink stem borer and germplasm group (data not shown).

Hybrids

There were significant differences among hybrids for days to silking, days to pollen shed, stem lodging, tunnel length, percentage of the stem damaged, and general appearance of the ear (data not shown). Individual analyses for grain yield did not detect differences among hybrids in 2002, but did in 2003 (data not shown). However, weather conditions, during corn growing period, in 2003 were atypical, allowing late materials to exceptionally complete their development. In 2003, the *Bt* genotype yielded significantly more than the other hybrids (Table 4). Among non *Bt* hybrids, the cross between the experimental inbred line EP77 and B93 showed significantly larger yield than other hybrids in 2003 and did not differ from the *Bt* hybrid in 2002. The hybrid EP77 × B93 also presented higher levels of stem and ear resistance compared to the susceptible check, INRA 260. Therefore, the good performance of this hybrid under pink stem borer infestation showed the success of the pedigree selection for resistance to pink stem borer because this hybrid involves an inbred line (EP77) derived from that selection program. However, the hybrid EP77 × B93 as well as the *Bt* hybrid could be too late for European Atlantic conditions as maturity and moisture data reveal (Tables 1 and 3). The other hybrid (EP79 × W552) which involves the inbred EP79 developed by selection for resistance to pink stem borer, differed significantly from A661 × EP42, described as tolerant in a previous work (Butrón et al 1999b), for stem and ear damage, but did not differ for yield under pink stem borer infestation. EP79 was obtained from the cross EP37 × CM109. The inbred EP79 was the earliest one and among the shortest inbreds because its parental inbred. Crosses to EP79 could be recommended for areas with short season, but never for areas with higher yield potential.

The coefficients of correlation confirmed the results obtained in the inbred trial (Table 2). Besides, the high correlation coefficients between the number of larvae of *Sesamia* and tunnel length (0.97, $P < 0.01$) and between the number of larvae of *Ostrinia* and general appearance of the ear (-0.96, $P < 0.01$) confirmed that *Sesamia* larvae prefer attacking corn stems rather than ears and larvae of *Ostrinia* are found on the ears rather than on the stem (Cordero et al. 1998). The high coefficient of correlation between stem lodging and tunnel length (0.98, $P = 0.0024$) agreed with observations made by Anglade (1961) who said that most of the larvae development takes place inside the corn plant and can provoke stem lodging.

As conclusions, new sources of resistance to pink stem borer have been detected among inbred lines improving the level of resistance presented by previously tested inbreds. Some of these lines were successfully developed by pedigree selection for resistance to pink stem borer.

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Noctuidae), and European corn borer (Lepidoptera: Pyralidae). J. Econ. Entomol. 84: 693-698.

Table 1. Means for several traits under pink stem borer infestation of 49 inbred lines evaluated in two years

Genotype	Germplasm group[1]	Days to silking	Days to pollen shed	Tunnel length	Percentage of damage on the stem	General appearance of the ear	Stem lodging
				cm	cm/1cm	(1- 9)[2]	%
Inbreds [3]							
CML349	O	100	99	6.4	0.07	7.1	
EP70	L	79	76	6.0	0.10	9.0	
W572	R	81	83	13.1	0.14	6.9	
CML240	O	88	87	20.8	0.18	7.3	
W552	CB	71	70	15.9	0.18	5.5	
W576	L	88	87	21.4	0.18	5.4	
Va26	L	78	76	25.4	0.19	6.8	
LH51	L	85	85	16.5	0.19	6.2	
TD16	O	81	83	19.9	0.20	6.3	
B52	CB	90	89	22.1	0.20	7.9	
B98	L	84	84	26.8	0.22	7.2	
B97	R	80	79	26.7	0.22	7.5	
A672	R	81	81	22.7	0.22	4.7	
EP72	CB	82	82	19.4	0.22	4.8	
TD25	O	89	86	24.0	0.22	8.4	
A682	L	73	72	23.0	0.22	5.5	
A671	L	74	74	23.9	0.23	6.7	

W570	R	81	80	25.8	0.23	6.2	
TD34	O	89	89	33.1	0.23	7.7	
IML6	O	85	88	29.2	0.24	6.8	
EP77	R	70	70	37.3	0.25	7.2	
EP68	CB	74	72	22.4	0.25	6.6	
BP1	O	78	78	22.8	0.26	5.4	
A677	L	71	71	27.9	0.26	5.7	
PB130	O	65	63	20.5	0.26	2.5	
BP2	O	78	78	36.0	0.26	7.6	
A635	R	79	80	19.3	0.27	4.3	
EP78	R	66	65	34.6	0.28	6.2	
A670	L	78	77	39.9	0.28	4.6	
B93	L	74	73	27.1	0.28	5.6	
IADS28	O	82	82	34.5	0.28	5.5	
A678	O	75	73	27.5	0.28	5.9	
EP79	R	64	65	36.2	0.51	5.8	
LSD		7	6	-	0.22	3.0	
Hybrids							
MEB531Bt		68	68	1.7	0.01	9.0	1.5
EP77 × B93		64	66	41.9	0.21	8.2	29.3
EP79 × W552		60	59	33.4	0.25	7.2	22.9
A661 × EP42		61	60	60.9	0.43	6.9	51.0
INRA 260		58	57	50.5	0.37	6.3	35.2
LSD		3	3	23.9	0.16	0.8	24.3

[1] Inbreds were classified in four germplasm groups: Reid (R), Lancaster (L), Corn Belt (CB), and Other (O). The Corn Belt group included all the American germplasm that was neither Reid nor Lancaster. Popcorn, European, CIMMYT, and Japanese germplasms made up the group Other.

[2] On a subjective visual scale from 1 (ear totally damaged) to 9 (without ear damage).

[3] Inbreds were ordered according to increasing percentages of damage on the stem.

Table 2. Coefficients of phenotypic correlation among traits evaluated in 49 inbreds and five hybrids in two years.

	Days to silking	Days to pollen shed	Tunnel length	Percentage of stem damaged
Inbred lines				
Days to pollen shed	0.99**			
Tunnel length	-0.44**	-0.43**		
Percentage of stem damaged	-0.60**	-0.58**	0.79**	
General appearance of the ear	0.46**	0.45**	0.04	-0.30*
Hybrids				
Days to pollen shed	0.99**			
Tunnel length	-0.51	-0.54		
Percentage of stem damage	-0.67	-0.72	0.96**	
General appearance of the ear	0.88*	0.92*	-0.77	-0.90*

*, ** Significant at 0.05, and 0.01 probability level.

Table 3. Means for grain yield and moisture under pink stem borer infestation of five maize hybrids evaluated in two years.

	Grain yield (Mg ha^{-1})		Grain moisture (g hg^{-1})	
	2002	2003	2002	2003
MEB531Bt	6.8	11.7a	31.2	32.1
EP77 × B93	6.0	8.0b	30.1	34.4
EP79 × W552	5.7	6.0c	29.3	29.5
A661 × EP42	7.3	5.5c	27.3	29.1
INRA 260	6.6	6.2c	28.4	29.1
LSD	-	1.7	-	-