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Evaluation of resistance to *Spodoptera frugiperda* in sweet and field corn genotypes

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Abstract

Maize can be affected by different pest damages throughout the crop cycle. Some insects are harmful, among which the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae), is considered the most deleterious pest, for attacking plants of both field and sweet corn plants in all stages. Thus, the use of resistant genotypes has become an important strategy in integrated pest management and highly valued in plant breeding programs. However, information regarding the impact of pests on sweet corn is scarce, requiring studies on possible resistant genotypes. The objective of this study was a laboratory evaluation of the resistance of 10 sweet corn genotypes and two controls of field corn (BRS 1030 and Zapalote Chico), as well as the damage caused by *Spodoptera frugiperda*, by means of multivariate analysis. A completely randomized experimental design with 27 replications was used. Eighteen traits were evaluated during the whole caterpillar cycle, including biological traits and indices of consumption and food use. After the multicollinearity diagnosis, only six traits were maintained for the canonical analysis and factor analysis as well as UPGMA clustering, which differentiated the 12 genotypes in three groups. The canonical analysis explained 89% of the total variation among the six traits and factor analysis divided the traits in two factors. The results showed that the sweet corn genotypes, Teea Dulce, MG 161, Doce Flor da Serra, Doce Cubano and Tropical Plus, tend to have antibiosis as resistance mechanism to fall armyworm due to the low consumed leaf area and the impaired insect development.

Introduction

Sweet corn (*Zea mays* L. *saccharata*) is classified as a specialty corn for having a sweeter flavor than field corn, due to the mutant genes that block the sugar-starch conversion in the endosperm (Creech, 1965; Kwiatkwowski and Clemente; 2007). The only difference of this corn type from field corn is the grain, which dries slowly after physiological maturity and becomes glassy, because of the crystallization of highly concentrated sugars, and wrinkled, due to the lower starch proportion in the endosperm (Stork and Lovato, 1991).

Sweet corn is considered one of the most important vegetables in the United States, Canada and Europe, where it is consumed both fresh and preserved. In Brazil, it is grown on about 36 thousand hectares, corresponding to 14.4% of the area cultivated in the United States, and a worldwide total of about 900 thousand hectares (Camilo et al., 2015).

The economic importance of maize requires more research efforts to develop lines or genotypes with higher yields and lower damage caused by pest insects. From sowing to harvest, maize is exposed to different attacks, resulting in damaged roots, stems, leaves, and ears (Rosa et al., 2010). One of the most harmful insects to the crop is the fall armyworm, *Spodoptera frugiperda* (J. E. Smith, 1797) (Lepidoptera: Noctuidae), causing damages that most frequently reach the level of economic damage (Grützmacher et al., 2000).

This caterpillar is considered the most deleterious pest, for attacking plants of both field and sweet corn plants in all stages, although it prefers leaf whorls of young plants (Giolo et al., 2002). The losses vary in intensity, according to the genotype, development stage, infestation degree, and environmental conditions. In Brazil, it is estimated that more than 25% of the losses caused by maize pests correspond to damage caused by *S. frugiperda* (Waquil and Vilella, 2003), which is the main pest in maize in this country (Farias et al., 2014).

Commonly, agrochemicals are sprayed to reduce the

pest damage. However, there is a need for a reduction in the use of energy and chemicals, minimizing undesirable residues in the environment and food, aside from reducing the risk of poisoning of field workers during crop management (Cunha et al., 2008; Crubelati-Mulati et al., 2014). In addition, *S. frugiperda* has developed resistance to several insecticides available on the market (Ayil-Gutiérrez et al., 2018). In this sense, one approach that can be used to control this pest is the development of resistant genotypes (Oliveira et al., 2018).

The use of resistant genotypes has been highly valued in plant breeding programs (Zhu et al., 2019), for representing an efficient method of pest control, by causing adverse effects on the biology of insects (antibiosis) or by causing insects to prefer one plant less than another for feeding (non-preference) (Smith, 2005), due to chemical and/or physical plant traits. In addition, the strategy contributed to reduction in agrochemical application. However, no research on the impact of pests on sweet corn is available. In this context, in this laboratory study the resistance of 10 sweet corn genotypes and two controls of field corn, against *S. frugiperda* was analyzed by multivariate analysis.

Materials and Methods

Experimental design and environmental conditions

The experiments were carried out at the Laboratory of Entomology of the Department of Agronomy, State University of Maringá (UEM) and in a greenhouse, on the experimental farm of Iguatemi-PR. The sweet and field corn seeds were kindly provided by the breeding program of specialty maize of the Department of Agronomy-UEM. A completely randomized design was used in the experiment with 12 treatments (Table 1) and 27 replications, and each caterpillar was considered one replication.

Sowing was performed in 14-L plastic pots, containing a mixture of soil, substrate (3:1 rate) and N-P-K fertilizer. Controlled irrigation was applied to the pots throughout the experiment. The laboratory experiment was carried out in a climate chamber under controlled conditions (25±1°C, 70 ± 10% RH; photophase 12h).

Fall armyworm rearing

After hatching, the larvae resulting from oviposition from mass rearing were individually isolated on transparent acrylic Petri dishes (diameter 9.0 cm, height 1.5 cm), lined with moistened filter paper. The larvae were maintained in a controlled environment during the larval phase, with freely fed with leaf blades collected from the middle third of the evaluated maize genotypes between the phenological stages V8 and V15 (8-15 expanded leaves), according to the scale of Ritchie et al. (1993). The food was exchanged daily and the same corn genotype was fed to the caterpillar of a treatment throughout the larval period.

The newly formed pupae were maintained in acrylic plates (diameter 9.0 cm, height 1.5 cm), lined with moistened filter paper, until adult emergence. The adults were confined in plastic pots (diameter 9 cm, height 18 cm) and were fed until death with a water: honey solution (10:1), exchanged every two days.

Traits under evaluation

The biological traits evaluated were the mean larval period (MLP), final larval biomass (FLB), mean larval biomass (MLB), pupal period (PP), mean pupal biomass (MPB), adult longevity (AL), and the biological cycle (BC).

The consumption and use of the sweet and field corn

Table 1: Genotypes and characteristics of sweet and field corn under study

Code	Genotypes	Grain type	Genetic characterization
1	BR-400	Sweet Corn	Variety
2	Milho Doce 1	Sweet Corn	Variety
3	MG162 - Amarelo Doce	Sweet Corn	Landrace
4	Tuc Blanco Dulce EEAOC	Sweet Corn	Variety
5	Doce Opaco	Sweet Corn	Variety
6	Teea Dulce EEAOC	Sweet Corn	Variety
7	MG 161 - Branco Doce	Sweet Corn	Landrace
8	PR030-Doce Flor da Serra	Sweet Corn	Landrace
9	Doce Cubano	Sweet Corn	Variety
10	Tropical Plus	Sweet Corn	Single-cross hybrid
11	BRS 1030	Field corn	Single-cross hybrid
12	Zapalote Chico [(1)]	Field corn	Variety

[(1)] described by several authors as source of fall armyworm resistance (Wiseman and Widstrom, 1986; Boiça et al., 1993).

genotypes by *S. frugiperda* were evaluated daily throughout the larval stage of the insect. To obtain the total feces weight (FW) of the larval period, the measurements were only initiated on the fifth day of life of the caterpillars, since FW was not measurable before. The measurements of caterpillar biomass were also initiated as of the 5th day of life of insects as: MLB = Σ B / N, where MLB is the mean caterpillar biomass, B is the daily caterpillar biomass and N is the duration of larval period in days.

The consumption and use indices of genotypes of sweet and field corn were calculated as proposed by Waldbauer (1968), with the modifications suggested by Scriber and Slansky Jr. (1981). The relative consumption rate (RCR), relative metabolic rate (RMR), relative growth rate (RGR), conversion efficiency of ingested food (CEI), apparent digestibility (AD), and the conversion efficiency of digested food (CED) were calculated as showing bellow:

$$RCR = CLW/ (Wm*T)$$

$$RMR = MF/ (Wm*T)$$

$$RGR = Wf/ (Wm*T)$$

$$CEI = (Wf/ CLW)* 100$$

$$AD = (AF/ CLW)* 100$$

$$CED = (Wf/AF)*100$$

where T is the duration of the larval period; CLW the weight of ingested (consumed) food during T; Wf is the final caterpillar weight; Wm is the mean caterpillar weight during T; FW are feces = undigested food + feces during T; AF is the assimilated food during T = CLW - FW; MF is food metabolized during T=AF - Wf.

Throughout the larval cycle, the total consumption of the leaves given to the caterpillars was evaluated. The consumed leaf area (CLA) was calculated as: CLA= SLA – LLA, where SLA is the supplied leaf area and LLA is the leftover leaf area after 24 h of supply to the insects.

Statistical analysis

The data were analyzed using a multivariate analysis of variance (MANOVA). The hypothesis of significant differences between vectors of means of each genotype was tested by the Wilks test ($p \leq 0.05$), using the GLM procedure, option MANOVA, in software SAS (SAS Institute, 2011).

The condition index (CI) and the variance inflation factor (VIF) were used as criterion to eliminate the problems of multicollinearity among the traits. Multicollinearity is considered weak when the CI <100 and VIF> 10 evidence substantial multicollinearity (Montgomery and Peck, 1981; Prunier et al., 2015). Therefore, in cases where CI and VIF were greater than 100 and 10, respectively, the traits were eliminated.

Then, canonical analysis, factor analysis and genotype grouping were used for the set of traits that were not discarded. Clustering was performed by the UPGMA method, based on Mahalanobis' generalized distance. In addition, the cophenetic correlation was calculated as proposed by Sokal and Rohlf (1962), to check the group consistency. These analyses were performed with software Genes (Cruz, 2006) and the packages "vegan" and "candisc" of R (R Development Core Team, 2016).

Results and discussion

Multivariate analysis improves the selection of traits in breeding programs

Considering all analyzed traits, significant differences ($p<0.0001$) between the vectors of means of the studied genotypes were confirmed by the Wilks test (means in Table 2). This result confirmed the existence of genetic variability among the genotypes. This genetic variation should be used to select and improve different genotypes in a breeding program based on a subset of traits, and another set of traits should be eliminated by problems, as well as, collinearity or correlation among them. In this study, the traits AF, MF, CED, CEI, FW, CLW, RGR, RMR, FLB, MLB, MPB and BC were discarded based on the multicollinearity diagnosis because showed CI and VIF higher than 100 and 10, respectively. Thus, only the traits CLA, AD, RCR, MLP, PP and LA remained, whose multicollinearity diagnosis resulted in CI of 60.76 (Table 3). As mentioned, the multicollinearity analysis, using VIF and CI criteria, is usually used in multivariate analysis and often justifies the removal of some traits (Prunier et al., 2015).

Oliveira et al. (2018) and Sanches et al. (2019) also used multivariate techniques to eliminate traits when evaluating the resistance of popcorn genotypes to *Spodoptera frugiperda*. Using multivariate analysis allowed a significant reduction in the number of study traits, increasing the chances of high selection efficiency, because according to Aaliya et al. (2016) the use of a lower number of traits, indicating the effective goals of the researcher, prevents the use of interrelated traits, avoiding redundancy in selection in a breeding program.

In this study, two factors were estimated by factor analysis, the first being a determinant factor for the traits CLA, AD, PP, and AL, while the second factor involved RCR and MLP (Table 4). Sanches et al. (2019) also reduced sixteen traits to five traits that were divided in two factors using factor analysis. Thus, they were able to concentrate the selection on five traits, facilitating the interpretation of the results.

Table 2: Mean of 18 traits evaluated in the 12 genotypes

	Evaluated traits [1]																	
	AF	MF	CLA	AD	CED	CEI	FW	CLW	RCR	RGR	RMR	MLP	FLB	MLB	PP	MPB	AL	BC
	(g)	(g)	(cm²)	(%)	(%)	(%)	(g)	(g)	(g/g)	(g/g)	(g/g)	(days)	(g)	(g)	(days)	(g)	(days)	(days)
BR 400	1.358	0.743	222.344	38.368	59.020	19.808	1.955	3.313	1.071	0.199	0.238	15.074	0.615	0.211	11.185	0.249	11.963	41.222
Doce1	1.831	1.192	289.678	44.169	40.648	16.482	2.195	4.026	1.212	0.192	0.362	16.000	0.639	0.210	11.444	0.260	12.519	42.963
MG162	1.993	1.339	290.493	44.121	42.255	15.876	2.294	4.287	1.255	0.192	0.384	16.556	0.654	0.207	12.667	0.251	12.630	44.852
TucBlanco	1.719	1.086	254.368	40.546	49.614	16.591	2.289	4.008	1.255	0.198	0.338	15.259	0.632	0.211	12.074	0.252	13.556	43.889
Doce Opaco	2.267	1.626	298.837	48.528	32.545	14.538	2.319	4.586	1.483	0.205	0.534	16.370	0.641	0.195	13.000	0.260	15.630	47.519
Teea Dulce	0.838	0.250	208.215	28.905	73.179	20.470	2.042	2.880	1.371	0.280	0.119	15.556	0.588	0.171	11.630	0.243	18.704	48.259
MG161	0.916	0.329	198.493	33.994	66.371	21.941	1.796	2.712	1.401	0.304	0.169	14.926	0.588	0.166	11.704	0.235	19.111	48.667
Doce Flor	0.812	0.235	189.327	31.130	71.612	22.106	1.813	2.625	1.402	0.310	0.125	14.630	0.576	0.165	11.037	0.227	20.370	49.000
Doce Cubano	0.913	0.318	228.119	31.317	67.880	20.581	2.000	2.913	1.438	0.292	0.150	15.000	0.595	0.174	11.074	0.246	23.000	52.148
Tropical Plus	0.803	0.232	204.036	31.406	74.088	22.336	1.800	2.603	1.418	0.317	0.126	15.074	0.570	0.158	11.000	0.240	23.556	51.926
BRS1030	0.905	0.324	155.210	38.208	68.401	24.562	1.486	2.391	1.021	1.375	0.752	16.778	0.581	0.141	11.926	0.229	15.444	47.333
Zapalote Chico	0.921	0.343	171.925	34.915	65.770	22.017	1.727	2.647	1.077	1.464	0.885	17.481	0.578	0.142	12.593	0.225	16.148	49.222

[1] AF: assimilated food; MF: metabolized food; CLA: consumed leaf area; AD: apparent digestibility; CED: conversion efficiency of digested food; CEI: conversion efficiency of ingested food; FW: feces weight; CLW: consumed leaf weight; RCR: relative consumption rate; RGR: relative growth rate; RMR: relative metabolic rate; MLP: mean larval period; FLB: final larval biomass; MLB: mean larval biomass; PP: pupal period; MPB: mean pupal biomass; AL: adult longevity; BC: complete biological cycle.

Acquaah et al. (1992), highlighted that factor analysis, in which the traits that best explain each biological phenomenon are united in each factor, require prior knowledge of the biological parameters to ensure a very careful choice of the target trait(s) of selection. Furthermore, O'Brien (2007) commented that factor analysis, through the commonality values, is adequate to select important traits. In this sense, factor analysis identified two factors that enable the detection of genotypes that are resistant and susceptible to *S. frugiperda* among the studied maize genotypes. The traits CLA, AD, PP and AL were united in the first factor (Table 4), with the highest contribution. Therefore, selection should be carried out based on these traits, simplifying result interpretation.

Table 3 : Multicollinearity diagnosis for consumed leaf area (CLA), apparent digestibility (AD), relative consumption rate (RCR), mean larval period (MLP), pupal period (PP) e adult longevity (AL) related to *Spodoptera frugiperda* resistance, evaluated in 10 sweet corn and two field corn genotypes

VIF		CI	
Diagonal		Order	Eigenvalue
1	7.079	1	3.585
2	9.658	2	1.588
3	2.727	3	0.839
4	0.641	4	0.065
5	1.289	5	0.059
6	8.258	6	0.136
Number of VIF≥ 10	0	CI(max/min)	60.76

Cluster analysis help to identify antibiosis mechanism of resistance to S. frugiperda

Canonical analysis explained 89% of the total variation among the six traits. The first canonical variable (Can1) was assigned 63.4% and the second canonical variable (Can2) was responsible for 25.6% of total variation (Figure 1). The UPGMA-based dendrogram grouped the 12 genotypes in three distinct groups: two with five sweet corn genotypes each (BR 400 (1), Doce 1 (2), MG 162 (3), Tuc Blanco (4) and Doce Opaco (5)) and (Teea Dulce (6), MG 161 (7), Doce Flor da Serra (8), Doce Cubano (9) and Tropical Plus (10)) and one with the controls (BRS 1030 (11) and Zapalote Chico (12)) (Figure 2).

The relationships between the 12 genotypes (Figure 2), based on a high cophenetic correlation (0.96), indicated an excellent fitting of the method (UPGMA), similarly with the study of Sanches et al. (2019). Three groups were formed in this dendrogram as well as in

Table 4 :Values of the factorial, initial (CFI) and final loadings (CFF) and commonalities obtained by factor analysis based on means of 10 sweet corn and two field corn genotypes, for the six selected traits

Trait	CFI		CFF		Commonality
	Factor 1	Factor 2	Factor 1	Factor 2	
CLA	0.620	-0.733	0.763	-0.583	0.922
AD	0.922	-0.263	0.957	-0.059	0.919
RCR	0.084	0.979	-0.128	0.974	0.965
MLP	0.717	0.644	0.562	0.783	0.929
PP	0.793	0.284	0.714	0.448	0.710
AL	-0.795	0.090	-0.796	-0.083	0.640

CLA: consumed leaf area; AD: apparent digestibility; RCR: relative consumption rate; MLP: mean larval period; PP: pupal period; and AL: adult longevity.

canonical analysis (Figure 1), based on six traits that can help identify possible resistance and/or susceptibility mechanisms in each genotype group.

According to our results, the primary traits were essential for selection of maize genotypes with *S. frugiperda* resistance. The results of canonical analysis and the clustering (UPGMA) analysis indicated that the controls BRS 1030 (11) and Zapalote Chico (12), were separated from the other genotypes for having a smaller consumed leaf area (155.21 and 171.93 cm^2, respectively), indicating the non-preference of larvae for these genotypes. In their studies, Viana and Potenza (2000) and Sanches et al. (2019) also observed

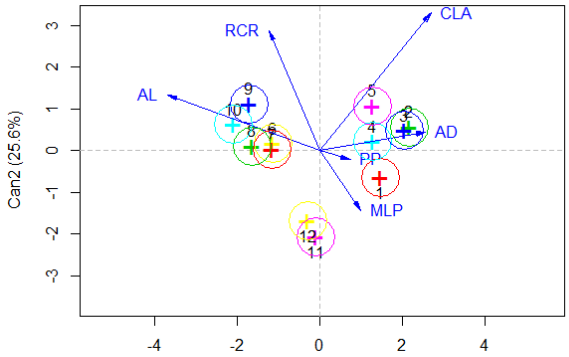

Fig. 1 - Biplot of the first two canonical variables showing the dispersion of 10 sweet corn and two field corn genotypes as to analyzed traits. 1: BR 400; 2: Doce 1; 3: MG 162; 4: Tuc Blanco;5: Doce Opaco; 6: Teea Dulce; 7: MG 161; 8: Doce Flor da Serra; 9: Doce Cubano; 10: Tropical Plus; 11: BRS 1030; 12: Zapalote Chico. CLA: consumed leaf area; AD: apparent digestibility; RCR: relative consumption rate; MLP: mean larval period; PP: pupal period; and AL: adult longevity.

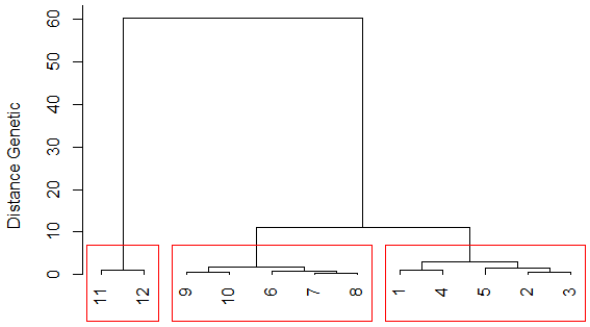

Fig. 2 - Dendrogram representative of genetic dissimilarity among 10 sweet corn and two field corn genotypes, obtained by the UPGMA clustering method based on Mahalanobis' generalized distance. 1: BR 400; 2: Doce 1; 3: MG 162; 4: Tuc Blanco;5: Doce Opaco; 6: Teea Dulce; 7: MG 161; 8: Doce Flor da Serra; 9: Doce Cubano; 10: Tropical Plus; 11: BRS 1030; 12: Zapalote Chico. Cophenetic correlation with dissimilarity matrix: 0,960.

a lower preference for Zapalote Chico than for the other genotypes.

On the other hand, for the genotypes BR 400 (1), Doce 1 (2), MG 162 (3), Tuc Blanco (4), and Doce Opaco (5), the consumed leaf area and apparent digestibility were highest, indicating the ease of fall armyworm to digest leaves of these genotypes, since digestibility represents the amount of food effectively used by the insect for metabolism and growth. According to Panizzi and Parra (2009), the physical properties (food hardness, surface, shape) and the allelochemicals and nutritional components are decisive for the insects' ability to consume and digest food.

For the genotypes Teea Dulce (6) MG 161 (7), Doce Flor da Serra (8), (9) Doce Cubano and Tropical Plus (10), of the other group, the values of apparent digestibility were lowest and adult longevity longest. These same genotypes showed low consumed leaf area and the pupal period of Doce Flor da Serra (8), Doce Cubano (9) and Tropical Plus (10) were the lowest obtained, suggesting an antibiosis resistance mechanism in these genotypes, because the consumption of food was low and the insect development was impaired. This effect of antibiosis can occur due a lack of essential nutrients in the plant, as well as by the presence of toxic inhibitors, making one plant less preferred than another (Smith, 2005).

Our results show that multivariate analysis, such as the canonical analysis and factor analysis are important tools to evaluate the resistance of maize to *S. frugiperda* in a breeding program, as mentioned by Wisser et al. (2011), Oliveira et al. (2018) and Sanches et al. (2019). The genotypes Teea Dulce (6), MG 161 (7), Doce Flor da Serra (8), Doce Cubano (9) and Tropical Plus (10), grouped with both UPGMA and canonical analysis, presented antibiosis as resistance mechanism to *S. frugiperda* and the controls (BRS 1030 (11) and

Zapalote Chico (12)), as expected, were the least preferred genotypes in the feeding of caterpillars.

Conclusions

The sweet corn genotypes, Teea Dulce, MG 161, Doce Flor da Serra, Doce Cubano and Tropical Plus, tend to have antibiosis resistance in tropical countries such as Brazil due to the low consumed leaf area and the impaired insect development. Thus, these genotypes can potentially be used in genetic breeding of sweet corn, in stacking genes, for example, in order to increase fall armyworm resistance.

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