



Insect-corn kernel interaction: Chemical signaling of the grain and host recognition by *Sitophilus zeamais*

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ABSTRACT

In living organisms, the cuticle has structural functions and is involved through chemical signaling in biological interactions such as plant-insect and provides protection against biotic and abiotic factors, thereby avoiding desiccation or the attack of predators. The objective of this study was to investigate the participation of the epicuticle in the maize kernel-*Sitophilus zeamais* interaction. The GC-MS analysis of the epicuticle extract demonstrated the presence of aliphatic hydrocarbons, alcohols, ethers, fatty acids, sterols and their derivatives. The results of bioassays show that the epicuticle of maize has a primordial role in its interaction with *S. zeamais*, and participates in the recognition and attraction to the food source, as well as regulating its reproduction. In addition the compounds present in the epicuticle extract may act as signal molecules and development regulators. This study reveals the effect of the maize kernel cuticle on *Sitophilus* behavior and contributes to the understanding of the interaction.

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1. Introduction

In natural and agronomic ecosystems, the interactions between plants and phytophagous insects are initially mediated by chemical signals such as volatiles from plants (Germinara et al., 2008). The silo is a new environment where a large number of biological interactions are produced and these cause significant economic losses (Cox, 2004; Cox and Collins, 2002). In this new environment, the insect *Sitophilus zeamais* (Motschulsky) is considered the principal and the most dangerous plague in tropical and subtropical climates of stored maize kernels (Tefera et al., 2011). This insect affects the harvest by lowering the quality of kernels and germination, also due to an increase in fungal infections by transporting spores and facilitating the penetration of hyphae through the damage done in the grain (Nesci et al., 2011; Yuya et al., 2009). Although numerous

investigations have studied damage to stored grains caused by insects in different biological systems, the signal that initiates the infection process is still unknown.

The first barrier of contact between organisms and the environment is the cuticle (Welti and Wang, 2004; Zunino and Zygadlo, 2005). In plants, this provides protection against the biotic and abiotic factors, and is involved in the plant-insect interactions affecting the behavior of predators and/or parasitoids (Bargel et al., 2006; Lemieux, 1996; Yeats and Rose, 2013). In general, the cuticle is composed of a complex mixture of long-chain non-polar compounds such as hydrocarbons, wax esters, aldehydes, ketones, long chain alcohols, fatty acids, terpenoids and sterols (Lemieux, 1996; Lucini et al., 2006). These compounds can act as precursors of hormones and pheromones, regulate development processes and/or modulate interactions between organisms (Kosma et al., 2010; Lemieux, 1996; Lucini et al., 2006). Although numerous studies have reported on the relationship between plant foliar waxes and insects (Braccini et al., 2015; Kosma et al., 2010; Li and Ishikawa, 2006; van Loon et al., 1992), little is known about the role of the kernel cuticle in grain-insect interactions. Several authors have observed that the cuticular waxes of the wheat grain play an

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important role in oviposition and alimentionation of *S. granarius* (Nawrot et al., 2010; Niewiada et al., 2005). However, there are few investigations about the effect of the cuticle on the maize kernel-*S. zeamais* interaction. García-Lara et al. (2003) observed that the hardness of the kernels is negatively correlated with the susceptibility index and damage caused by *S. zeamais*. Moreover, the polyphenolic compounds of the cuticle of maize kernel were related with resistance to attack by *S. zeamais* (García-Lara et al., 2003; Panagabko et al., 2000; Sen et al., 1994). These results suggest a participation of the cuticle kernel in the interaction with the insect. Based on this, the aim of the present work was to investigate the participation of the epicuticle of the maize kernel as a chemical signal in the interaction with *S. zeamais*, which provided findings towards a better management of this pest in the silo, translating this into a lesser economic loss for farmers.

2. Methods and materials

2.1. Kernels and insects

Maize kernels were obtained from Manfredi Experimental Station (INTA, Córdoba, Argentina) and kept in closed containers at $-20\text{ }^{\circ}\text{C}$ and $70 \pm 5\%$ relative humidity (r.h.). The varieties used were CV: ACA468MGRR2 N° station: 229 (ACA) and Illinois CV: 1767 MG Rep 2. N° station: 222 (ILLI), of which were harvested in 2014. These maize varieties show resistance (ACA) and susceptibility (ILLINOIS) to fungal infection, allowing us to suppose that this could affect the insect's response. *Sitophilus zeamais* adults, without differentiation of age or sex (except in the progeny assay), were reared on maize kernels and maintained under laboratory conditions ($28 \pm 2\text{ }^{\circ}\text{C}$ and $70 \pm 5\%$ r.h.) until being used in the bioassays.

2.2. Kernel epicuticle extraction and GC-MS analyses

The epicuticle components of the two varieties of kernels were removed using a methodology of Russin et al. (1997), with some modifications. Briefly, the extraction was carried out using chloroform (3:2 kernels/ml of chloroform) for 30 s, for washing only the most superficial components of the grain (epicuticle), then the solvent was evaporated and the extract was resuspended, and its weight was quantified.

The GC-MS analysis of the epicuticle extract composition was performed on a Clarus SQ 8T GC/Mass Spectrometer (Perkin Elmer). The methodology used was that reported by Nawrot et al. (2010) with some modifications of equipment. A Perkin Elmer Elite 5MS column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$ of thick) was used. The GC oven temperature was programmed from $40\text{ }^{\circ}\text{C}$ to $320\text{ }^{\circ}\text{C}$ at a rate of $4\text{ }^{\circ}\text{C}/\text{min}$, followed by a 20 min isothermal run. Helium was used as the carrier, and the injector temperature was $300\text{ }^{\circ}\text{C}$ in splitless mode. The mass spectrometer was fitted with an electron ionization source operated at 70 eV, the source temperature was $230\text{ }^{\circ}\text{C}$, and the interface temperature was $280\text{ }^{\circ}\text{C}$ with a solvent delay of 6 min. Mass spectra were recorded from m/z 45–400 amu in the full scan mode. Identification of the compounds was carried out using the NIST2005 library.

2.3. Thickness of the epicuticle

To compare the epicuticle thicknesses, digital photographs of the kernels of both varieties, with or without the epicuticle, were taken using a magnifying glass Olympus SZX16 coupled to an Olympus camera DP71. To carry this out, the methodology of Jacobsen et al. (1971) with some modifications, was used. The kernels of both varieties, with or without epicuticle, were boiled for 15 min in distilled water and placed in a solution of

hydrochloric acid (20%) for 36 h. Then, the kernels were washed with distilled water and cut transversely with a knife. To visualize the epicuticle, the sections were stained with Sudan IV, washed and then observed. Four replicates of each treatment were used.

2.4. Repellent/attraction activity bioassay

To determine the effect of the epicuticle components in the kernels, the behavior of the *S. zeamais* was compared for kernels with (control) or without the epicuticle (treatment), using a two-choice olfactometer bioassay according to Herrera et al. (2015). Briefly, two flasks (250 ml) were connected by a glass tube ($30\text{ cm} \times 1\text{ cm}$ diameter) with a small hole ($1\text{ cm} \times 1\text{ cm}$) in the middle (15 cm from the two flasks), with entry points between the flasks and the tube being sealed with rubber plugs, which were covered with parafilm to prevent gas leakage. Before connecting the flasks and the tube, 14 maize kernels were placed in the flasks (flask A was control: kernels with epicuticle; and flask B was the treatment: kernels without epicuticle). Then for each experimental set, groups of twenty insects, deprived of food for at least 24 h, were released in the hole of the glass tube and tested for 2 h in a climatic chamber. The experiments being carried out between 10:00 and 16:00 h and the response index (RI) was calculated (see below). The position of the flasks was changed at each replication. To discount the effect of the solvent on the response of the insect, a solvent control was performed using 14 kernels with epicuticle in one flask vs 28 kernels (14 with and 14 without epicuticle) in other flask, under the same conditions using both maize varieties. After this, the same two-choice olfactometer was used to evaluate preference for increasing concentrations of epicuticle extract compared with the control.

The experiments were performed seven times for each assay, with each group of insects only being used once. For each trial, the RI was calculated from equation 1:

$$RI = \left[\frac{(T - C)}{Tot} \right] \times 100$$

where, T was number of insects responding to the treatment; C was number of insects responding to the control and Tot was total number of insects responding to the bioassay. In this case, insects in the flask with kernels with an epicuticle or the extract were considered treatment values and insects in the flask with kernels without an epicuticle or solvent were considered control values. Insects that did not show any response in the experiment were not taken into account (Phillips et al., 1993). Positive values of RI indicated attraction to the treatment, while negative ones revealed repellence. For the statistical analysis, the *paired-sample t-test* was used for the choice of insects and ANOVA for the comparison of the response indexes of both varieties.

2.5. Susceptibility of kernels to insect attack. Determination of kernel damage and loss of weight

This experiment was carried out for 20 days under laboratory conditions in 250 mL-flasks simulating store conditions. In each flask, 14 kernels (treatment or control), previously weighed, were introduced and 20 insects were released. After 20 days the kernels were weighed and the percentage of damage was determined by counting the number of perforated grains. For the statistical analysis the one-way ANOVA was used comparing the results obtained, for each determined variable, between both treatments (with and without epicuticle). The experiment was performed seven times.

2.6. Progeny assay of *S. zeamais*

To determine if the epicuticle components exerted an effect on the reproduction or development of *S. zeamais*, bioassays of progeny were carried out according to Nawrot et al. (2010), with some modifications. A 250 mL-flask containing 150 kernels (with or without epicuticle), with two pairs of *S. zeamais* per flask, were maintained under laboratory conditions for 31 days. Then, the adults were removed and the flask was incubated for 11 more days, after which, the emerging insects were counted. The experiment was performed seven times. For the statistical analysis the one-way ANOVA was performed comparing the progeny obtained in each treatment (with and without epicuticle).

3. Results

3.1. Thickness, content and composition of the epicuticle

The amount (weight) of epicuticle extracted was not significantly different between kernel varieties ($P > 0.05$), with the kernels of the ILLI and ACA varieties possessing on average 0.014 ± 0.005 mg/kernel of epicuticle. After the extraction process, a decrease in the thickness of the epicuticle of the kernels of both varieties was observed with respect to the kernels that had not undergone the extraction process (Fig. 1). In kernels without the epicuticle (Fig. 1 b; d) this decrease in thickness was observed as a reduction in the outer layer width of the kernels, whereas in the ILLI variety a greater reduction of the epicuticle could be observed. However, on average, both varieties showed a similar epicuticular

extract yield.

In the chemical composition of the epicuticular extract of both varieties of kernels, 35 compounds were identified (Table 1). In general, the varieties ACA and ILLI presented similar profiles, which were enriched by alkanes (50.98% and 51.36% respectively), fatty acids (19.43% and 0.58%), sterols (6.53% and 15.95%) aromatic compounds (3.65% and 5.85%), alcohols (4.15% and 2.31%) and aldehydes (1.38% and 1.66%). The main compounds in the extracts of both varieties were the alkanes *Tetradecane*, *Hexadecane*, *Octadecane*, *Eicosane* and *Heneicosane*, the aromatic compound *1,2-dichlorobenzene* and the sterols *Simiarenol* and γ -*Sitosterol*. The compound *Hexadecane* was present at greater percentages, than the other compounds, in the epicuticular extract of both corn varieties, with the ACA variety having the highest relative percentage (11.11%) than ILLI variety (9.96%). The compounds *4-ethyl-tetradecane*, *4,22-stigmastadiene-3-one* and *Stigmast-4-en-3-one* were only identified in the ILLI variety, while the ACA variety revealed 8 compounds that were not present in the ILLI variety. Anyway the three main components are present in both varieties.

3.2. Repellent/attraction activity bioassay

The repellent/attraction activities of kernels with or without the epicuticle were investigated to determine their effects on the food choice of *S. zeamais*. For both maize varieties, the insects were significantly more attracted to kernels with the epicuticle ($P < 0.05$) (Fig. 2). The RI for varieties ACA and ILLI was 21.72 ± 13.43 and 39.47 ± 13.84 respectively, with the positive values recorded indicating the attractive effect of the kernels with the epicuticle. To

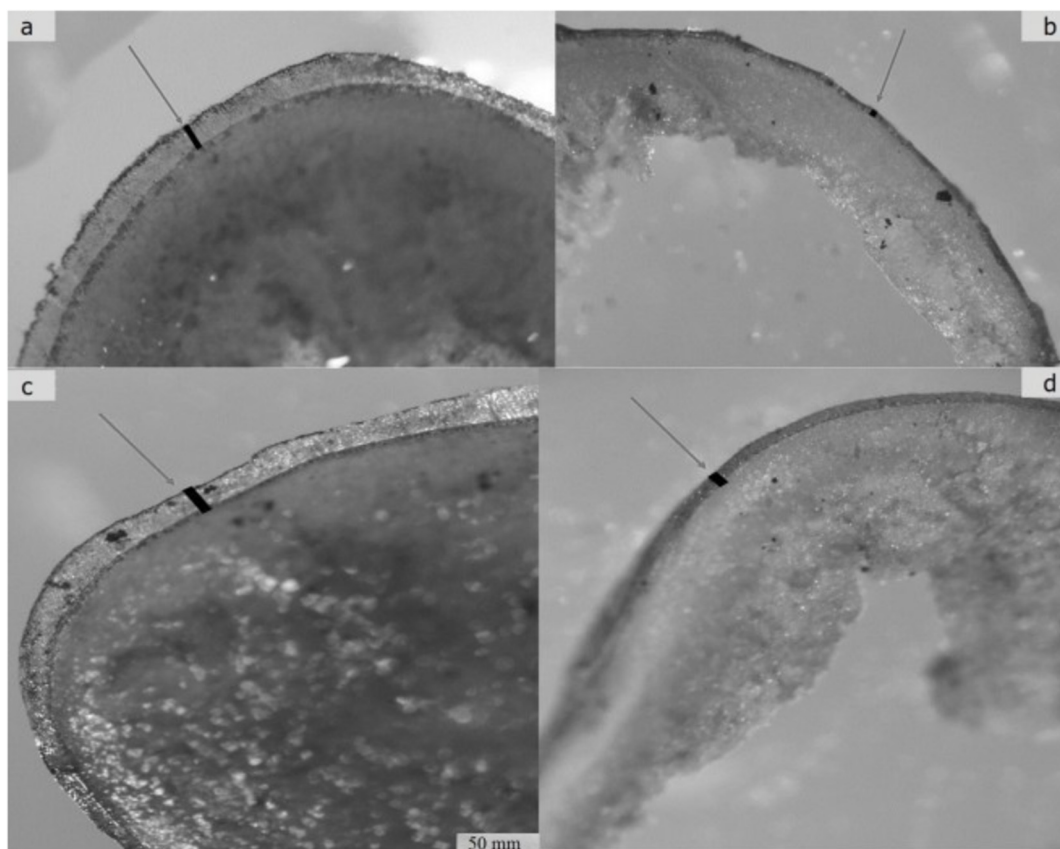


Fig. 1. Cross-section of maize kernel of both varieties. (a) Section of maize kernel of variety ACA468MGRR2 with epicuticle. (b) Section of maize kernel of variety ACA468MGRR2 without epicuticle. (c) Section of maize kernel of variety ILLINOIS CV: 1767 MG with epicuticle. (d) Section of maize kernel of variety ILLINOIS CV: 1767 MG without epicuticle. The arrow and the segment indicate the thickness of the epicuticle. Magnification: $\times 5$.

Table 1
Composition of epicuticle extract of both varieties of maize analyzed by GC-MS (relative percentage).

Kovats Index	Compounds	%ACA	%ILLI
991.69	1,3,5-trimethylbenzene	tr	0.55
1000.71	Decane	1.24	1.80
1012.71	1,4-dichlorobenzene	1.06	1.42
1030.79	1,2-dichlorobenzene	2.59	3.35
1397.73	Diphenyl ether	0.59	0.84
1402.4	Tetradecane	8.73	8.64
1448.08	2-methoxy-naphthalene	tr	0.52
1600.68	Hexadecane	11.11	9.96
1740.38	2-(phenylmethylene)- octanal	1.38	1.66
1749.81	4-ethyl- tetradecane	–	0.80
1757.74	Tetradecanoic acid	tr	0.58
1800.59	Octadecane	9.77	9.25
1800.59	1-Octadecanol	1.12	1.15
1814.89	Heneicosane, 5-methyl-	0.61	–
1899.21	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	–	0.67
1910.65	n-Hexadecanoic acid	8.12	–
1945.03	1-Eicosanol	1.07	–
1976.70	Eicosane	7.50	7.84
1989.57	3-ethyl-5-(2-ethylbutyl)- octadecane	0.67	–
2033.34	(Z,Z)-9,12-Octadecadienoic acid	3.46	–
2038.70	Oleic Acid	3.15	–
2057.63	Octadecanoic acid	4.70	–
2080.02	Heneicosane	5.74	5.96
2158.56	1-Docosanol	1.96	1.16
2211.54	Tetracosane	3.80	–
2225.11	17-Pentatriacontene	0.15	0.95
2434.85	Heptacosane	tr	4.42
2631.97	tetracontane	1.06	0.91
2675.34	Stigmasterol	1.12	1.26
2698.55	(3 α ,4 α)- 4-methyl-cholesta-8,24-dien-3-ol	–	0.68
2704.2	Sitosterol	1.97	2.07
2734.95	4,22-stigmastadiene-3-one	–	1.14
2752.14	Simiarenol	3.44	3.76
2764.44	Stigmast-4-en-3-one	tr	2.44
	Alkanes	50.98	51.36
	Aromatic compounds	3.65	5.85
	Alcohols	4.15	2.31
	Aldehydes	1.38	1.66
	Fatty acid	19.43	0.58
	Sterols and derivates	6.53	15.95

*Components are listed in order of elution in the db-5 column. Percentages were calculated from the peak area without correction. KI: Kovats Index: retention index relative to homologous alkanes. tr: traces (<0.05%); "–": Compound not present.

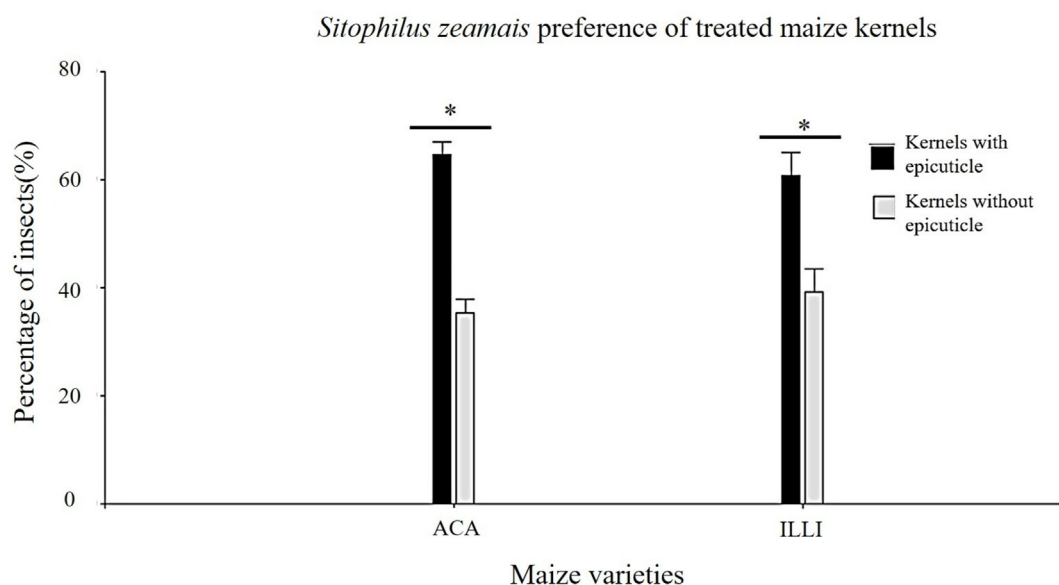


Fig. 2. Percentage of *S. zeamais* individuals that responded to kernels with or without epicuticle in two-choice olfactometer bioassay. Asterisk (*) indicates statistical difference for a paired-sample *t*-test ($P < 0.05$).

Table 2
Percentage of *S. zeamais* individuals that responded to 14 kernels with epicuticle and 28 kernels (14 with and 14 without epicuticle) in two-choice olfactometer bioassay.

Maize Variety	Percentage of Insects in flask with 14 kernels with epicuticle	Percentage of Insects in flask with 14 kernels with epicuticle +14 kernels without epicuticle
ACA	60.86 ± 17.78	39.14 ± 17.78
ILLI	59.88 ± 7.86	40.13 ± 7.86

discard a possible solvent effect on the choice of the insect, an additional experiment was performed. For both varieties, no significant differences were found between flasks ($P > 0.05$), indicating that the repellency of the kernels without the epicuticle was not due to the presence of a residue of solvent remains (Table 2).

The effects of different amounts of epicuticle extract on the behavior response to *S. zeamais* were determined (Fig. 3). For both maize varieties, significant differences were observed for 0.14 and 0.56 mg of epicuticle extract ($P < 0.05$) (Fig. 3). In general, the percentage of insects was higher in flasks with the epicuticle extract, as shown by the positive response index. However, at other concentrations, no dose-dependent response was observed ($P > 0.05$). Finally, when the RIs of each variety were compared, significant differences were obtained ($P < 0.05$).

3.3. Susceptibility of kernels to insect attack and progeny assay

The effect of the epicuticle of the kernels on the feeding and development of *S. zeamais* is shown in Fig. 4 and Fig. 5. After twenty days of incubation, the kernels with an epicuticle were more damaged than the kernels without one ($P = 0.0008$) (Fig. 4), with the former presenting $39.85 \pm 4.59\%$ damage, which was significantly higher than the latter ($25.65 \pm 2.30\%$ damage). In agreement with this result, the percentage of weight loss was significantly higher in the kernels that retained their epicuticle with respect to those without one ($P < 0.05$) (6.73% and 4.13%, respectively) (Fig. 4).

The number of offspring of *S. zeamais* was also significantly different between kernels with or without epicuticle. When

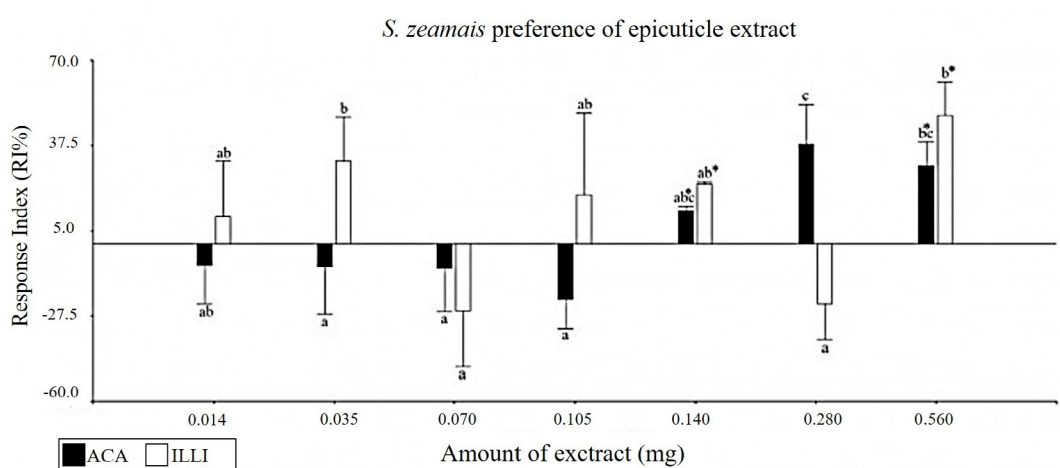


Fig. 3. Response Index (RI) of *S. zeamais* to epicuticle extract of ACA and ILLI kernels in two-choice olfactometer bioassay. Asterisk (*) indicates statistical difference for a paired-sample *t*-test ($P < 0.05$). In each variety, different letters indicate statistical difference for an LSD Fisher Post hoc test ($P < 0.05$).

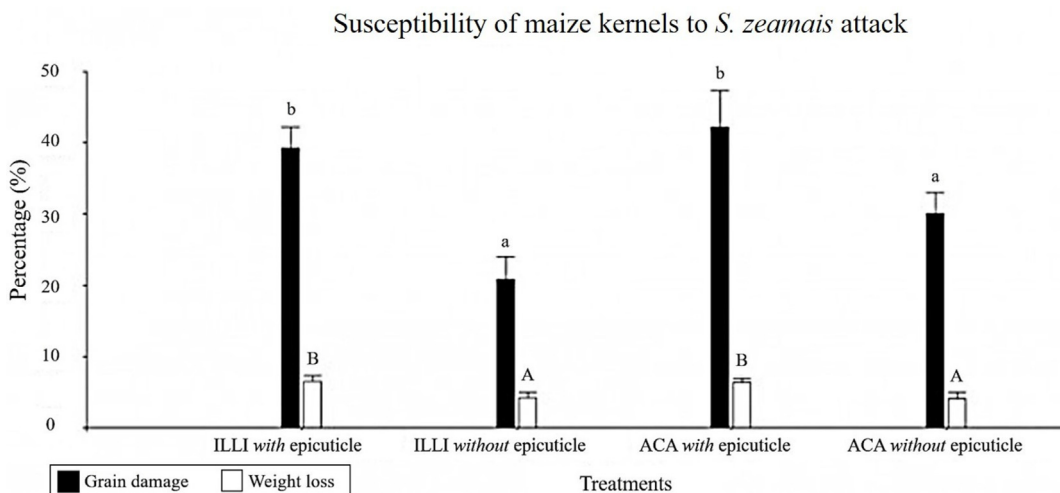


Fig. 4. Kernel damage and weight loss caused by *S. zeamais* expressed as a percentage. Different letters indicate statistical differences for ANOVA ($P < 0.05$) with an LSD Fisher Post hoc test.

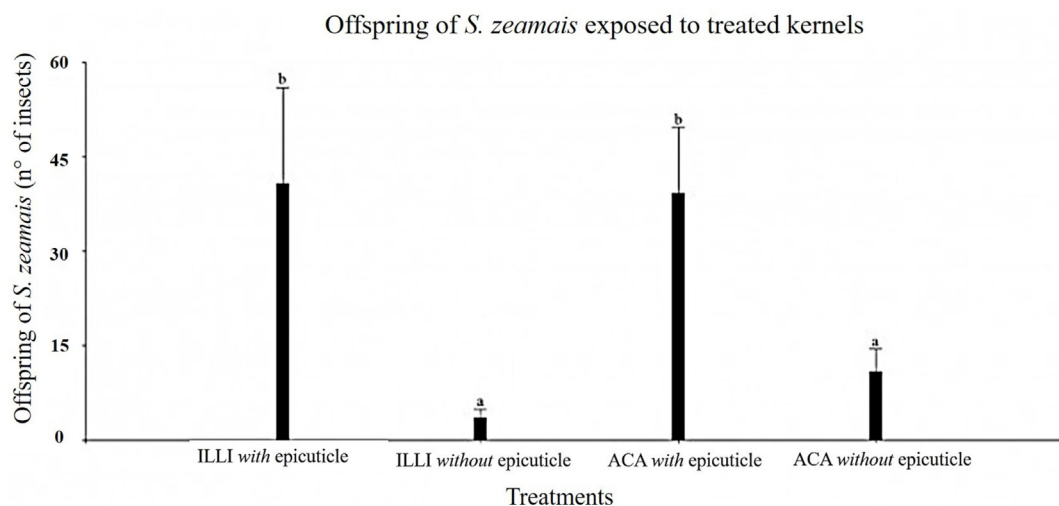


Fig. 5. Offspring of *S. zeamais* exposed to two varieties of kernels with or without epicuticles. Different letters indicate statistical difference for ANOVA ($P < 0.05$) with an LSD Fisher Post hoc test.

S. zeamais was found in an environment made up of kernels with an epicuticle, the number of emergent insects (32.5 ± 7.93 insects) was significantly higher (4-fold) than that of emergent insects in kernels without epicuticle (7.56 ± 2.39 insects) ($P < 0.05$) (Fig. 5).

4. Discussion

In phytophagous insects, such as *Sitophilus* spp, the search for food has been reported to involve two complementary stages: the reaction of insects to the volatile organic compounds (VOCs) emitted by the food source, and the testing of surface by the insects through the gustatory and tactile receptors (Chapman, 2003; Kostromytska et al., 2018; Ukeh et al., 2010). This would allow to suggest that the epicuticle may participate in these complementary search stages. The results obtained in the present study are in agreement with this hypothesis, because maize kernels without epicuticle were less preferred and consequently less damaged by *S.zeamais*.

In our investigation, the volatile attraction stage was demonstrated by the attraction response shown by the insects exposed to the epicuticle extracts of maize kernels. The epicuticle was found to be enriched in alkanes, fatty acid, alcohols, sterols and aldehydes, and to our knowledge, there are no previous reports on the chemical composition of maize cuticle kernels. However, Niewiada et al. (2005) proposed that individual compounds such as those found in the present work, may be involved in the recognition of kernels by insects. In addition, maize cuticle compounds such as *Heneicosane*, *Eicosane*, *Oleic Acid*, *Octadecadienoic Acid* have been reported to be attractive for a wide variety of insects such as *Callosobruchus maculatus* (Fabricius), *Diabrotica virgifera virgifera* (LeConte), and *Aedes aegypti* (Adhikary et al., 2014; Hibbard et al., 1994; Mendki et al., 2000).

Our findings have demonstrated that kernels without the epicuticle were less damaged than kernels with this feature. Tipping et al. (1988) reported that the resistance of kernels is associated with the surface relief, as there were fewer egg plugs observed in kernels with smooth surfaces. This is in agreement with the decrease in the thickness of the epicuticle after the extractive process used in our investigation, associating this decrease of the epicuticle with a possible disappearance of clusters of waxes and, thus, the roughness of the grain surface. Thus, the results presented here suggest that washing the epicuticle with

organic solvent decreased the roughness of the kernel, thereby rendering it less attractive to the insect. Nevertheless, the second stage of recognition implies that testing the surface by means of the gustatory and tactile receptors may be complemented by the presence of phagostimulating compounds, such as *Sitosterol*, *Stigmasterol*, *Octadecanoic Acid*, *Heptacosane* (Doss et al., 1982; Doss and Shanks, 1984; Harada, 1985; Lawrance, 2016), which were found in the maize kernel cuticle. This suggests that the attractive effect of the maize cuticle might be related to the joint action of the cuticular relief and the chemical composition.

Another biological factor that affects plants is the capacity of the insect to produce offspring. The results found in our study demonstrated that insects exposed to kernels without an epicuticle had fewer offspring than those exposed to kernels with one. This is in agreement with Nawrot et al. (2010), who observed that the reproduction rate of the insect *S. granarius* was significantly lower in washed grains of wheat than in the respective controls. Moreover, Howard (2001) reported that the parasitoid *Pteromalus cerealellae* (Boucek) could sense the cuticle hydrocarbons of the insect *Sitotroga cerealella* (Olivier) and thus detect the oviposition sites. Hence, the fewer offspring found in insects exposed to kernels without an epicuticle may be explained by a reduction in oviposition stimulant compounds, such as hydrocarbons, during the cuticle washing (Niewiada et al., 2005). In the present work, some compounds found in the kernel maize cuticle, such as *Friedelinol*, *Heptacosane*, *1-Docosanol*, *Tetracosane*, *Oleic Acid*, have been previously reported to act as markers of oviposition (Braccini et al., 2015; Oshima et al., 1973; Trabalón and Assi-Bessekon, 2008; Wegener et al., 2001). Furthermore, Tipping et al. (1988) demonstrated that grain relief can also affect the oviposition of the insect *S. zeamais*. Taking the above results together, it can be hypothesized that the reproduction of the insect is regulated by both the relief and the chemical composition of the epicuticle of the maize kernels.

Moreover, it should be noted that the response of the insect for all measured parameters did not present a difference between the two varieties despite their differences in chemical composition.

5. Conclusion

In conclusion, the epicuticle of maize kernels, the composition and the relief, have a primordial role in the interaction with *S. zeamais*, by participating in the recognition and attraction to the

food source and regulating its reproduction. According to our knowledge, this work is the first investigation to report on the epicuticle components of the maize kernels and their effects on the behavior and reproduction of *S. zeamais*. This study contributes to a better understanding of the participation of the epicuticle in the kernel-insect interaction to find an integral solution to the problem of infestation in the silos.

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References

- Adhikary, P., Mukherjee, A., Barik, A., 2014. Role of surface wax alkanes from *Lathyrus sativus* L. seeds for attraction of *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). *J. Stored Prod. Res.* 59, 113–119.
- Bargel, H., Koch, K., Cerman, Z., Neinhuis, C., 2006. Structure–function relationships of the plant cuticle and cuticular waxes — a smart material? *Funct. Plant Biol.* 33, 893.
- Braccini, C.L., Vega, A.S., Coll Aráoz, M.V., Teal, P.E., Cerrillo, T., Zavala, J.A., Fernandez, P.C., 2015. Both volatiles and cuticular plant compounds determine oviposition of the Willow Sawfly *Nematus oligospilus* on Leaves of *Salix* spp. (Salicaceae). *J. Chem. Ecol.* 41, 985–996.
- Chapman, R.F., 2003. Contact chemoreception in feeding by phytophagous insects. *Annu. Rev. Entomol.* 48, 455–484.
- Cox, P.D., 2004. Potential for using semiochemicals to protect stored products from insect infestation. *J. Stored Prod. Res.* 40, 1–25.
- Cox, P.D., Collins, L.E., 2002. Factors affecting the behaviour of beetle pests in stored grain, with particular reference to the development of lures. *J. Stored Prod. Res.* 38, 95–115.
- Doss, R.P., Luthi, R., Edelman, D.L., Hrutford, B.F., 1982. Sitosterol and quercetin 3-galactoside, obscure root weevil feeding stimulants from *Rhododendron*. *J. Agric. Food Chem.* 30, 1079–1082.
- Doss, R.P., Shanks, C.H., 1984. Black vine weevil, *Otiorhynchus sulcatus* (Coleoptera: Curculionidae), Phagostimulants from 'Alpine' strawberry. *Environ. Entomol.* 13, 691–695.
- García-Lara, S., Burt, A.J., Serratos, J.A., Pontones, D.M.D., Arnason, J.T., Bergvinson, D.J., 2003. Defensas Naturales en El Grano de Maíz Al Ataque de *Sitophilus zeamais* (Motsch, Coleoptera: Curculionidae): mecanismos Y bases de La Resistencia. *Rev. Educ. Bioquímica (REB, Mex)* 22, 138–145.
- Germinara, G.S., De Cristofaro, A., Rotundo, G., 2008. Behavioral responses of adult *Sitophilus granarius* to individual cereal volatiles. *J. Chem. Ecol.* 34, 523–529.
- Harada, K., 1985. Feeding attraction activities of amino acids and lipids for juvenile yellowtail. *Bull. Japanese Soc. Sci. Fish* 51, 453–459.
- Herrera, J.M., Pizzolitto, R.P., Zunino, M.P., Dambolena, J.S., Zygadlo, J.A., 2015. Effect of fungal volatile organic compounds on a fungus and an insect that damage stored maize. *J. Stored Prod. Res.* 62, 74–80.
- Hibbard, B.E., Bernklau, E.J., Bjostad, L.B., 1994. Long-chain free fatty acids: semi-chemicals for host location by western corn rootworm larvae. *J. Chem. Ecol.* 20, 3335–3344.
- Howard, R.W., 2001. Cuticular hydrocarbons of adult *Pteromalus cerealellae* (Hymenoptera: Pteromalidae) and two larval hosts, *Angoumois grain Moth* (Lepidoptera: Gelechiidae) and *Cowpea weevil* (Coleoptera: Bruchidae). *Ann. Entomol. Soc. Am.* 94, 152–158.
- Jacobsen, J.V., Knox, R.B., Pyliotis, N.A., 1971. The structure and composition of aleurone grains in the barley aleurone layer. *Planta* 101, 189–209.
- Kosma, D.K., Nemacheck, J.A., Jenks, M.A., Williams, C.E., 2010. Changes in properties of wheat leaf cuticle during interactions with Hessian fly. *Plant J.* <https://doi.org/10.1111/j.1365-3113.2010.04229.x>.
- Kostromytska, O.S., Rodriguez-Saona, C., Alborn, H.T., Köppenhöfer, A.M., 2018. Role of plant volatiles in host plant recognition by *Listronotus maculicollis* (Coleoptera: Curculionidae). *J. Chem. Ecol.*
- Laurance, A., 2016. Orientation of Polyphagous lepidoptera to Hostplant Kairimones. University of Illinois at Urbana-Champaign.
- Lemieux, B., 1996. Molecular genetics of epicuticular wax biosynthesis. *Trends Plant Sci.* 1, 312–318.
- Li, G., Ishikawa, Y., 2006. Leaf epicuticular wax chemicals of the Japanese knotweed *Fallopia japonica* as oviposition stimulants for *Ostrinia latipennis*. *J. Chem. Ecol.* 32, 595–604.
- Lucini, E.L., Zunino, M.P., Lopez, M.L., Zygadlo, J.A., 2006. Effect of monoterpenes on lipid composition and sclerotial development of *Sclerotium cepivorum* Berk. *J. Phytopathol.* 154, 441–446.
- Mendki, M.J., Ganesan, K., Prakash, S., Suryanarayana, M.V.S., Malhotra, R.C., Rao, K.M., Vaidyanathaswamy, R., 2000. Heneicosane: an oviposition-attractant pheromone of larval origin in *Aedes aegypti* mosquito. *Curr. Sci.* 78, 1295–1296.
- Nawrot, J., Gawlak, M., Szafranek, J., Szafranek, B., Synak, E., Warchalewski, J.R., Piasecka-Kwiatkowska, D., Błaszczak, W., Jeliński, T., Fornal, J., 2010. The effect of wheat grain composition, cuticular lipids and kernel surface microstructure on feeding, egg-laying, and the development of the granary weevil, *Sitophilus granarius* (L.). *J. Stored Prod. Res.* 46, 133–141.
- Nesci, A., Barra, P., Etcheverry, M., 2011. Integrated management of insect vectors of *Aspergillus flavus* in stored maize, using synthetic antioxidants and natural phytochemicals. *J. Stored Prod. Res.* 47, 231–237.
- Niewiada, A., Nawrot, J., Szafranek, J., Szafranek, B., Synak, E., Jeleń, H., Wąsowicz, E., 2005. Some factors affecting egg-laying of the granary weevil (*Sitophilus granarius* L.). *J. Stored Prod. Res.* 41, 544–555.
- Oshima, K., Honda, H., Yamamoto, I., 1973. Isolation of an oviposition marker from Azuki bean weevil, *Callosobruchus chinensis* (L.). *Agric. Biol. Chem.* 37, 2679–2680.
- Panagabko, C., Chenier, D., Fixon-Owoo, S., Atkinson, J.K., 2000. Ion-pair HPLC determination of hydroxycinnamic acid monoconjugates of putrescine, spermidine and spermine. *Phytochem. Anal.* 11, 11–17. [https://doi.org/10.1002/\(SICI\)1099-1565\(200001/02\)11:1<11::AID-PCA479>3.0.CO;2-0](https://doi.org/10.1002/(SICI)1099-1565(200001/02)11:1<11::AID-PCA479>3.0.CO;2-0).
- Phillips, T.W., Jiang, X.-L., Burkholder, W.E., Phillips, J.K., Tran, H.Q., 1993. Behavioral responses to food volatiles by two species of stored-product coleoptera, *Sitophilus oryzae* (Curculionidae) and *Tribolium castaneum* (Tenebrionidae). *J. Chem. Ecol.* 19, 723–734.
- Russin, J.S., Guo, B.Z., Tubajika, K.M., Brown, R.L., Cleveland, T.E., Widstrom, N.W., 1997. Comparison of kernel wax from corn Genotypes resistant or susceptible to *Aspergillus flavus*. *Phytopathology* 87, 529–533.
- Sen, A., Bergvinson, D., Miller, S.S., Atkinson, J., Fulcher, R.G., Arnason, J.T., 1994. Distribution and Microchemical detection of phenolic acids, flavonoids, and phenolic acid amides in maize kernels. *J. Agric. Food Chem.* 42, 1879–1883.
- Tefera, T., Mugo, S., Likhayo, P., Beyene, Y., 2011. Resistance of three-way cross experimental maize hybrids to post-harvest insect pests, the larger grain borer (*Prostephanus truncatus*) and maize weevil (*Sitophilus zeamais*). *Int. J. Trop. Insect Sci.* 31, 3–12.
- Tippling, P., Legg, D., Rodriguez, J., Poneleit, C., 1988. Influence of maize pericarp surface relief on resistance to the maize weevil (Coleoptera: Curculionidae). *J. Kans. Entomol. Soc.* 61, 237–241.
- Trabalon, M., Assi-Bessekon, D., 2008. Effects of web chemical signatures on intraspecific recognition in a subsocial spider, *Coelotes terrestris* (Araneae). *Anim. Behav.* 76, 1571–1578.
- Ukeh, D.A., Birkett, M.A., Bruce, T.J., Allan, E.J., Pickett, J.A., Mordue Luntz, A.J., 2010. Behavioural responses of the maize weevil, *Sitophilus zeamais*, to host (stored-grain) and non-host plant volatiles. *Pest Manag. Sci.* 66, 44–50.
- van Loon, J.J.A., Blaakmeer, A., Griepink, F.C., van Beek, T.A., Schoonhoven, L.M., de Groot, A., 1992. Leaf surface compound from *Brassica oleracea* (Cruciferae) induces oviposition by *Pieris brassicae* (Lepidoptera: Pieridae). *Chemoecology* 3, 39–44.
- Wegener, R., Schulz, S., Meiners, T., Hadwich, K., Hilker, M., 2001. Analysis of volatiles induced by oviposition of Elm leaf beetle *Xanthogaleruca luteola* on *Ulmus minor*. *J. Chem. Ecol.* 27, 499–515.
- Welti, R., Wang, X., 2004. Lipid species profiling: a high-throughput approach to identify lipid compositional changes and determine the function of genes involved in lipid metabolism and signaling. *Curr. Opin. Plant Biol.* 7, 337–344.
- Yeats, T.H., Rose, J.K.C., 2013. The formation and function of plant cuticles. *Plant Physiol.* <https://doi.org/10.1104/pp.113.222737>.
- Yuya, A.I., Tadesse, A., Azerefegne, F., Tefera, T., 2009. Efficacy of combining Niger seed oil with malathion 5% dust formulation on maize against the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae). *J. Stored Prod. Res.* 45, 67–70.
- Zunino, M.P., Zygadlo, J.A., 2005. Changes in the composition of Phospholipid fatty acids and sterols of maize root in response to Monoterpenes. *J. Chem. Ecol.* 31, 1269–1283.