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Evaluation of diets from various maize hybrids reveals potential tolerance traits against *Spodoptera littoralis* (Boisd) as measured by developmental and digestive performance

Shirin Alekaram[1], Seyed Ali Hemmati[1], Masumeh Ziaee[1] and Lukasz L. Stelinski[2]

[1]Department of Plant Protection, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran and [2]Department of Entomology and Nematology, Citrus Research and Education Center, University of Florida, Lake Alfred, FL, 33850, USA

Research Paper

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Corresponding author:
Seyed Ali Hemmati;
Email: sa.hemmati@scu.ac.ir

Abstract

Spodoptera littoralis (Boisd) (Lepidoptera: Noctuidae) is a highly polyphagous insect that significantly reduces agricultural production of several food staples. We evaluated performance of *S. littoralis* on several meridic diets based on various maize hybrids, including Oteel, Simon, Valbum, SC703, and SC704. Growth, feeding behaviours, and activity of digestive enzymes of *S. littoralis* were examined under laboratory conditions. In addition, selected biochemical characteristics of maize hybrid seeds were evaluated, including starch, protein, anthocyanin, as well as phenolic and flavonoid contents, to examine relationships between plant properties and digestive performance of *S. littoralis*. Performance of *S. littoralis* on maize hybrids, as measured by nutritional indices, was related to both proteolytic and amylolytic activities quantified using gut extracts. Larval *S. littoralis* reared on SC703 exhibited the highest efficiency of conversion of digested food, while the lowest was recorded in those fed on the Oteel hybrid. *S. littoralis* reared on SC703 and Oteel also exhibited the highest and lowest relative growth rates, respectively. The highest levels of proteolytic activity in *S. littoralis* were measured from larvae reared on the SC703 hybrid, while the lowest levels occurred on the Oteel and Valbum hybrids. Amylolytic activity was lowest in larvae reared on SC703 and Valbum hybrids and highest in larvae reared on the Oteel hybrid. Our results suggest that the SC703 hybrid was the most suitable host for *S. littoralis*, while the Oteel hybrid demonstrated the greatest level of tolerance against *S. littoralis* of those evaluated. We discuss the potential utility of maize hybrids exhibiting tolerance traits against this cosmopolitan pest with reference to cultivation of tolerant varieties and identification of specific tolerance traits.

Introduction

Maize, *Zea mays* L., is a monocotyledonous annual crop cultivated globally given its many uses, high nutritional value, and adaptability to diverse climates (especially with the production of new hybrids). Maize ranks third in the world after wheat and rice in terms of cultivated area and total production (Khodabandeh, 2003; Erenstein *et al*., 2022). Maize yield potential per unit area is high allowing harvest of 15 to 20 tons of seeds per hectare at the commercial level. Maize has been called the king of grains due to its capacity for seed production (Scott and Emery, 2016).

One of the important pests of maize is the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd) (Lepidoptera: Noctuidae), which significantly reduces maize production annually (Gouinguené *et al*., 2003; Khanjani, 2006; El-latif, 2014; Zamani Fard *et al*., 2022). Also, *S. littoralis* is one of the most important pests of other high value crops such as cotton, beans, alfalfa, clover, sugar beet, and vegetables in southern Europe, Africa, and the Middle East (Hegazi and Schopf, 1984; Hosseini Mousavi *et al*., 2023). *S. littoralis* larvae damage vegetative and reproductive parts of agricultural crops, including cotton, tomato, maize, and beans (Sneh *et al*., 1981; El-latif, 2014; Ismail, 2020). The larvae feed collectively on the parenchyma of young and old leaves, which become reticulated, and can be completely skeletonised under high population densities (Khanjani, 2006; Lanzoni *et al*., 2012).

Typically, farmers use broad-spectrum insecticides to control *S. littoralis* (Ismail, 2020; Hosseini Mousavi *et al*., 2023). Insecticide overuse can cause harmful side effects, including evolution of resistance, destruction of natural enemies, reduced crop quality, and secondary pest outbreaks (Ongley, 1996; Smagghe *et al*., 1999; Gacemi *et al*., 2019). Therefore, alternative approaches for management of *S. littoralis* have been prioritised in many regions. At present, identification of tolerance traits to breed for pest resistant crops represents one environmentally sound approach to managing this pest that can also be economical for growers in developing nations (Panda and Khush, 1995; Liu *et al.*, 2004; Tsai and Wang, 2006; Hemmati *et al.*, 2022). Also, incorporating resistant plant cultivars can supplement chemical and biological control methods in IPM programmes (Sharma and Ortiz, 2002; Lacey *et al.*, 2015; Jafari *et al.*, 2022, 2023).

The growth, development, and reproduction of insects strongly depend on the quality and quantity of their food (Scriber and Slansky, 1981; Razmjou *et al.*, 2014). In response to insect herbivory, plants produce a series of biochemical metabolites, which may reduce digestibility as well as deterrents compounds, which can inhibit feeding (Smith and Clement, 2012; Zamani Fard *et al.*, 2022). For example, plant protease inhibitors (PIs) interfere with the activity of digestive function in insects, thereby reducing assimilation of diets and resulting in nutrient deficiency, deceased development, and increased larval mortality (Stevens *et al.*, 2012; Hemati *et al.*, 2012a). Previous studies have indicated that seeds from cereals (Gramineae), such as maize, are a rich source of antifeedant compounds and PIs from a diverse group of feeding inhibitor families (Franco *et al.*, 2002; Gomes *et al.*, 2005; Silva *et al.*, 2007; El-latif, 2014). Furthermore, PI-producing plant cultivars show promise when incorporated as part of pest management programmes due to their tolerance traits (Gatehouse and Gatehouse, 1998; Mehrabadi *et al.*, 2012).

Tolerance against *S. littoralis* has been investigated previously among various legume cultivars. For example, cowpea Mashhad exhibits considerable tolerance against this pest (Shishehbor and Hemmati, 2022). Zamani Fard *et al.* (2022) also investigated the digestive enzyme activities and feeding efficiency of *S. littoralis* on different mung bean varieties and reported that the VC6371 variety was a comparatively vulnerable cultivar to this pest. Recently, Hosseini Mousavi *et al.* (2023) investigated performance of *S. littoralis* on various leafy vegetables, and found that coriander was a suitable host, while purslane exhibited tolerance.

The objectives of this study were to quantify the performance of *S. littoralis* on meridic diets derived from various maize hybrids and to measure the response of major digestive enzyme groups in this insect after feeding on these potential hosts. Moreover, we measured various primary and secondary metabolites including protein, carbohydrate, and phenolic content as well as flavonoids and anthocyanins among seeds of these maize hybrids. Finally, we examined relationships between growth indices or digestive enzyme responses of *S. littoralis* and the primary and secondary metabolite levels found among the hybrids evaluated. Our results provide a basis for selection of available maize hybrids for cultivation based on their level of tolerance against *S. littoralis*. Incorporating plant tolerance into management programmes where *S. littoralis* causes significant damage should be a useful additional consideration, particularly in areas where economic means limit availability of chemical tools.

Material and methods

Maize seeds

Maize seeds from the hybrids Oteel, Simon, Valbum, SC703, and SC704, commonly cultivated for commercial purposes in Iran, were obtained from the Safiabad Dezful Agriculture and Natural Resources Research and Education Center, Dezful, Iran. The seeds were individually ground and stored at 4°C before use in the experiments. Meridic diets were separately prepared from seed powder of each plant hybrid (250 g) and with the addition of wheat germ (30 g) for supplemental protein and carbohydrate sources, sorbic acid (1.1 g) as an antimicrobial agent, ascorbic acid (3.5 g) as a vitamin source, sunflower oil (5 ml) as a lipid source, agar (14 g) as a moisturiser, methyl *p*-hydroxybenzoate (2.2 g), formaldehyde 37% (2.5 g), and distilled water (650 ml). The meridic diets were prepared based on the method described by Shorey and Hale (1965) and were stored in the refrigerator for no longer than two weeks prior to use.

Insect rearing

The initial population of *S. littoralis* was collected from maize fields in Khuzestan province of southwestern Iran, and reared in a growth chamber at 25 ± 1°C, 60 ± 5% RH, and a of 16:8 h (L:D) photoperiod. The collected larvae were placed in plastic containers (diameter 9 cm, depth 1 cm) and reared on the various maize-based meridic diets. Adults that emerged were kept in plastic containers (15 cm diameter × 20 cm height) and provided wet cotton soaked in honey solution (10%). Prior to initiating experiments, larvae were reared on diets consisting of all five maize hybrids for two generations to create a homogenous cohort.

Food consumption and growth indices of Spodoptera littoralis

Forty *S. littoralis* eggs of the same age were selected from the main culture for subsequent rearing per maize hybrid diet. Until third instar larvae appeared, first and second instars were reared as groups of 40 larvae in 25 × 15 cm (height × width), plastic containers. After third instars emerged, 25 larvae were chosen at random for each maize hybrid diet and placed individually into 8 × 1 cm Petri dishes with diet to prevent cannibalism. Petri dish lids were modified by drilling 2 cm diameter holes in the lids that were securely covered with a net cloth to allow ventilation. Humidity was maintained by adding cotton soaked with deionised water in the rearing container. Recording of nutritional indices for *S. littoralis* was initiated after emergence of third instar larvae. Daily weight measurements were taken, including the weight of the larvae before and after eating, the weight of the frass produced, and the weight of the initial and leftover food. *S. littoralis* were also weighed at pre-pupal and pupal stages within 24 h of appearance. All weights were measured using a digital scale with an accuracy of 0.001 g. Moreover, the dry weights of the meridic diet samples, as well as larvae and their frass, were calculated by first weighing 25 samples per maize cultivar, then oven-drying the samples at 60°C for 48 h, and then re-weighing those samples again.

Nutritional performance of *S. littoralis* larvae on meridic diets based on various maize hybrids was estimated by calculating several indices, including approximate digestibility (AD), a consumption index (CI), the efficiency of conversion of digested food (ECD), the efficiency of conversion of ingested food (ECI), relative growth rate (RGR) and relative consumption rate (RCR), using the formulas by Waldbauer (1968):

$$CI = [(E/A)];\ AD = [(E - F)/E];\ ECI = [(P/E) \times 100];$$
$$ECD = [(P/E - F) \times 100];\ RCR = [(E/W_0 \times T)];$$
$$\text{and } RGR = [P/W_0 \times T].$$

where A = average of larval dry weight over time (mg), E = dry weight of the food consumed (mg), F = dry weight of faeces produced, P = dry weight gain of larvae (mg), T = the feeding duration (day), and W_0 = primary weight of larvae (mg).

Moreover, to calculate the standardised insect-growth index (SII), pupal weight (P_w) was divided by the larval duration (T) (Itoyama *et al*., 1999). In order to determine the index of plant quality (IPQ) for the maize hybrids investigated here, pupal weight was divided by the dry weight of insect frass (Koricheva and Haukioja, 1992).

Preparation of midgut extracts from S. littoralis *larvae*

Sixth instar *S. littoralis* larvae were anesthetised on ice after 24 h of rearing on each of the maize hybrid diets and dissected under a stereomicroscope. Twenty larvae were randomly selected from each maize hybrid. Extraneous tissues were removed and haemolymph was rinsed with precooled, distilled water. The midguts were then added to the distilled water and homogenised on ice. The homogenates were then centrifuged at 14,000 ***g*** for 10 min at 4°C, and the clear supernatants were aspirated and stored at 20°C until completion of enzymatic assays.

Amylase activity

Amylase activity was assessed with the dinitrosalicylic acid (DNSA) method with 1% starch as a substrate in a universal buffer (10 mM succinate-glycine-2, morpholinoethan sulfunic acid) at pH 10. Midgut extract mixtures with 1% starch were incubated at 37°C for 30 min. The enzymatic reaction was stopped by adding 50 µl of DNSA reagent and heated for 15 min in boiling water. After cooling on ice, the mixture's adsorption was measured at 540 nm (Bernfeld, 1955). The amount of maltose released during the α-amylase assays was calculated using the standard curve created by using known amounts of maltose.

Protease activity

Proteolytic activity was measured using azolacasein (1.5%) as the substrate in universal buffer (50 mM sodium phosphate-borate) at pH 11. Fifty µl of midgut extract mixtures were combined with 80 µl of the substrate, which was then incubated at 37°C for 50 min. Thereafter, 100 µl of 30% trichloroacetic acid was added to the mixture, which was then centrifuged for 10 min at 14,000 ***g*** after being chilled for 30 min at 4°C. The supernatant was mixed with an equal volume of 2 M NaOH, and the absorbance was measured at 440 nm (Elpidina *et al*., 2001). The Bradford (1976) protein assay was also used to determine protein concentrations. Known amounts of bovine serum albumen were used to generate the standard curve.

Biochemical properties of maize hybrids

Biochemical characteristics of seeds from the various maize hybrids investigated were quantified to explore possible relationships between these variables and nutritional or enzymatic activities measured from *S. littoralis*. Assessment of each maize hybrid was performed in three replications and distilled water served as the negative control. All phytochemicals from the hybrids were measured using powdered seeds, which were used as the basis of each meridic diet.

The protein content of seeds was estimated using the Bradford technique. In brief, 100 µl of the homogenate was combined with 3 ml of Bradford reagent after 200 mg of the powdered seeds from each hybrid were homogenized in 10 ml of distilled water. The samples absorbance was measured at 595 nm using a standard of bovine serum albumin (Bradford, 1976).

The amount of starch in seeds was determined using Bernfeld's method. Powdered seeds (200 mg) from each hybrid investigated were mixed with 35 ml of distilled water before heating the samples to boiling point. Afterwards, 2.5 ml of iodine reagent (0.2% KI and 0.02% I_2) was added to 100 µl of each sample, and the absorbance was measured at 580 nm (Bernfeld, 1955).

Total phenolic content was ascertained using the Slinkard and Singleton (1997) method. The supernatants from the crude seed extracts were added to 1.5 ml of Folin-Ciocalteau reagent after being centrifuged for 15 min at 12,000 ***g***. Thereafter, 1.4 ml of 7% sodium carbonate was added to the mixture and was allowed to sit in the dark for 30 min. The standard utilised was gallic acid. Reagents were combined with distilled water, which also served as the blank. The absorbance of standards and samples was measured at 765 nm using a spectrophotometer. For each hybrid, assays were completed in three replicates.

Total anthocyanin and flavonoid levels were quantified with the method described by Kim *et al*. (2003). Two g of maize hybrid seeds were placed in a mortar with 3 ml of acidified ethanol (1:100 acetic acid:ethanol). The seed samples were crushed and centrifuged for 15 min at 12,000 ***g***. The extract was filtered through Whatman filter paper No. 1 and then boiled in a water bath for five minutes at 80°C. After cooling, absorbance of the extracts was measured at 415 nm for total flavonoids and 520 nm for total anthocyanins using a UV-visible spectrophotometer.

Statistical analysis

The data obtained from calculating digestive enzyme activities, nutritional indices and phytochemical content were examined for normality using the Shapiro–Wilk test before being subjected to one-way multivariate analysis of variance (MANOVA). The Tukey's HSD test was used to examine the statistical variances across means with a $P < 0.01$ threshold. Furthermore, a cluster analysis was carried out to find groups of maize hybrids with similar traits using the growth parameters and enzyme activity levels from *S. littoralis* larvae as variables. We determined the contribution of these variables to performance of *S. littoralis* on maize hybrids by a two-step cluster approach using Ward's minimum-variance hierarchical clustering method. The relationships between the nutritional indices or enzyme activities of *S. littoralis* and the biochemical properties of the maize hybrids evaluated were examined with Pearson's correlation analysis. All analyses were conducted in SPSS version 22.0 statistical software.

Results

Feeding and growth of S. littoralis

Table 1 displays the feeding performance of *S. littoralis* third to sixth instar larvae reared on diets from various maize hybrids. The highest consumption index (CI) index was recorded on the Oteel hybrid, while, the lowest was observed on the SC703 hybrid ($F_{4,\ 120} = 52.643$; $P < 0.01$). The maximum values of approximate digestibility (AD) occurred on SC704, Valbum, and Oteel hybrids, while the lowest values were recorded on SC703 and Simon hybrids ($F_{4,\ 120} = 7.396$; $P < 0.01$). The highest efficiency of conversion of ingested food (ECI) occurred on SC703, while the lowest was on Oteel ($F_{4,120} = 138.769$; $P < 0.01$). The highest efficiency of conversion of digested food (ECD) was exhibited by larvae

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Table 1. Nutritional indices (mean ± SE) of third to sixth instar *Spodoptera littoralis* reared on various maize hybrids

Hybrid	CI	AD	ECI	ECD	RCR	RGR
SC703	1.758 ± 0.037d	37.638 ± 0.673b	21.244 ± 0.323a	60.146 ± 1.595a	0.586 ± 0.012c	0.08 ± 0.001a
SC704	1.978 ± 0.04c	44.34 ± 1.42a	17.054 ± 0.436b	42.676 ± 2.155b	0.591 ± 0.012c	0.069 ± 0.001b
Valbum	2.273 ± 0.042b	44.451 ± 1.487a	13.715 ± 0.385c	37.275 ± 2.018b	0.669 ± 0.013b	0.067 ± 0.001b
Oteel	2.72 ± 0.078a	44.909 ± 1.279a	9.173 ± 0.279d	28.146 ± 1.977c	0.785 ± 0.023a	0.053 ± 0.001d
Simon	1.989 ± 0.048c	38.967 ± 1.349b	14.307 ± 0.44c	43.096 ± 2.058b	0.587 ± 0.015c	0.062 ± 0.001c

CI, consumption index; AD, approximate digestibility; ECI, efficiency of conversion of ingested food; ECD, efficiency of conversion of digested food; RCR, relative consumption rate; RGR, relative growth rate.
Means followed by different letters in the same column are significantly different (Tukey, $P < 0.01$).

reared on hybrid SC703, while the lowest value was recorded from larvae reared on the Oteel hybrid ($F_{4,120} = 35.096$; $P < 0.01$). The highest relative consumption rate (RCR) index was recorded from larvae reared on the Oteel hybrid, which was significantly higher than that observed from larvae reared on SC703, SC704 or Simon hybrids ($F_{4,120} = 31.211$; $P < 0.01$). The lowest relative growth rate (RGR) was exhibited by larvae fed on the Oteel hybrid ($F_{4,\ 120} = 65.692$; $P < 0.01$). In contrast, larvae reared on the SC703 hybrid exhibited the highest RGR ($F_{4,120} = 65.692$; $P < 0.01$) (Table 1).

Larval weight was greatest following rearing on the on the SC703 hybrid, and lowest on the Oteel hybrid (fig. 1) ($F_{4,\ 120} = 34.755$; $P < 0.01$). Total mass of diet consumed was highest on SC704 and lowest on Simon ($F_{4,\ 120} = 7.820$; $P < 0.01$). Larvae produced the greatest amount of frass on the SC703 hybrid, and significantly less frass was produced on the Valbum, Oteel, and Simon hybrids ($F_{4,\ 120} = 5.661$; $P < 0.01$). The highest weight gain occurred in larvae that were fed on the SC703 hybrid and the lowest occurred on the Oteel hybrid ($F_{4,\ 120} = 98.424$; $P < 0.01$). Furthermore, our results revealed that *S. littoralis* had the shortest larval period when reared on the SC703 and SC704 hybrids, whereas the longest larval period was obtained on the Oteel hybrid ($F_{4,\ 120} = 19.471$; $P < 0.01$) (fig. 1).

The greatest pre-pupal weight was observed in *S. littoralis* fed on the SC703 hybrid while the lowest weight occurred on the Oteel hybrid ($F_{4,\ 120} = 4.560$; $P < 0.01$). Moreover, the highest and lowest pupal weights of *S. littoralis* occurred on the SC703 and Oteel hybrids, respectively ($F_{4,\ 120} = 14.589$; $P < 0.01$) (fig. 2).

There were significant differences in the standardised insect-growth index (SII) and the index of plant quality (IPQ) on different maize hybrids evaluated (fig. 3). The highest SII ($F_{4,\ 120} = 14.186$; $P < 0.01$) was quantified when the larvae were reared on the SC703 hybrid, whereas the lowest value occurred for larvae reared on the Oteel hybrid. The lowest IPQ value ($F_{4,\ 120} = 14.589$; $P < 0.01$) was observed on the SC703 hybrid and the highest occurred on the Oteel hybrid (fig. 3).

Proteolytic and amylolytic activity in S. littoralis

Proteolytic activity was significantly higher in midguts of *S. littoralis* reared on SC703 than that observed in larvae reared on Oteel or Valbum hybrids ($F_{4,\ 10} = 76.89$; $P < 0.01$). However, amylolytic activity was greatest among larvae fed on the Oteel hybrid, and was significantly higher than that observed among larvae reared on SC703 and Valbum hybrids ($F_{4,10} = 17.96$; $P < 0.01$) (fig. 4).

Cluster analysis

Various maize hybrids were grouped by visual inspection of the dendrogram presented in fig. 5. Individual clusters define maize hybrid phenotypes based on the responses of *S. littoralis* as measured by the nutritional indices, growth parameters, and enzymatic activities. Two main clusters (A and B) of *S. littoralis* emerged in the dendrogram with a single phenotype (hybrid SC703) in cluster B exhibiting the highest suitability to *S. littoralis*. Sub-cluster A_1 included Valbum, Simon and SC704 hybrids, which were relatively suitable to *S. littoralis*. The Oteel hybrid separated into sub-cluster A_2, which was a relatively unsuitable host for *S. littoralis* (fig. 5).

Biochemical properties of different maize hybrids

The primary and secondary metabolites of maize hybrid evaluated are given in table 2. There were significant differences among the metabolites quantified; starch content was greatest in the SC703 hybrid and lowest in the Oteel hybrid ($F_{4,10} = 17.34$; $P < 0.01$) (table 2). The Oteel hybrid was characterised by the lowest protein content, which was significantly lower than that recorded from all of the other hybrids investigated ($F_{4,10} = 29.36$; $P < 0.01$). The SC703 hybrid exhibited the lowest phenolic content, while the Oteel hybrid was characterised by the highest total phenolic content ($F_{4,10} = 20.15$; $P < 0.01$). Furthermore, total flavonoids were highest in the Simon hybrid, whereas the lowest flavonoid content was detected in the SC704 hybrid ($F_{4,10} = 25.18$; $P < 0.01$). Total anthocyanin content was highest in the Oteel hybrid and lowest in the SC703 and SC704 hybrids ($F_{4,10} = 6.67$; $P < 0.01$) (table 2).

Correlation analysis

Pearson's correlations investigating associations between consumption or growth of *S. littoralis* and digestive enzymatic activity on various maize hybrids are presented in table 3. Standardised insect-growth index, index of plant quality, relative growth rate, efficiency of conversion of ingested food, and proteolytic activity were positively correlated with protein and starch contents and negatively correlated with total phenolic and anthocyanin contents ($P < 0.01$). Furthermore, there was a significant negative correlation between the relative consumption rate of *S. littoralis* and protein or starch content in the various maize hybrids (table 3). Amylolytic activity was also significantly negatively correlated with both protein and starch contents of maize hybrids (table 3). In addition, total phenolic and anthocyanin contents were positively correlated with RCR and amylolytic activity ($P < 0.01$) (table 3). Flavonoid content measured in the various hybrids was not correlated ($P > 0.05$) with any of the nutritional or growth indices or digestive enzyme activities, while it was negatively correlated with the index of plant quality (table 3).

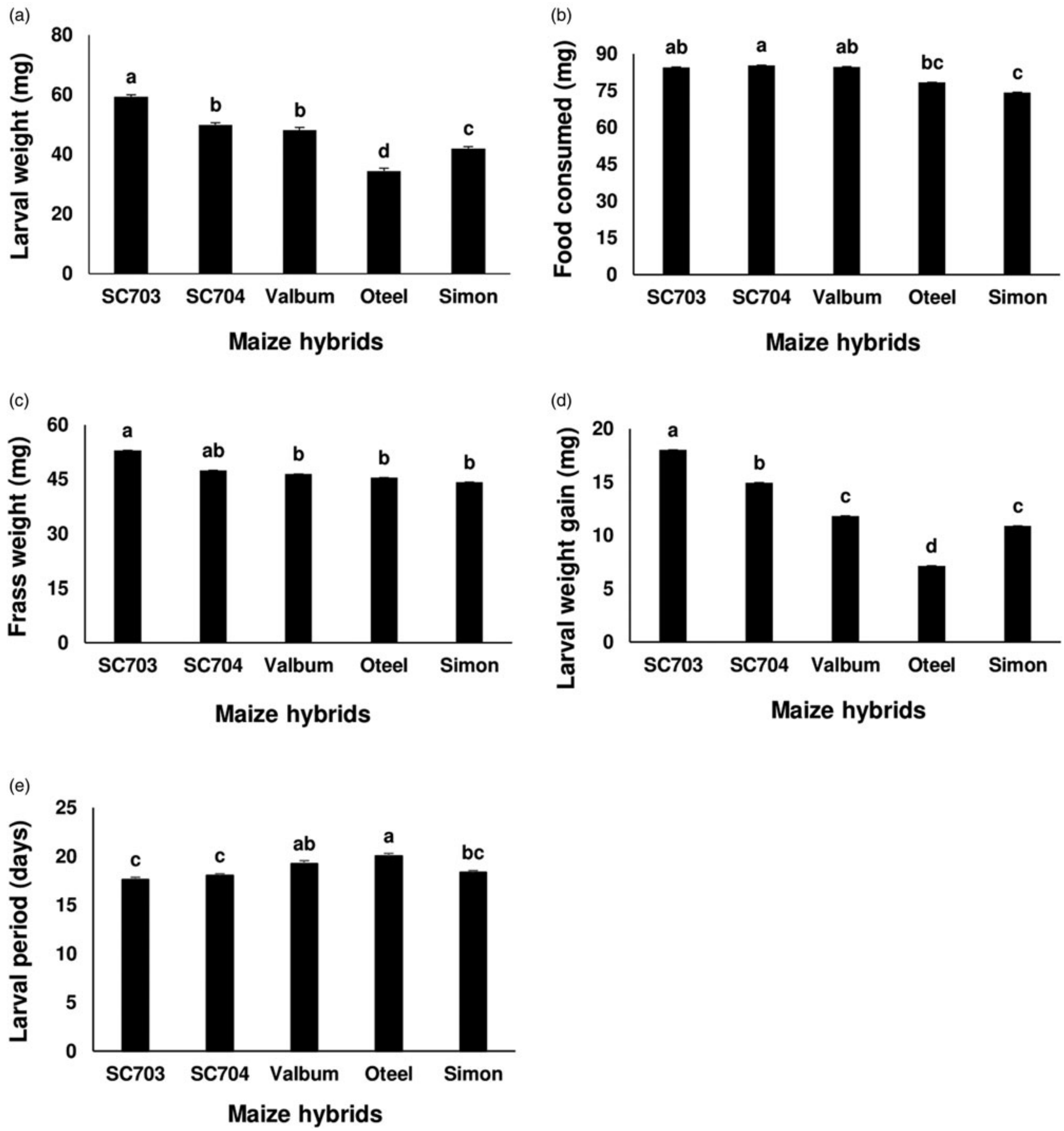

Figure 1. (a) Mean larval weight, (b) food consumed, (c) frass produced, (d) larval weight gain, and (e) larval period of *Spodoptera littoralis* reared on various maize hybrids.

Discussion

Insect feeding behaviour and digestive function are influenced by food quality, and these responses can serve as measures of plant tolerance to pests. The significance of plant nutritional quality, phytochemical metabolite composition, as well as plant physical properties are all well-known variables affecting plant-insect interactions (Kennedy *et al*., 1987; Abedi *et al*., 2020; Shishehbor and Hemmati, 2022). Our results indicate that while *S. littoralis* was able to utilise all of the maize hybrids evaluated here as hosts, there was considerable variability between hybrids in terms of their quality as resources and resultant development by *S. littoralis*. Previous investigations have revealed considerable variation in digestive enzyme response of *S. littoralis* depending on host plant quality and with regard to variation in both primary and secondary metabolites (Ladhari *et al*., 2013; Khafagi *et al*., 2016; Gacemi *et al*., 2019; Shishehbor and Hemmati, 2022; Zamani Fard *et al*., 2022; Hosseini Mousavi *et al*., 2023).

Our findings indicate that maize hybrid genotype affect *S. littoralis* feeding rates and development. Larval *S. littoralis* reared on the Oteel hybrid exhibited the highest approximate digestibility (AD value); however, it appears that a high AD index could not

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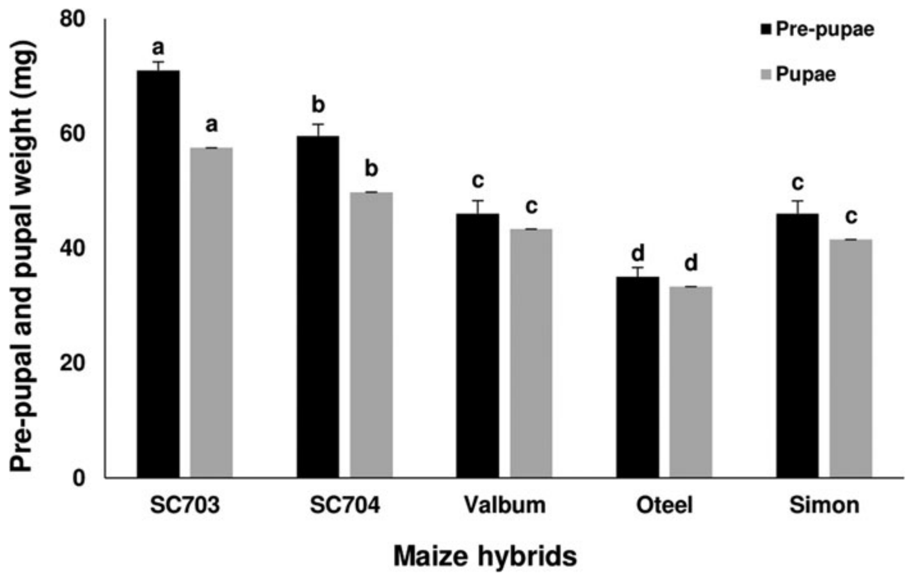

Figure 2. Pre-pupal and pupal weight (mg) of *Spodoptera littoralis* reared on various maize hybrids.

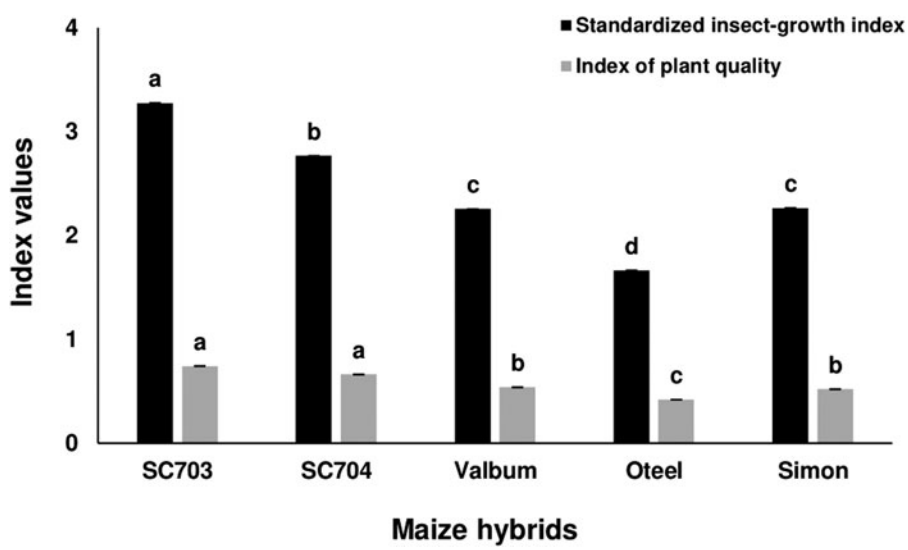

Figure 3. Index of plant quality (IPQ) and standardised insect-growth index (SII) of *Spodoptera littoralis* reared on various maize hybrids.

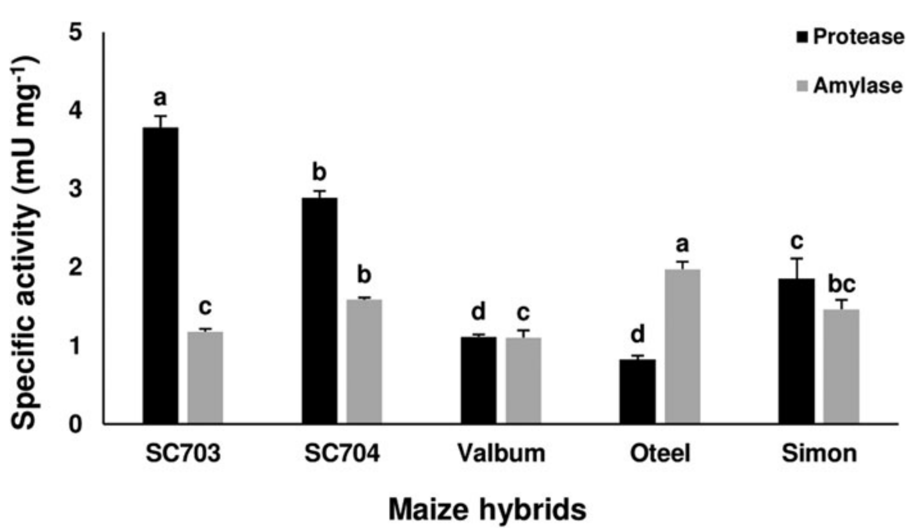

Figure 4. General proteolytic and amylolytic activity in midgut extracts from sixth instar larvae of *Spodoptera littoralis* reared on various maize hybrids.

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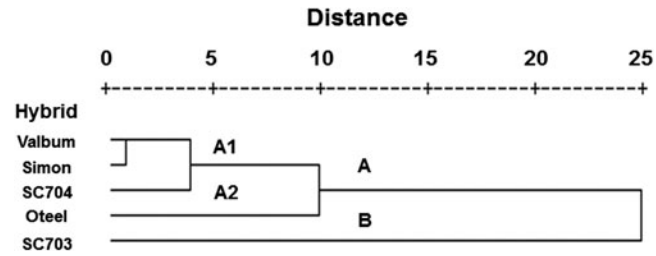

Figure 5. Dendrogram of various maize hybrids based on nutritional indices and enzymatic activities of *Spodoptera littoralis* reared on various maize hybrids.

compensate for the low efficiency of conversion of digested food (ECD index) observed on this hybrid, which resulted in diminished larval growth. It is possible that the larval period was extended on this hybrid, increasing instar duration, where larger amounts of ingested food must be allocated to maintain metabolism (Scriber and Slansky, 1981).

The efficiency of conversion of ingested food (ECI) is an indirect measure of food source quality and assesses the capacity of insects to consume food for growth and development (Batista Pereira *et al*., 2002). The low ECI value observed for *S. littoralis* reared on the Oteel hybrid indicates that this genotype was not readily converted into biomass. The total phenolic and anthocyanin contents of the maize hybrids were inversely related to quantified ECI values for *S. littoralis* developing on them. For example, high total phenolic and anthocyanin contents measured in the Oteel hybrid were coincident with low ECI values quantified for *S. littoralis* reared on this genotype.

Variation in the efficiency of conversion of digested food (ECD) can be attributed to differences in the biochemical properties of the consumed food (Nathan *et al*., 2005). The Oteel hybrid displayed the lowest ECD value for *S. littoralis* larvae, indicating that this host was unfavourable for development of *S. littoralis* and may have increased metabolic costs associated with catabolism and excretion (Scriber and Slansky, 1981). Furthermore, the duration of the feeding period in *S. littoralis* larvae is a significant determinant of both relative growth rate (RGR) and relative consumption rate (RCR) (Hemati *et al*., 2012b). Insect consumption rate is often positively correlated with insect body mass (Nathan *et al*., 2005). The maize hybrids on which *S. littoralis* exhibited the lowest RCR of were SC703, SC704, and Simon, suggesting that these genotypes are nutritionally sub-optimal for this species. In contrast, the highest RGR value for *S. littoralis* was observed on the SC703 hybrid indicating that this hybrid caused the largest rate of weight gain. The lowest RGR value for *S. littoralis* larvae occurred on the Oteel hybrid and this may have been related to allocation of ingested food for maintenance of metabolism. The differences in primary and secondary plant metabolites between the various maize hybrids evaluated may explain the observed variation in the RGR index.

Our results indicate that the Oteel hybrid was a relatively poor host for *S. littoralis* as evidenced by the relatively long larval periods and low pupal weights observed on this genotype, which may be related to the low standardized insect-growth index measured on this genotype. In addition, the relatively low index of plant quality (IPQ) observed with the Oteel hybrid can be attributed to the relatively low pupal weight of larvae reared on this genotype.

Polyphagous herbivores can rapidly adjust profiles of their digestive enzymes in response to ingested plant PIs. This adjustment involves both the reduction and augmentation of gut proteases (Jongsma and Bolter, 1997; Hemmati *et al*., 2021). In the present investigation, our measurements focused on proteases and amylases, which constitute the principal digestive enzymes within the midgut of *S. littoralis*. The role of digestive proteases in insect physiology encompasses two key functions: neutralisation of protein toxins assimilated during feeding and breakdown of proteins into essential amino acids that facilitates growth and development (Nation, 2002). The abundance of proteins in maize seeds could potentially impact major insect digestive enzymes such as proteases (Biggs and McGregor, 1996). The inhibition of digestive enzymes through the presence of inhibitors results in the suppression of gut proteases and other digestive enzymes, including amylases. This suppression subsequently gives rise to suboptimal nutrient utilisation, hindered growth, and ultimately, mortality due to starvation (Hemmati *et al*., 2021).

Our results confirmed a positive correlation between proteolytic activity in the midgut of *S. littoralis* and the protein content present among the in maize hybrids evaluated. Proteases are likely the main digestive enzymes in the midgut of *S. littoralis* and these were elevated in larvae that fed on hybrids characterised by greater protein content. It is likely that feeding on protein-rich hybrid genotypes stimulates heightened production of these digestive enzymes in *S. littoralis*. In contrast, the amylolytic activity of larvae feeding on these hybrids exhibited a negative correlation with starch content. Notably, plants often produce amylase inhibitors that hinder the breakdown of starch molecules by digestive amylases. Remarkably, *S. littoralis* larvae feeding on the Oteel hybrid demonstrated the most elevated amylase activity when compared to other maize hybrids. This result may have been caused by a particularly rich store of amylase inhibitors in the Oteel hybrid, resulting in reduced starch utilisation. Consequently, this inhibition-induced scarcity of starch may have prompted an upsurge in amylase synthesis as a compensatory response to the inhibitory influence (Bouayad *et al*., 2008; Hemmati *et al*., 2021).

Table 2. Biochemical characteristics (mean ± SE) (mg ml^{-1}) of various maize hybrids

Hybrid	Starch content	Protein content	Total phenolic content	Flavonoid content	Anthocyanin content
SC703	2.020 ± 0.219a	0.183 ± 0.014a	5.937 ± 0.885c	25.444 ± 1.635bc	3.794 ± 0.0260c
SC704	1.695 ± 0.028ab	0.178 ± 0.008a	7.299 ± 0.625bc	16.802 ± 1.076d	4.038 ± 0.349c
Valbum	1.186 ± 0.190b	0.115 ± 0.005b	9.646 ± 0.514b	20.481 ± 1.759cd	5.750 ± 0.527bc
Oteel	0.524 ± 0.041c	0.056 ± 0.007c	13.641 ± 0.380a	30.642 ± 3.079ab	12.350 ± 0.685a
Simon	1.115 ± 0.093bc	0.112 ± 0.009b	9.010 ± 0.730b	31.790 ± 2.383a	6.665 ± 0.401b

Means followed by different letters in the same column are significantly different (Tukey, $P < 0.01$).

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Table 3. Pearson's correlation coefficients (r) between nutritional and physiological characteristics of *Spodoptera littoralis* with biochemical traits of various maize hybrids

Parameter	Starch content	Protein content	Total phenolic content	Flavonoid content	Anthocyanin content
Standardised insect-growth index	0.928 (0.000)	0.925 (0.000)	−0.940 (0.000)	−0.486 (0.066)	−0.870 (0.000)
Index of plant quality	0.932 (0.000)	0.938 (0.000)	−0.921 (0.000)	−0.563 (0.029)	−0.868 (0.000)
Relative growth rate	0.904 (0.000)	0.878 (0.000)	−0.919 (0.000)	−0.480 (0.070)	−0.875 (0.000)
Relative consumption rate	−0.760 (0.001)	−0.809 (0.000)	0.876 (0.000)	0.349 (0.202)	0.883 (0.000)
Efficiency of conversion of ingested food	0.920 (0.000)	0.913 (0.000)	−0.950 (0.000)	−0.435 (0.105)	−0.874 (0.000)
Amylolytic activity	−0.595 (0.019)	−0.545 (0.036)	0.688 (0.005)	0.274 (0.323)	0.754 (0.001)
Proteolytic activity	0.876 (0.000)	0.885 (0.000)	−0.879 (0.000)	−0.403 (0.137)	−0.755 (0.001)

Correlations were evaluated based on Pearson's correlation test ($P < 0.05$).
Numbers in parenthesis represent P values.

The application of cluster analysis delineated three discrete groups of maize hybrids based on similarity of performance and enzymatic activity of *S. littoralis* reared on them. Cluster A1 was comprised of the Valbum, Simon, and SC704 hybrids, which can be characterised as intermediately suitable hosts of *S. littoralis*. The Oteel hybrid segregated from the others in clusters A or B as a relatively unsuitable genotype in sub-cluster A2. This positioning of the Oteel hybrid may be attributed to certain unique characteristics observed in larvae that developed on it including the lowest index of performance (IPQ) along with the highest values of secondary metabolites among the hybrids evaluated. Finally, cluster B was comprised of the SC703 hybrid, which is characterised as a relatively suitable genotype for development and performance of *S. littoralis* with reference to the evaluated parameters.

Our results confirmed an inverse relationship between the quantities of secondary metabolites among a diversity of maize hybrids and multiple nutritional and growth attributes exhibited by *S. littoralis* developing on them. These findings substantiate the deleterious impact of secondary compounds in these hybrids on the growth and performance of *S. littoralis*. Furthermore, our results suggest that the Oteel hybrid could be a useful candidate for cultivation in areas where options for chemical control of *S. littoralis* are limited or within the context of integrated pest management of maize fields, where plant tolerance traits are exploited as a component of the overall management program.

In summary, the present study characterised differences among several maize hybrids with respect to performance and growth of *S. littoralis* larvae, as well as their digestive enzyme responses after feeding. Our results indicate that the Oteel hybrid may be particularly rich in amylase inhibitors and exerted a particularly strong antibiosis effects against *S. littoralis*. Further research is needed to pinpoint the precise plant compound(s) responsible for the reduced growth and development of *S. littoralis* observed on certain hybrids such as Oteel. Identification of potential digestive enzyme inhibitors could be useful for development of resistance or tolerance in maize to chewing pests such as *S. littoralis* via traditional breeding or transgenic approaches. Moreover, it would be useful to investigate a broader range of plant genotypes with *S. littoralis* to potentially identify cultivars exhibiting even greater tolerance traits. A comprehensive exploration encompassing biochemical and molecular analyses of midgut proteases and carbohydrases could provide insights into the adaptive responses of *S. littoralis* larvae when exposed to various maize cultivars in their diet.

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