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THE INFLUENCE OF COWPEA CULTIVAR ON THE HOST PREFERENCES OF COWPEA BRUCHIDS [*CALLOSOBRUCHUS MACULATUS* (F.)]

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ABSTRACT

Callosobruchus maculatus (F.) is a major storage pest that causes significant quantitative and qualitative losses during storage and sale of cowpea (*Vigna unguiculata* L. Walp.). In this study, the behavioral response of cowpea bruchids (*C. maculatus*) to five local (‘Gombe’, ‘Drum’, ‘Sokoto’, ‘Big’, and ‘Honey’) and improved (ITI6K-1966-1, ITI5K-2377-1, ITI6K-1984-1, ITI4K-1903-1, and ITI5K-2386-1) cowpea cultivars was investigated using a Y-tube olfactometer. Gas chromatography-mass spectrometry (GC-MS) was used to analyze and identify the compounds of cowpea oils extracted using n-hexane, ethanol, and water solvents. The results showed that cowpea genotype had a significant effect on the decision time for attraction to the cowpea. Although mated female bruchids generally showed shorter decision times than male bruchids, gender significantly affected the attractiveness of only two cultivars. The solvent used for extraction had no significant effect on the time taken to decide on the attractiveness of different cowpea genotypes. Of the 20 bioactive compounds identified in local cultivars and 19 in improved cultivars, 13 were common to both cultivar groups. Bioactive compounds present in the highest percentages may be used as effective baits for monitoring and control of cowpea bruchids.

Key words: behavioral response, bioactive compounds, Y-tube olfactometer, *Vigna unguiculata*, volatile attractants

INTRODUCTION

In most African countries, including Nigeria, food security remains a challenge due to pest infestations in stored products. The cowpea bruchid, *Callosobruchus maculatus*, is a serious pest of cowpea seeds, causing significant losses in the quality and quantity of stored cowpeas (Hamzavi et al. 2022; Kalpna et al. 2022). The degree of susceptibility or resistance of cowpea seeds to cowpea bruchid infestation depends on certain characteristics of the seeds (Kosini et al. 2019; Nyarko et al. 2022). Some cowpea genotypes have built-in defense mechanisms that prevent or reduce the extent of cowpea bruchid infestation. Resistance traits can be physical (seed coat strength), chemical (secondary metabolites or allelochemicals), or genetic. They have been shown to affect the ability of insects to consume cowpea seeds, oviposit, or complete their life cycle on them (Arotolu et al. 2018). Cowpea genotypes with harder seed coats or containing certain chemical compounds can prevent female cowpea bruchids from laying eggs on seeds (Togola et al. 2017; War et al. 2017).

Callosobruchus exhibits a variety of behavioral responses to cowpea seeds, mainly involving

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oviposition, feeding, and mating (Arnold et al. 2012). For oviposition, female cowpea bruchids use sensory cues, such as visual and olfactory signals, to locate suitable cowpea seeds. The chemical signals emitted by insects depend on the intrinsic properties of the seed, such appearance, odor, and chemicals secreted by the seed (Anukiruthika et al. 2021). Usually, seeds that attract *C. maculatus* are not damaged, providing a safer environment for their offspring to develop. Both adults and their larvae feed on cowpea seeds, but most damage to cowpea seeds is caused by larval feeding (Baidoo et al. 2015; Tiroesele et al. 2015). Some cowpea genotypes may contain chemical substances that are toxic or unappetizing to cowpea bruchids, which can discourage both adults and larvae from feeding.

Cowpea cultivars are known to produce certain chemicals called semiochemicals that can modulate the behavior of *C. maculatus* (Ajayi et al. 2015; Osei-Owusu et al. 2020). The potential of plant-produced semiochemicals to modulate insect behavior has been exploited in the management of insect pest infestations of crops both in the field and in storage (Agelopoulos et al. 1999; Abd El-Ghany 1999; Sharma et al. 2019). Cowpea cultivars differ in their biochemical composition, so the extent to which they can influence insect behavior may vary (Jacob et al. 2016; Arotolu et al. 2018). Therefore, this paper aims to evaluate the behavioral responses of *C. maculatus* to ten different cowpea genotypes (5 local and 5 improved cultivars) and to determine the bioactive components of their extracts.

MATERIALS AND METHODS

Source of cowpea cultivars

A total of ten cowpea genotypes, comprising five local and five improved cultivars, were used in this study. Local cultivars ('Gombe', 'Drum', 'Sokoto', 'Big', and 'Honey') were purchased from the local market. Improved cultivars (ITI6K-1966-1, ITI5K-2377-1, ITI6K-1984-1, ITI4K-1903-1, and ITI5K-2386-1) were obtained from the Institute of Agricultural Research, Kano, Nigeria.

Laboratory culturing of *C. maculatus*

C. maculatus was cultured at the Entomology Laboratory, Department of Biology, Federal University of Technology, Akure, Ondo State, Nigeria, following the procedures described by Magagula and Maina (2012) and Beck and Blumer (2014). Cowpea bruchids were obtained from parent stock that had previously been maintained on a susceptible local cultivar, 'Black Eye'. Cowpea seeds were properly harvested to remove contaminants, dust particles, plant remains, and damaged and immature seeds. Healthy seeds were then disinfested by freezing for 72 hours in a freezer (Thermocool) at −4 °C. Disinfested seeds were allowed to acclimatize to ambient laboratory temperature for 24 hours before use in the experiment. Approximately 200 g of seeds were weighed into a plastic container, and five pairs of adult *C. maculatus* were introduced into each container and covered with a muslin cloth to provide oxygen and prevent insect escape. The containers were then placed in an insect rearing chamber under ambient laboratory conditions (temperature: 28 ± 2 °C, relative humidity: 65 ± 5%, photoperiod 12:12 h). The culture was maintained for 28 ± 2 days until adults emerged and then they (0–2-day-old) were used for experiments.

Behavioral response of *C. maculatus* to cowpea cultivars

The behavioral response of cowpea bruchids was determined according to the method described by Webster et al. (2008) and Ahuchaogu and Ojiako (2020) in Y-tube olfactometer bioassays. The Y-tube olfactometer has two arms: one is connected to a jar containing cowpea seeds of one cultivar (50 g), and the other is connected to a jar without seeds (control). A single bruchid was released and allowed to choose between two arms within 15 minutes of being introduced to the olfactometer. Bruchids that did not enter either arm within 15 minutes were considered nonresponders and were not included in the analysis. The olfactometer was cleaned with acetone after testing 20 insects, and the arms were rotated 90° to minimize positional effects. Each bruchid was tested once and discarded. For each cowpea cultivar, 80 respondents (40 male and 40 female) were used.

Extraction of crude oil from selected cowpea cultivars

Following previous studies on the behavioral response of *C. maculatus* to local and improved cowpea genotypes, three best local (‘Gombe’, ‘Big’, and ‘Drum’) and improved (ITI6K-1966-1, ITI5K-2377-1, and ITI6K-1984-1) cowpea cultivars were selected and used for extraction. Crude aqueous, ethanolic, and n-hexane extracts were prepared according to the extraction method used by Oyeniyi et al. (2021). Cowpea seeds were separately pulverized into powder (Mascot Mixer Grinder, Titan Scales, India; ISO 9001:2000). The powders were sieved through a 180 μm sieve and stored individually in prelabeled glass jars with an airtight lid. To the seed powder (30 g) of each cowpea cultivar, 120 mL of distilled water, ethanol (70%), and n-hexane (70%) were added separately and mixed in a shaker for 24 hours to prepare aqueous, ethanolic, and n-hexane extracts, respectively. The extracts were filtered using Whatman No. 1 filter paper. The filtrates were concentrated on a rotary evaporator (Rotavapor R-IIA; Buchi, Switzerland). Crude oil extracts were stored in amber glass bottles and kept refrigerated (4 °C) until subsequent experiments.

Olfactory response of cowpea bruchids to crude extracts of cowpea cultivars

The behavioral response of cowpea bruchids to cowpea seed extracts was performed using a Y-tube olfactometer bioassay as previously described for cowpea seed cultivars, with minor modifications. Two drops of each crude oil extract were applied onto a clean cotton wool and placed on one arm of the olfactometer, while the other arm contained cotton wool without extracts. Insects were introduced into two arms of a Y-tube olfactometer and were allowed to make decisions within 15 minutes of their introduction into the olfactometer. The cotton wool impregnated with the extracts was replaced after every five trials. The olfactometer was cleaned with acetone after testing 20 insects, and the arms were rotated (90°) to minimize positional effects. Each bruchid was tested once and discarded. A total of 40 male and 40 female respondents were used in the study.

Analysis of crude extracts of cowpea cultivars by gas chromatography-mass spectrometry (GC-MS)

Bioactive components of extracts of cowpea genotypes were determined using GC-MS (Agilent), according to the method of Oyeniyi et al. (2024). GC-MS analysis was performed using a Varian 3800/4000 GC-MS spectrometer equipped with an HP-5MS capillary column (Restek, USA; bonded and cross-linked with 5% phenyl methyl polysiloxane, 30 mm long, 0.25 mm in diameter, and 0.25 μm coating thickness). The carrier gas was nitrogen at a flow rate of 1 $mL \cdot min^{-1}$. The injection sample volume was 1 μL in splitless mode. The GC temperature program was set as follows: initial temperature of 50 °C held for 3 minutes, then increased to 150 °C at 5 $°C \cdot min^{-1}$ and held for 3 minutes, a second increase of 2.5 $°C \cdot min^{-1}$ to 220 °C held for 3 minutes, and a final increase of 25 $°C \cdot min^{-1}$ to 300 °C held for 2 minutes. The mass spectrometer (MS) was operated in electron impact ionization (EI) mode at 70 eV with a detector voltage of 1.2 kV. The MS detector scanned from m/z 50 to 1000 at a scan speed of 2 $scans \cdot s^{-1}$. Both the transfer line and ion source were held at 280 °C. The MS spectrum of each peak (corresponding to FAME) was identified using library matching software from the National Institute of Standards and Technology (NIST).

Data analysis

Data obtained in the Y-tube olfactometer test (attraction of male and female bruchids to cowpea seeds or extracts) were analyzed using a two-sided binomial test. Decision time data for the two cultivar types (local or improved) were subjected to two-way analysis of variance (ANOVA) separately, and means were separated using Duncan’s new multiple range test. A general linear model was used to determine the effects of the two types of cultivars (local or improved) and gender on the attraction decision time of the exposed *C. maculatus*. All analyses were performed using the Statistical Package for the Social Sciences (SPSS, version 26.0).

RESULTS

Effect of cowpea genotype and gender on *C. maculatus* decision time of on the attractiveness in the Y-tube test

Tables 1–4 show the effects of cowpea cultivar and gender on the *C. maculatus* decision to be attracted to seeds and extracts. Although bruchid decision time varied with cultivar, extraction solvent, and gender, these differences were generally insignificant. In the case of local cultivars, significantly ($F_{4, 390} = 4.61$; $p = 0.001$) the longest (4.90 min) and the shortest (2.70 min) attraction decision time was observed in male and female bruchids exposed to 'Sokoto' and 'Drum' seeds, respectively (Table 1). Similarly, significantly ($F_{4, 390} = 4.02$; $p = 0.003$) the longest (4.08 min) and the shortest (2.70 min) attraction decision time was observed in male bruchids exposed to ITI5K-2377-1 and ITI4K-1903-1, respectively (Table 2). Bruchid gender had a significant effect on decision time only for local cultivars ($F_{1, 390} = 5.63$; $p = 0.018$) (Table 1 & 2). The interaction of cultivar and gender had no significant effect on the behavioral response of bruchids to seeds of local ($F_{4, 390} = 1.82$; $p = 0.125$) (Table 1) and improved ($F_{4, 390} = 1.60$; $p = 0.175$) (Table 2) cowpea cultivars.

Table 1. Attraction decision time (minutes) of male and female cowpea bruchids to five local cowpea cultivars

Cultivar	Gender	Decision time	Source	df	F	*P*
'Honey'	male	3.40±0.39[ab]	Intercept	1	974.23	<0.0001
	female	3.73±0.39[ab]				
'Big'	male	3.32±0.31[ab]	Cultivar (C)	4	4.61	0.001
	female	2.90±0.26[ab]				
'Sokoto'	male	4.90±0.55[c]	Gender (G)	1	5.63	0.018
	female	3.50±0.34[ab]				
'Gombe'	male	3.88±0.36[ab]	Cultivar × Gender (C × G)	4	1.82	0.125
	female	2.98±0.26[ab]				
'Drum'	male	2.90±0.26[ab]				
	female	2.70±0.20[a]				

Means in the column marked with the same superscript letters are not significantly different ($p > 0.05$) using Duncan's new multiple range test; df – degree of freedom

Table 2. Attraction decision time (minutes) of male and female cowpea bruchids to five improved cowpea cultivars

Cultivar	Gender	Decision time	Source	df	F	*P*
ITI6K-1966-1	male	3.38±0.36[abc]	Intercept	1	1106.17	<0.0001
	female	2.93±0.31[ab]				
ITI5K-2377-1	male	4.08±0.43[c]	Cultivar (C)	4	4.02	0.003
	female	3.78±0.36[bc]				
ITI4K-1903-1	male	2.70±0.20[a]	Gender (G)	1	2.58	0.109
	female	3.08±0.25[ab]				
ITI6K-1984-1	male	3.05±0.27[ab]	Cultivar × Gender (C × G)	4	1.60	0.175
	female	3.08±0.27[ab]				
ITI5K-2386-1	male	3.20±0.30[abc]				
	female	2.80±0.21[ab]				

Note: see Table 1

The decision time of bruchid attraction to the three extracts (aqueous, ethanolic, and n-hexane) of local and improved cowpea cultivars are presented in Tables 3 and 4, respectively. Irrespective of the cowpea cultivar and the solvent used for extraction, there was no significant difference ($p > 0.05$) in the attraction time for both sexes exposed to local (Table 3) and improved (Table 4) cultivars. Only female bruchids exposed to the local cultivar 'Drum' showed a significantly shorter ($p < 0.05$) attraction time (2.94 min) to volatile organic compounds (VOCs) in the ethanolic extract compared to those exposed to aqueous and n-hexane extracts (Table 3). In the case of the improved cultivar, the attraction time (3.35 min) of male bruchids to the VOCs in n-hexane extracts of ITI6K-1966-1 was significantly longer ($p < 0.05$) than that in the aqueous extract (Table 4). However, no significant difference ($p > 0.05$) was found in the attraction time of male and female bruchids to all ITI6K-1984-1 and ITI5K-2377-1 extracts (Table 4).

Olfactory response of *C. maculatus* to cowpea seeds and volatile organic compounds (VOCs) in crude extracts of local and improved cowpea cultivars in the Y-tube test

The olfactory response of *C. maculatus* to cowpea seeds of local and improved cultivars is shown in Figure 1 and to the different extracts in Figures 2 and 3. Generally, both sexes of adult *C. maculatus* were significantly ($p < 0.001$) attracted to local (Fig. 1A) and improved (Fig. 1B) cowpea cultivars, compared to the control. In the Y-tube test, of 40 females tested for each local cultivar, 36–40 (90–100%) female bruchids were significantly ($p < 0.001$) attracted to all five local cowpea cultivars, compared to the control (Fig. 1). In addition, 30 male bruchids were attracted significantly ($p < 0.01$) to 'Sokoto', compared to the control (Fig. 1A). However, only 'Gombe' showed complete attraction (100%) of female bruchids, compared to the control (Fig. 1A). In the improved cultivars, 37 male and 37 female bruchids (92.5%) were significantly ($p < 0.001$) attracted to the cowpea seeds of ITI6K-1984-1 and ITI6K-1966-1 in the Y-tube olfactometer test, compared to the control (Fig. 1B). However, 31 male bruchids (77.5%) were significantly ($p < 0.01$) attracted to ITI4K-1903-1, compared to the control (Fig. 1B).

Similarly, both male and female bruchids showed significant ($p < 0.001$) attraction to aqueous, ethanolic, and n-hexane extracts of local (Fig. 2) and improved (Fig. 3) cowpea cultivars. Regardless of gender, out of 80 bruchids (40 pairs) tested separately for each extract, 36–38 (90–95%) bruchids significantly ($p < 0.001$) preferred the 'Gombe' aqueous, ethanolic, and n-hexane extracts, compared to the control (Fig. 2A–C). In the improved cultivars, the highest number of 38 (95%) female bruchids chose the aqueous (Fig. 3A) and ethanolic (Fig. 3B) extracts of ITI6K-1966-1, while 37 (92.5%) chose the n-hexane (Fig. 3C) extract of ITI5K-2377-1, compared to the control. However, significantly ($p < 0.01$) the lowest number of 31 (77.5%) female bruchids chose VOCs in the aqueous (Fig. 3A) and n-hexane (Fig. 3C) extracts of ITI6K-1984-1, compared to the control. Furthermore, significantly ($p < 0.001$) the lowest number of male bruchids – 32 (80%) – chose the aqueous (Fig. 3A) and n-hexane extracts of ITI6K-1984-1 (Fig. 3C), as well as the n-hexane extract of ITI5K-2377-1 (Fig. 3C), compared to the control. However, significantly ($p < 0.001$) the highest number of male bruchids – 37 (92.5%) – chose the aqueous (Fig. 3A) and ethanolic (Fig. 3B) extracts of ITI5K-2377-1, compared to the control.

Table 3. Attraction decision time (minutes) of male and female cowpea bruchids to crude oil extracts (volatile organic compounds, VOCs) of three local cultivars

Cultivar	Gender	Extract		
		aqueous	ethanolic	n-hexane
'Big'	male	$3.13±0.16^{a\,(A)}$	$3.20±0.18^{a(A)}$	$3.21±0.17^{b(A)}$
	female	$3.17±0.17^{a(A)}$	$3.09±0.14^{a(A)}$	$3.35±0.20^{ab(A)}$
'Gombe'	male	$2.92±0.15^{a(A)}$	$2.78±0.14^{a(A)}$	$2.76±0.14^{ab(A)}$
	female	$3.15±0.16^{a(A)}$	$3.01±0.16^{a(A)}$	$3.21±0.17^{b(A)}$
'Drum'	male	$2.85±0.15^{a(A)}$	$2.87±0.15^{a(A)}$	$3.43±0.19^{b(A)}$
	female	$3.27±0.16^{a(B)}$	$2.94±0.17^{a(A)}$	$3.63±0.19^{b(C)}$

Means in the column marked with the same lowercase letter and in the row with the same uppercase letter are not significantly different ($p > 0.05$) using Duncan's new multiple range test

Table 4. Attraction decision time (minutes) of male and female cowpea bruchids to crude oil extracts (volatile organic compounds, VOCs) of three improved cultivars

Cultivar	Gender	Extract		
		aqueous	ethanolic	n-hexane
ITI6K-1966-1	male	3.98±0.16$^{a(A)}$	3.18±0.16$^{a(AB)}$	3.35±0.17$^{a(B)}$
	female	3.05±0.16$^{a(A)}$	3.44±0.19$^{a(B)}$	3.21±0.17$^{a(AB)}$
ITI6K-1984-1	male	3.07±0.15$^{a(A)}$	3.02±0.17$^{a(A)}$	2.99±0.17$^{a(A)}$
	female	3.03±0.16$^{a(A)}$	3.02±0.16$^{a(A)}$	3.05±0.20$^{a(A)}$
ITI5K-2377-1	male	3.31±0.20$^{a(A)}$	3.35±0.17$^{a(A)}$	3.14±0.18$^{a(A)}$
	female	3.28±0.19$^{a(A)}$	3.28±0.17$^{a(A)}$	3.00±0.16$^{a(A)}$

Note: see Table 3

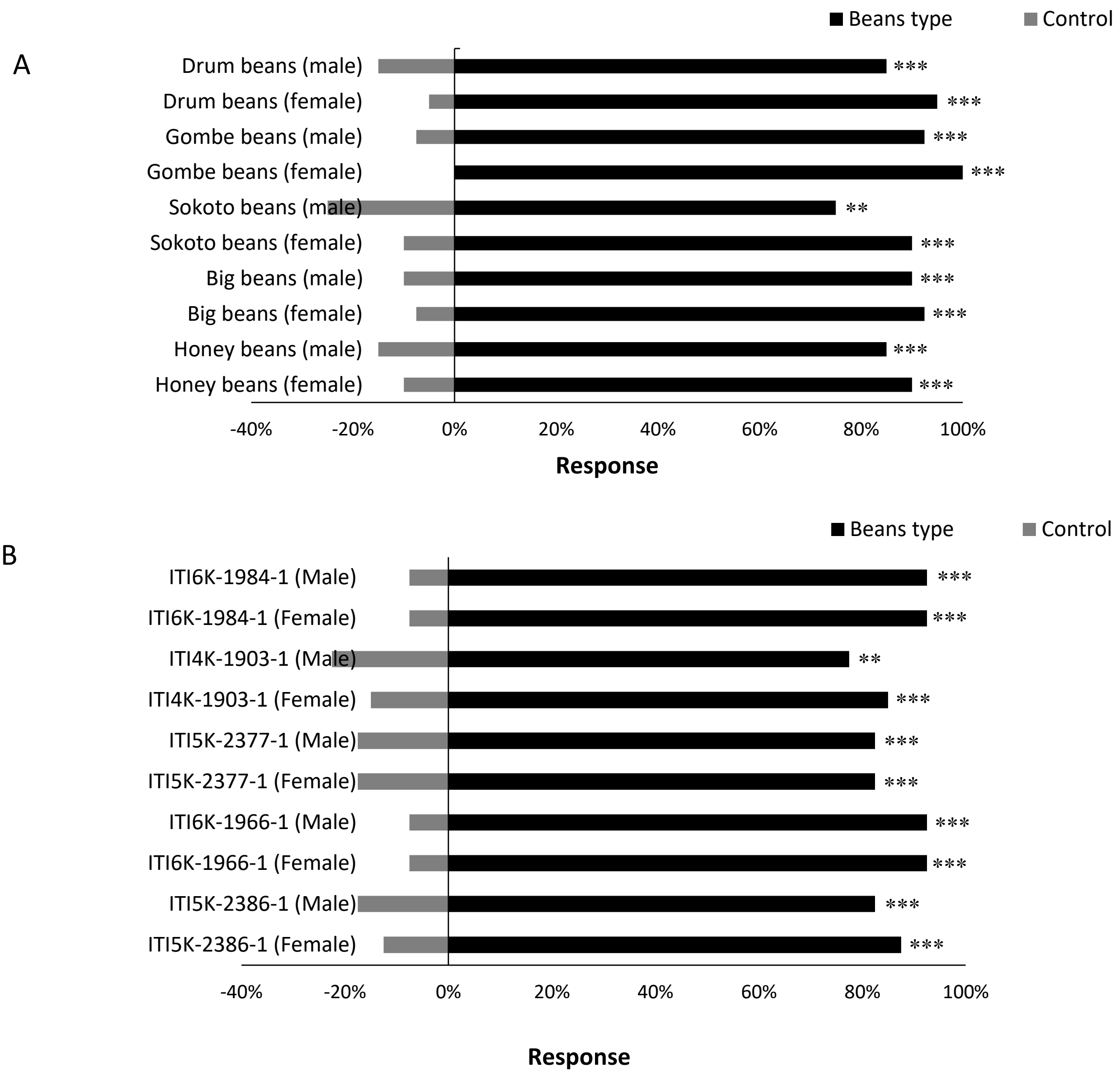

Figure 1. Attraction of male and female cowpea bruchids in the Y-tube olfactometer to different types of cowpea cultivars – local (A) and improved (B), compared to the control (n = 80)
(*) p < 0.05, (**) p < 0.01, (***) p < 0.001, (ns) p > 0.05 (two-sided binomial test, α = 0.05)

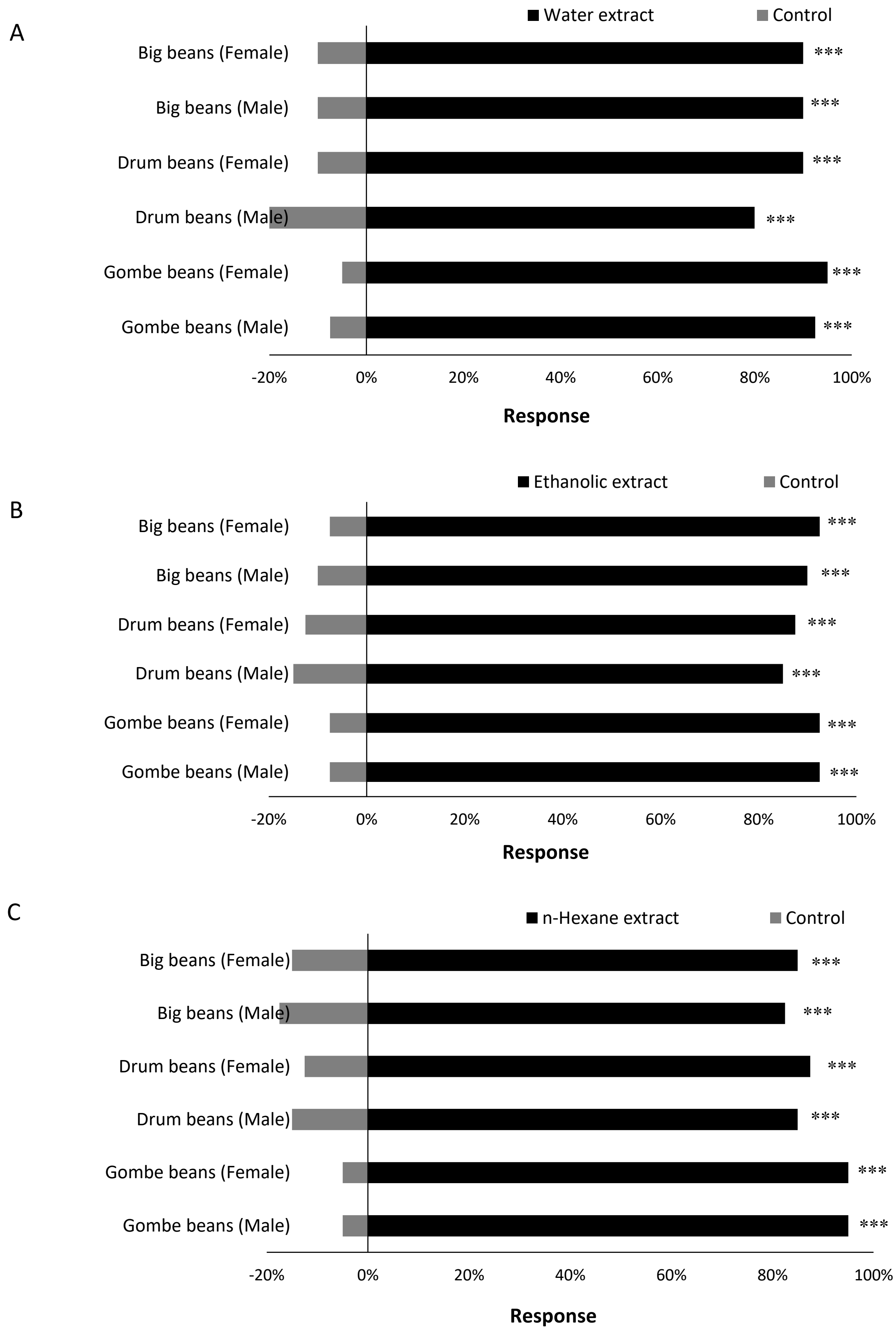

Figure 2. Attraction of male and female cowpea bruchids in the Y-tube olfactometer to aqueous (A), ethanolic (B), and n-hexane (C) extracts of local cowpea cultivars, compared to the control (n = 80)

Note: see Figure 1

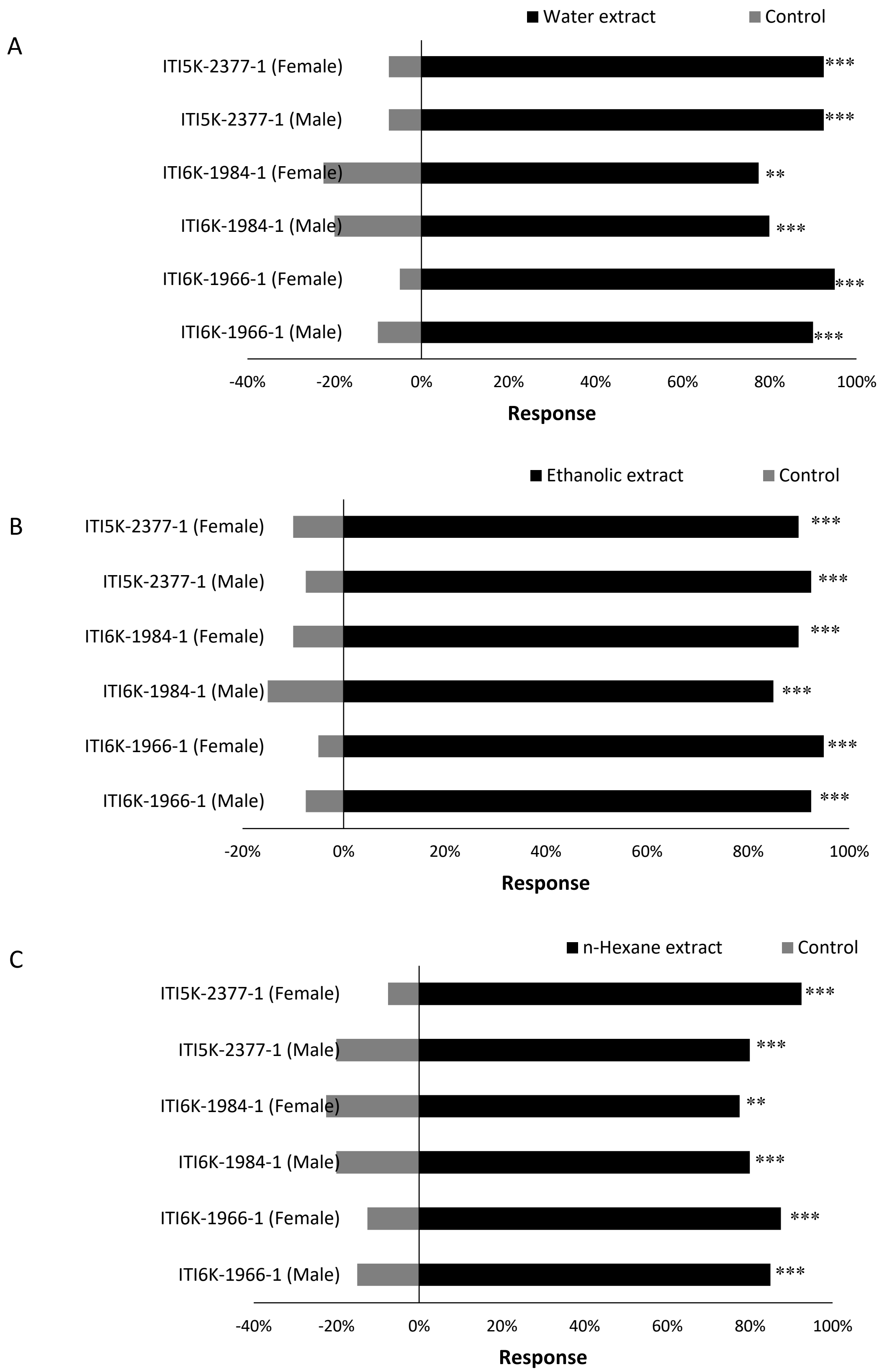

Figure 3. Attraction of male and female cowpea bruchids in the Y-tube olfactometer to aqueous (A), ethanolic (B), and n-hexane (C) extracts of improved cowpea cultivars, compared to the control (n = 80)

Note: see Figure 1

Bioactive compounds detected in aqueous, ethanolic, and n-hexane extracts of local and improved cowpea cultivars

Twenty (20) and nineteen (19) bioactive compounds were detected in the three extracts of the local (Table 5) and improved (Table 6) cowpea cultivars, respectively. However, only twelve (12) compounds were detected and identified in all three extracts of local cowpea cultivars (Table 5): p-xylene, 1-ethyl-3-methylbenzene, n-hexadecanoic acid, methyl salicylate, (R)-linalool, elaidolinolenic acid, methyleugenol, (E)-4,8-dimethylnona-1,3,7-triene, oleic acid, β-ionone, linoleic acid, and pentadecanoic acid. Similarly, out of 19 compounds identified in improved cultivars (Table 6), only 12 compounds were detected in all three extracts: β-pinene, 4-methoxycinnamaldehyde, n-hexadecanoic acid, methyl salicylate, (R)-linalool, elaidolinolenic acid, methyleugenol, (E)-4,8-dimethylnona-1,3,7-triene, oleic acid, β-ionone, linoleic acid, and pentadecanoic acid. nine identified compounds had a higher percentage composition (≥ 6.0) than other compounds: 1-ethyl-3-methylbenzene, n-hexadecanoic acid, methyl salicylate, (R)-linalool, elaidolinolenic acid, oleic acid, β-ionone, linoleic acid, and pentadecanoic acid.

Table 5. Compounds detected in extracts of local cowpea cultivars

No.	Compound	Molar mass ($g \cdot mol^{-1}$)	Molecular formula	Local cowpea seed extract
1	n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	all extracts
2	methyl salicylate	152	$C_8H_8O_3$	all extracts
3	pentadecanoic acid	242	$C_{15}H_{30}O_2$	all extracts
4	(R)-linalool	154	$C_{20}H_{32}O_2$	all extracts
5	elaidolinolenic acid	200	$C_{12}H_{24}O_2$	all extracts
6	methyleugenol	178	$C_{11}H_{14}O_2$	all extracts
7	(E)-4,8-dimethylnona-1,3,7-triene	150	$C_{11}H_{18}$	all extracts
8	oleic acid	282	$C_{18}H_{34}O_2$	all extracts
9	β-ionone	192	$C_{13}H_{20}O$	all extracts
10	linoleic acid	280	$C_{18}H_{32}O_2$	all extracts
11	squalene	410	$C_{22}H_{44}O_2$	aqueous extract
12	p-cymene	134	$C_{10}H_{14}$	n-hexane extract
13	ricinoleic acid	298	$C_{18}H_{34}O_3$	n-hexane extract
14	azulene	128	$C_{18}H_8$	aqueous and n-hexane extract
15	p-xylene	106	C_8H_{10}	all extracts
16	1-ethyl-3-methylbenzene	120	C_9H_{12}	all extracts
17	campesterol	400	$C_{28}H_{48}O$	n-hexane extract
18	benzeneethanamine	121	$C_8H_{11}N$	aqueous and ethanolic extract
19	4-hydroxy-7,8-dihydro-β-ionone	210	$C_{13}H_{22}O_2$	aqueous and ethanolic extract
20	quercetin	302	$C_{15}H_{10}O_7$	aqueous and ethanolic extract

Table 6. Compounds detected in extracts of improved cowpea cultivars

No.	Compound	Molar mass ($g \cdot mol^{-1}$)	Molecular formula	Improved cowpea seed extract
1	n-hexadecanoic acid	256	$C_{16}H_{32}O_2$	all extracts
2	methyl salicylate	152	$C_8H_8O_3$	all extracts
3	pentadecanoic acid	242	$C_{15}H_{30}O_2$	all extracts
4	(R)-linalool	154	$C_{20}H_{32}O_2$	all extracts
5	elaidolinolenic acid	200	$C_{12}H_{24}O_2$	all extracts
6	methyleugenol	178	$C_{11}H_{14}O_2$	all extracts
7	(E)-4,8-dimethylnona-1,3,7-triene	150	$C_{11}H_{18}$	all extracts
8	oleic acid	282	$C_{18}H_{34}O_2$	all extracts
9	β-ionone	192	$C_{13}H_{20}O$	all extracts
10	linoleic acid	280	$C_{18}H_{32}O_2$	all extracts
11	squalene	410	$C_{22}H_{44}O_2$	aqueous extract
12	p-cymene	134	$C_{10}H_{14}$	n-hexane extract
13	ricinoleic acid	298	$C_{18}H_{34}O_3$	n-hexane extract
14	azulene	128	$C_{18}H_8$	aqueous and n-hexane extract
15	4-methoxycinnamaldehyde	120	$C_{10}H_{10}O_2$	all extracts
16	β-pinene	136	$C_{10}H_{16}$	all extracts
17	eicosanoic acid	308	$C_{20}H_{40}O_2$	n-hexane extract
18	gallic acid	170	$C_7H_6O_5$	aqueous and ethanolic extract
19	myricetin	318	$C_{15}H_{10}O_8$	aqueous and ethanolic extract

DISCUSSION

Understanding host–insect interactions is crucial to effectively managing the variety of insect pests that attack stored products. This study investigated how different cowpea cultivars modulate the host preferences of the cowpea bruchids, *C. maculatus*. This study showed that the attraction decision time and olfactory response of *C. maculatus* differed depending on cowpea genotype, bruchid gender, and solvents used to extract volatile organic compounds (VOCs). All cowpea seeds used in this study were susceptible to *C. maculatus* infestation, which is consistent with several previous studies that confirmed the susceptibility of some local and improved cowpea seed cultivars to cowpea bruchids (Oyeniyi et al. 2015a, b; Musa & Adeboye 2017). Although mated female bruchids generally needed less time to determine the attractiveness of cowpea seeds than males, differences due to gender or extraction solvent in decision time were generally insignificant. It was shown that the local and improved cowpea cultivars used in this study may have not been very diverse in their genetic composition. The laboratory population of bruchids used in this study was maintained in our laboratory for more than 30 generations. It is also possible that bruchids may have exhibited genetic uniformity and showed a high rate of acclimatization to laboratory ambient conditions over time, which could have influenced their attraction decision time. If the cowpea seeds had little genetic variation, this could also be the reason for the insignificant differences in attraction decision time.

However, a significant number of male and female bruchids were significantly attracted to seeds and extracts of local and improved cowpea cultivars compared to the control in Y-tube olfactometer bioassays. Similar observations were reported by Ajayi et al. (2015) for mated female bruchids on three legume cultivars. The strong preference of female bruchids for the odors contained in the seeds of local and improved cultivars confirms the suitability of these cultivars for consumption and oviposition. Female bruchids seek out legume seeds as a suitable site for oviposition, while males are usually attracted to cowpea seeds that are approached by females suitable for mating (Nojima et al. 2007; Arnold et al. 2012). Three local ('Big', 'Gombe', and 'Drum') and improved (ITI6K-1966-1, ITI5K-2377-1, and ITI6K-1984-1) cultivars of cowpea showed considerably high attractiveness in the Y-tube bioassays. However, the local 'Gombe' and improved ITI6K-1966-1 cultivars strongly attracted female bruchids in the Y-tube olfactometer. The seeds of cowpea cultivars 'Gombe' and ITI6K-1966-1 are larger than those of other cultivars. Female bruchids prefer cowpea seeds with a relatively large surface area for laying and distributing eggs (Arong & Usua 2006; Oyeniyi & Adegoke 2024). This could have contributed to the greater attraction to the seeds of these cultivars among females compared to others. Furthermore, differences in the attraction of male and female bruchids could be linked to the semiochemical cues released from the seeds of the different cowpea genotypes used in this study (War et al. 2017; Ajayi et al. 2018; Kpoviessi et al. 2019). Therefore, the preference of *C. maculatus* for cowpea seeds could also have been influenced by differences in chemical cues between cultivars. This shows that the chemical cues used are important for *C. maculatus* to locate and select preferred cowpea seeds, and this mainly depends on their genotype. *C. maculatus* gender had no significant effect on bruchid behavioral response to improved cowpea cultivars, unlike to the local cultivar. This indicates that chemical cues affect both male and female bruchids equally in this group of cultivars. Therefore, improved cultivars may have had less genetic differences than local cultivars. The observed result could also have been influenced by the negative impact on the odor and chemical composition of improved cowpea cultivars, which could have been caused by attempts to enhance traits such as yield, pest resistance, and nutritional value (Oyeniyi et al. 2015a; Boukar et al. 2020).

Although the type of solvent used for extraction (distilled water, ethanol, or n-hexane) had no considerable effect on the attraction decision time of bruchids to VOCs in the three local ('Big', 'Gombe', and 'Drum') and improved (ITI6K-1966-1, ITI5K-2377-1, and ITI6K-1984-1) cowpea cultivars, it did influence bruchid attraction and amount of compounds observed in the seeds. Among the 20 and 19 compounds identified in local and improved cowpea cultivars, respectively, only 10 compounds showed significant abundance over others, irrespective of the extraction solvent and cowpea cultivar. Eight of these ten compounds (n-hexadecanoic acid, methyl salicylate, (R)-linalool, elaidolinolenic acid, methyleugenol, (E)-4,8-dimethylnona-1,3,7-triene, β-ionone, and linoleic acid) were identified as insect attractants in previous studies (Saeidi & Adam 2011; Tamiru et al. 2015; Tewari et al. 2015; Ajayi et al. 2018; Huff & Pitts 2019; Salamanca et al. 2019; Paparella et al. 2021; Tobing et al. 2022). Similarly, synthetic forms of some of these eight compounds have different odors and have been reported to attract cowpea bruchids (Adhikary et al. 2015; Ajayi et al. 2015). This indicates that the infestation of cowpea seeds by *C. maculatus* could be mediated by the odor of these compounds. The high rate of bruchid attraction could therefore be linked to the presence of mixtures of these eight compounds identified in the cowpea cultivars. Mixtures of different VOCs usually attract more insects than the individual components (Beck et al. 2012; Li et al. 2012). However, the n-hexane extract contained the highest levels of VOCs, regardless of the cowpea cultivar, which is consistent with the observations of Phillips (1997). The greatest attractiveness of the local 'Gombe' and improved ITI6K-1966-1 cultivars for female bruchids could therefore be linked to the synergistic effect of known insect attractants observed in their aqueous, ethanolic, and n-hexane seed extracts, such as n-hexadecanoic acid, methyl salicylate, methyleugenol, (E)-4,8-dimethylnona-1,3,7-triene, and β-ionone (Saeidi & Adam 2011; Tamiru et al. 2015; Salamanca et al. 2019; Paparella et al. 2021). However, further studies are needed to determine the actual composition of 'Gombe' and ITI6K-1966-1 to whose seed extracts female bruchids respond with strong attraction. The most attractive for *C. maculatus* females were n-hexane and aqueous extracts of 'Gombe' and ethanolic and aqueous extracts of ITI6K-1966-1 cowpea seeds. This suggests that water could be used as a cheap and environmentally friendly solvent for in cowpea seed extracts.

CONCLUSIONS

All cowpea seed genotypes studied were susceptible to cowpea bruchid attack, attracting female and male bruchids to varying degrees. Female *C. maculatus* showed a greater attraction to cowpea seeds than males, although the differences were mostly insignificant. The greatest attractiveness for cowpea bruchids was shown by the local cultivar 'Gombe' and the improved ITI6K-1966-1. The study showed that distilled water can be a suitable solvent for extracting potential insect attractants. Chemical cues contained in cowpea seeds may be a mediator in attracting cowpea bruchids. The study also showed that there may be little genetic diversity among the genotypes studied, especially the improved ones, due to the insignificant effect of gender and solvent type on the attraction decision time of bruchids. Further research is therefore needed to confirm the above information and to determine the exact mix of compounds that attracts *C. maculatus* and its potential as an effective bait for their control in storage facilities and retail establishments.

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