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Effects of Host and Non-Hosts Plant Volatiles on the Behaviour of the Lesser Grain Borer, *Rhyzopertha dominica* (Fab.)

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Abstract: Single choice behavioural responses of the lesser grain borer *Rhyzopertha dominica* (Coleoptera: Bostrichdae) to host and non-hosts plant volatiles was studied in a four-choice airflow olfactometer in the laboratory. The host plants odour sources were 30 g maize seeds and 30 g winter wheat kernels, while the non-hosts plant odour sources were 30 g dried *Piper guineense* and 30 g dried *Monodora myristica* seeds, respectively. Also 10% (w/w) of each non-host plant was incorporated with host plant grains and tested against *R. dominica* male and female adults. Results showed that both male and female adult *R. dominica* were significantly ($p<0.001$) repelled by *P. guineense* and *M. myristica* alone and also when 10% (w/w) of these plant seeds were incorporated with 30 g maize and winter grains, respectively. Both sexes also significantly ($p<0.0001$) preferred maize and winter wheat seeds to the control in the mean time spent and the number of entries made to their odour zones.

Key words: *Rhyzopertha dominica*, *Piper guineense*, *Monodora myristica*, repellence, olfactometer

INTRODUCTION

Farmers in many parts of Africa traditionally dry and store grains and cereals in open storage facilities holding between 500-1500 kg of harvested products. Storing grain is not only an activity to increase food security, it can also be considered, from the farmer's perspective, as an investment of time and material from which a profit is expected. In West Africa, grains sold 6 months after harvest, when grain is relatively scarce, generally commands a much higher price than grains sold at the time of harvest, when grains and other foods are plentiful (Meikle *et al*., 2002; Law-Ogbomo and Enobakhare, 2006). More than 600 species of beetles and 70 of moths among the insects, 355 species of mites, 40 species of rodents and 150 species of fungi have been reported to be associated with various stored products (Rajendran, 2005). Although the diversity of insects is high in the granaries in the sub-region, *Rhyzopertha dominica* (Fab.) has been reported as one of the major pests of stored grains such as maize, wheat, rice, sorghum, legumes and dried cassava (Haines, 1991; Arthur *et al*., 2006). This insect can penetrate many types of packaging material such as jute bags and both larvae and adults consume grain-based products resulting in fragmented kernels, powdery residues and a characteristic pungent odour. The complete life history of *R. dominica* has been reported to be approximately one month under optimum conditions (Howe, 1950; Rajendran, 2005). Unlike *Sitophilus zeamais* (Mostch.), there is little evidence that *R. dominica* infests ripening grains in the field (Hagstrum, 2001); therefore infestation in storage emanates from either a failure to destroy residual populations from the granaries or from dispersing individuals exploiting unprotected stored grains. This rapid colonization behaviour,

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strong flight ability and broad polyphagy, coupled with the fact that *R. dominica* has been trapped in diverse environments, including wood-lands substantial distances from grain stores (Cogburn, 1988), suggests the movement of this insect pest between potential natural habitats and grain storage facilities. This movement by *R. dominica* is enhanced by the male-produced aggregation pheromones and host plant volatiles in storage houses (Landolt, 1997; Bashir *et al*., 2001). Males produce the aggregation pheromone upon location of a suitable food source for feeding and breeding (Edde *et al*., 2005, 2007). The aggregation pheromones of *R. dominica* were identified as (S)-(+)-1-methylbutyl (E)-2-methyl-2-heptanoate and (S)-(+)-1-methylbutyl (E)-2,4-dimethyl-2-pentanoate (Khorramshahi and Burkholder, 1981; Williams *et al*., 1981; Razkin *et al*., 1996). The two pheromone blends are commonly referred to as Dominicalure-1 or DL1 and Dominicalure-2 or DL2, respectively. Both pheromones are equally attractive to both sexes of the beetle in the field and the laboratory resulting in local accumulations of *R. domonica* on food hosts, most importantly on stored cereals (Bashir *et al*., 2001; Edde *et al*., 2005). Annual post-harvest pest losses world over have been estimated at 10%. Weight losses (dry matter loss) in the range of 0.5-17% in cereals and up to 50% in pulses have been reported (Pantenius, 1988; Rees, 2004). In Nigeria, the loss of grains during storage due to storage insect pests has long been a serious problem to the farmers. Inputs in the form of human power and finances invested in the production of the crop are wasted. Insect contaminants such as excreta (uric acid) exuviate (cast skins) and dead bodies, webbing and secretions in food commodities pose a quality-control problem for food industries. Processing and end-use qualities of food commodities are also affected by insect infestation, as are cash value and marketability of products (Umoeteok *et al*., 2004).

Current pest management programs for *R. dominica* as recommended by the Nigerian Ministry of Agriculture and Stored product agencies specify sanitation of empty storage facilities and the use of residual surface treatment, insecticide seed treatments, grain temperature management, routine insect monitoring using grain probes and fumigation when an insect infestation develops (Umoeteok *et al*., 2004). Methyl bromide and aluminium phosphide are the most common fumigants extensively used all over the world, particularly in developing countries as grain preservatives in larger storage containers. However, some reports have appeared in the literature in which serious toxic effects of these substances to lungs, heart and blood vessels causing pulmonary oedema, shock and arrhythmias have been reported (Singh *et al*., 1991; Khosla *et al*., 1992; Abder-Rahman, 1999). Thus, it is highly imperative to look for cheap, less toxic and environmentally friendly natural products for reducing *R. dominica* damage in storage. Plants, plant parts or extracts with repellent or toxic properties can be used to protect stored products against pest attack (Adler *et al*., 2000; Boeke *et al*., 2004).

The objective of this study was to investigate the repellent properties of West African black pepper, *Piper guineense* Thonn and Schum (Piperaceae) and African nutmeg, *Monodora myristica* Dunal (Annonaceae) against *R. dominica* using a four-arm airflow olfactometer in the laboratory. These plants were selected on the basis of their ethnomedical studies and endemicity as their seeds are used in the traditional African medicine for the treatment of headache, constipation, rheumatism, fever, diarrhoea, sores and guinea worm infections. The ripe fresh or dried fruits of these plants are sold in most markets in Nigeria as a spice use for flavouring soups and sauces (Oyedeji *et al*., 2005; Adewoyin *et al*., 2006).

MATERIALS AND METHODS

Insect Culture

R. dominica was obtained from stock culture maintained by Central Science Laboratory, Sand Hutton, York, United Kingdom and reared on winter wheat “conqueror” variety seeds in a Constant Temperature and Humidity (CTH) room running at 25°C, 65% relative humidity on a 12:12 DL photoperiod. Fifty pairs of adult *R. dominica* sexed following the methods of Ghorpade and Thyagarajan (1980) were introduced into 300 g wheat seeds in bell jars. The adults were allowed to feed on wheat grains in the bell jars for 12 days (optimum oviposition time) after which they were removed and discarded. A sieve size of 2 mm, (Endecotts Ltd., England) was used to sieve out the emerged insects daily and records were kept of their sexes and dates of emergence. Beetles whose responses were to be tested in the four-arm airflow olfactometer were kept singly for 6 days and then starved for 24 h before being used for the bioassays.

Plant Material Collection and Preservation

Two plant materials namely; *Piper guineense* and *Monodora myristica* were obtained from fields around Akamkpa (situated between latitude 5°00′ and 5°15′ North and longitude 8°04′ and 8°25′ East) in southern Nigeria in December 2006 and identified at the department of Crop Science, University of Calabar, Nigeria. The matured seeds of these plants were dried in the shade for 3 days before transportation.

Bioassays

The four-arm airflow olfactometer was obtained from Rothamsted Research (Harpenden, Hertfordshire, England). It consisted of a four-pointed star-shaped exposure chamber was milled into a circular transparent plastic plate measuring 12×12×1.2 cm, with a hole (3 mm diameter) drilled into the walls at each of the four cardinal points. Another plastic plate (10.2×10.2×0.6 cm) served as the floor and another transparent plastic plate of the same size but with a hole (4 mm diameter) in its centre, served as a cover. Since *R. dominica* cannot walk on smooth glass surfaces a sheet of Springfield filter paper 125 mm (Springfield Mill, Maidstone, Kent, England) was used as a floor covering. The olfactometer side arms made of socket glass were inserted through the holes of the chamber walls. The air stream through the olfactometer was supplied by the Air entrainment system (KNF Neuberger, Germany) through Teflon tubing measuring 3.2×1.5 mm (Camlab Ltd., UK). Immediately after the pump, the air was divided through 2 carbon rods to clean and dry it. From each carbon rod, the air stream was then further divided and pushed through two flow meters (GPE Ltd., Leighton Buzzard, UK) to give a total of four airflows going into the behaviour chambers at the rate of 200 mL min^{-1}. Each air stream then passed through a glass side arm, which contained either, the odour/volatile source or clean filter paper, which served as a control. The air was pulled from the chamber through the central hole in the cover plastic plate. The different odour sources used to compare the responses of naïve adult male and female *R. dominica* were; (1) host volatiles emanating from 30 g of whole, sound, grains of white maize or winter wheat seeds "conqueror" variety alone, (2) non-host volatile odours from 30 g partially crushed *P. guineense* or *M. myristica* seeds alone, (3) incorporation of 10% (w/w) of each non-host + host plant volatiles combination. Single choice repellent and attraction bioassays consisting of *P. guineense* and *M. myristica* as non-host and White maize and winter wheat grains as host plant volatiles listed above were conducted in the CTH room. The olfactometer was coded as five areas: one square shaped central area and four rectangular areas corresponding to the four arms of the olfactometer, each area was marked with a number between 1 and 5. For these bioassays, the arm with the test odour(s) was given number 1; the 3 controls numbered 2, 3 and 4, respectively, while the central arena was given number 5. 30 g partially crushed seeds of the test material was weighed and placed in arm number 1 while the other 3 arms contained clean filter papers and served as controls. Each beetle was observed for ten min using a stopwatch and each bioassay was replicated 10 times. During each replicate, a fresh insect and olfactometer were used, while odour samples were replaced after every 2 replications. All experiments were conducted between 9.00 am and 12.00 pm in the CTH room. After starting the experiment by the release of the beetle into the centre of the olfactometer, the insect was followed visually and considered to have entered a given field when its entire thorax crossed the zone boundary. A computer programme for collecting and analysing behavioural data with the four-armed olfactometer (commonly referred to as OLFA programme) developed by Francesco Nazzi (33100 Udine, Italy) was used to obtain data. The data recorded includes the time spent by the insect in the different areas of the olfactometer and the number of entries into each area or odour zone.

Statistical Analysis

For statistical analysis the software package MINITAB 14 was used. A pair-wise t-test was used to test for significant differences between the treated arm and the mean of the control arms. The time spent and numbers of entries (visits) by the beetle into different odour zones of the olfactometer were the parameters chosen for assessment of the difference between plant volatiles and the control.

RESULTS

The results of the repellent plants bioassay showed that all pair wise comparisons of test with control were significantly different ($p<0.0001$) from the control. The males spent significantly shorter time in the olfactometer arms emitting volatiles of *P. guineense* seeds ($t = 12.97$, $p<0.0001$) and *M. myristica* ($t = 11.75$, $p<0.0001$) than the control arms receiving clean air. The females also preferred control arms to the treated ones for *P. guineense* ($t = 11.6$, $p<0.0001$) spending a mean 3.04 and 0.62 min, respectively. To *M. myristica* treated arm, females spent 0.59 min and 3.14 in the control ($t = 10.47$, $p<0.0001$). For the number of entries made to each arm, the males significantly preferred control arm to *P. guineense* and made 3.57 and 1.8 visits, respectively ($t = 6.38$, $p<0.0001$), while the mean number of visits to *M. myristica* arm were 1.5 and the control 3.53 ($t = 8.28$, $p<0.0001$). The mean number of entries made by females to *P. guineense* arm were 1.8 and control 3.33 ($t = 5.71$, $p<0.0001$) and to M. *myristica* arm 1.7 and control 3.43, respectively ($t = 7.31$, $p<0.0001$) (Table 1).

For the host plants bioassay, the males were significantly more attracted to maize grains ($t = 26.58$, $p<0.0001$) and winter wheat grains ($t = 22.79$, $p<0.0001$) than the control. Males spent means of 6.2 and 6.3 min, respectively in the maize and winter wheat treated arms compared to control arm with mean time of 1.15 and 1.16 min, respectively. For the females attraction to maize grains was 5.95 min and control 1.23 min ($t = 45.64$, $p<0.0001$) and winter wheat seeds 6.2 min and control 1.11 min ($t = 32.84$, $p<0.0001$) (Table 2). For the number of entries or visits made to the treated and control arms, the responses of males to maize grains was 4.89 entries while the control arm was 1.52 ($t = 11.18$, $p<0.0001$) and to winter wheat seeds 4.2 entries and control 1.77 entries ($t = 8.16$, $p<0.0001$). The females responded to maize grains by making a mean number 5.11 entries and control 1.63 entries ($t = 12.04$, $p<0.0001$) and to winter wheat 4.4 entries with the control arm receiving 1.67 entries ($t = 11.52$, $p<0.0001$) (Table 2).

For the 10% repellent plants and maize seed volatiles bioassay results, the males were significantly repelled by *P. guineense* ($t = 8.9$, $p<0.001$) and *M. myristica* ($t = 5.42$, $p<0.001$) in the mean time spent than the control. The females also showed significant repulsion from *P. guineense* ($t = 7.44$, $p<0.001$) and *M. myristica* ($t = 7.43$, $p<0.001$) compared to control arms (Table 3). For the number of entries made between treated and control arms, the males were significantly repelled from *P. guineense* ($t = 3.02$, $p<0.014$) but not repelled by *M. myristica* ($t = 1.91$, $p<0.089$) than control arms. Females were significantly repelled by *P. guineense* ($t = 2.49$, $p<0.034$) and *M. myristica* ($t = 3.67$, $p<0.005$) treated arms than control arms (Table 3).

When 10% repellent plant volatiles were combined with winter wheat seeds, males were significantly repelled by *P. guineense* ($t = 10.03$, $p<0.0001$) and *M. myristica* ($t = 10.86$, $p<0.0001$) in the mean time spent than controls. Females were also repelled by *P. guineense* ($t = 10.14$, $p<0.0001$) and *M. myristica* ($t = 11.76$, $p<0.0001$) in the mean time spent than control arms (Table 4). For the

Table 1: Mean time spent in the test or average of three control arms out of 10 min and mean number of entries made by *R. dominica* adults in response to 30 g *P. guineense* or 30 g *M. myristica* seeds in the airflow olfactometer

	Time spent (min)		No. of entries	
Treatments	Test	Control	Test	Control
P. guineense				
Male *R. dominica*	0.60±0.14	3.14±0.06	1.8±0.33	3.57±0.20
Female *R. dominica*	0.62±0.14	3.04±0.07	1.8±0.25	3.33±0.10
M. myristica				
Male *R. dominica*	0.91±0.13	2.93±0.05	1.5±0.17	3.53±0.16
Female *R. dominica*	0.59±0.18	3.14±0.06	1.7±0.26	3.43±0.22

Table 2: Mean time spent in the test or average of three control arms out of 10 min and mean number of entries made by *R. dominica* adults in response to 30 g Winter wheat or 30 g maize grains in the airflow olfactometer

	Time spent (min)		No. of entries	
Treatments	Test	Control	Test	Control
Winter wheat				
Male *R. dominica*	6.30±0.17	1.16±0.06	4.20±0.25	1.77±0.09
Female *R. dominica*	6.20±0.13	1.11±0.04	4.40±0.22	1.67±0.09
Maize				
Male *R. dominica*	6.20±0.15	1.15±0.05	4.89±0.26	1.52±0.09
Female *R. dominica*	5.95±0.07	1.23±0.04	5.11±0.26	1.63±0.13

Table 3: Mean time spent in the test or average of three control arms out of 10 min and the mean number of entries made by *R. dominica* adults in response to 30 g Maize + 10% *M. myristica* or 30 g maize + 10% *P. guineense*

	Time spent (min)		No. of entries	
Treatments	Test	Control	Test	Control
10% *M. myristica* + 30 g maize				
Male *R. dominica*	1.78±0.12	2.67±0.05	2.9±0.23	3.3±0.10
Female *R. dominica*	1.59±0.11	2.72±0.05	2.6±0.22	3.4±0.07
10% *P. guineense* + 30 g maize				
Male *R. dominica*	1.85±0.06	2.57±0.03	2.4±0.27	3.37±0.08
Female *R. dominica*	1.99±0.05	2.52±0.03	2.7±0.21	3.37±0.08

Table 4: Mean time spent in the test or average of three control arms out of 10 min and the mean number of entries made by *R. dominica* adults in response to 30 g wheat + 10% *M. myristica* or 30 g winter wheat + 30 g *P. guineense*

	Time spent (min)		No. of entries	
Treatments	Test	Control	Test	Control
10% *M. myristica* + 30 g winter wheat				
Male *R. dominica*	1.57±0.08	2.84±0.04	2.4±0.16	3.47±0.14
Female *R. dominica*	1.35±0.09	2.86±0.03	2.3±0.26	3.50±0.10
10% *P. guineense* + 30 g winter wheat				
Male *R. dominica*	1.36±0.11	2.84±0.04	2.4±0.27	3.67±0.14
Female *R. dominica*	1.42±0.10	2.78±0.04	2.5±0.22	3.63±0.14

number of entries, the males made significantly less entries to *P. guineense* ($t = 4.20$, $p<0.002$) and *M. myristica* ($t = 4.40$, $p<0.002$), respectively. Females significantly frequented control arms than *P. guineense* ($t = 4.74$, $p<0.001$) and *M. myristica* ($t = 5.13$, $p<0.001$) as well (Table 4).

DISCUSSION

Based on the results of this study, adult *R. dominica* demonstrated clear orientation choices between the volatiles generated by host and non-host plants and a combination of volatiles emitted by both. Adult *R. dominica* that were given the choice between control and *P. guineense* or *M. myristica* seeds in the four arm airflow olfactometer significantly preferred the control arms. This suggests that *R. dominica* are able to detect these repellent plants through olfaction and avoid them when given the choice. This could explain at least in part, how the application of *P. guineense* and *M. myristica* protects grain from insect infestation in storage. These repellent plants may contain certain active volatile component that elicits antifeedant behaviour by the visiting insect. The compositions of the volatile oil from the seeds (berries) of these plants have been investigated: the major components of *P. guineense* seeds are dominated by monoterpenoids and moderate sesquiterpenoids including β-Pinene, β-Caryophyllene, Bicyclogermacrene, α-Pinene, Germacrene, δ-3-Carene, E-α-Bisabolene, α-Cubebene, Sabinene, Camphor, α-Phellandrene, β-Phellandrene, χ-Muurolene, Z-Nerolidol, Z-β-Ocimene, Myrcene, Ishwarane, Myristicin, Dillapiole, Elemicin, Limonene and Camphene (Salgueiro *et al*., 1998; Oyedeji *et al*., 2005). *M. myristica* essential oil components include α-Thuyene, α-Pinene, Myrcene, Limonene, α-Phellandrene, *p*-Cymene, Isopugegol, Aromandendrene, α-Terpineol, Carvacrol, Thymol and cis-Sabinol (Cimanga *et al*., 2002; Tatsadjieu *et al*., 2003). These essential oil components could be responsible for the repellent activity of *P. guineense* and *M. myristica* seeds against *R. dominica* in the airflow olfactometer. Similar findings have been reported by Oparaeke *et al*. (2006) that aqueous mixtures of *P. guineense* with garlic (*Allium sativum*) bulb at 10:10 and 20:10% w/v significantly reduced the population of the cowpea flower thrips, *Megalurothrips sjostedti* and increased the pod density compared to cypermethrin plus dimethoate which was the synthetic check. Umeh and Ivbijaro (1999) reported that the crude seed oil of *P. guineense* at 10% concentration significantly reduced damage by the termites *Macrotermes bellicosus* and *M. subhyalinus* in maize plots and increased yield. *M. myristica* has been reported to exhibit antibacterial properties against *Bacillus subtilis*, *Citrobacter* sp., *Escherichia coli*, *Proteus vulgaris*, *Salmonella typhimurium* and *Shigella flexneri* (Cimanga *et al*., 2002) and antifungal activity against *Aspergillus flavus* (Tatsadjieu *et al*., 2003). On the bioactivity of botanicals against *R. dominica*, Hassanali *et al*. (1997) reported that the bioactivity of materials derived from the leaves and succulent stems of *Ocimum kenyense* evoked high repellency against *S. zeamais*, moderate repellency against *R. dominica* and low repellency against *Sitotroga cerealella* in the laboratory. Also Belmain *et al*. (2005) reported that *Securidaca longepedunculata* (Polygalaceae) root powder, its methanol extract and the main volatile component, methyl salicylate, exhibited repellent and toxic properties to stored products insect pests. Methyl salicylate vapour also showed fumigant effect against *R. dominica*, *S. zeamais* and *Prostephanus truncatus*. The fact that insect response to particular plant volatiles can be markedly influenced by the concentration of that volatile is common in chemical ecology (Chhabra *et al*., 1999; Belmain *et al*., 2005). Only recently, Ukeh *et al*. (2007) reported that plantain inflorescence ash and crude neem products were effective in the prevention of insect pests' colonization of *Solanum melongena* in the field, suggesting that plant allelochemicals exert a wide range of effects on insects as repellents, deterrents and antifeedants.

Maize and winter wheat kernels were found attractive to *R. dominica* in the single choice olfactometer bioassay. Pike *et al*. (1994) identified the main volatile compound of maize seeds as hexanoic acid, nonanoic acid, nonanal, decanal, 2-phenylethanol and vanillin. The study showed that both male and females spent more time in the olfactometer arms emitting maize and wheat volatiles and could mean that the enhancement of attraction could be expected with other cereals. It might be thought that the attraction of the beetle to host volatiles was in response of both sexes to their proximity to a food source and an increased pheromone output by the males. These results confirmed the reports of Bashir *et al*. (2001) on the behavioural responses of *R. dominica* to blends of host volatiles and male-produced aggregation pheromone in the four-arm airflow olfactometer. They concluded that males spent significantly more time than females in the zone with only maize volatiles and females spent significantly more time in the higher maize volatiles zone. This could suggest that males are more likely to attract mates to a food source resulting to the formation of leks. In this study, no significant differences were observed between the sexes in their response to maize or winter wheat volatiles. Ako *et al*. (2003) also reported no sexual behavioural differences when adult male and female

S. zeamais were presented with maize seeds infested with *Fusarium verticillioides* and healthy maize kernels in a four-arm airflow olfactometer. Similarly, no sexual behavioural differences were reported between both sexes of another Coleopteran, *Scyphophorus acupunctatus* to their host plant *Agave tequilana* volatiles although they were attractive to the host volatiles in the Y-tube olfactometer bioassays (Altuzar *et al*., 2007).

These studies have reported the viability of the use of semiochemicals (behaviour modifying chemicals) for the monitoring and prevention of *R. dominica* in stored products. Repellents have the potential for the exclusion of stored product pests from grain and have been used to prevent insect feeding and oviposition. They represent an important part of the integrated pest management strategies in Africa because they are locally available, less expensive and safe to the environment.

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