Responses of *Bactrocera invadens* (Diptera:Tephritidae) to Volatile Emissions of Fruits from Three Hosts

Fikira Kimbokota1* Peter George Nganga Njagi2 Baldwyn Torto3 Sunday Ekesi4 and Ahmed Hassanali5

1. Faculty of Science, Mkwawa University College of Education, P.O. Box 2513, Iringa, Tanzania
2. Biological Sciences Department, University of Kabianga, P.O. Box 2030, 20200 Kericho, Kenya
3. International Centre of Insects Physiology and Ecology (icipe), P.O. Box 30772, Nairobi, Kenya
4. Department of Chemistry, Kenyatta University, P.O. Box 43844-00100, Nairobi, Kenya

* E-mail of the corresponding author: kimbokota@yahoo.com

Abstract

*Bactrocera invadens* is an invasive fruit fly species of Asian origin that was first detected in Kenya in 2003 and now has been reported in many parts of the African continent. The responses of *B. invadens* towards odour blends emitted from three host fruits, namely mango (*Mangifera indica*), marula (*Sclerocarya birrea*), and Indian almond (*Terminalia catappa*) growing in Nguruman and Embu in Kenya, were monitored in a dual choice olfactometer. Three mango varieties were tested (sensation, apple and kent), each at immature, mature unripe and ripe stages. Marula and Indian almond fruits were tested when mature, both when unripe and ripe. Results showed that, both male and female *B. invadens* were attracted equally to mature unripe and ripe mango fruits. However, in all cases attraction to immature fruits was significantly lower (P < 0.05). Two-way comparisons showed no significant difference between sensation and apple, but both attracted significantly more flies than kent. There were no significant different in attraction when *M. indica, T. catappa* and *S. birrea* were compared between each other. The results indicate that mature host fruits emit volatile constituents that are attractive to the fruit flies and if characterized may be useful in monitoring and managing *B. invadens*.

Keywords: *Bactrocera invadens*, fruit hosts, attraction, 2-choice wind tunnel

1. Introduction

Fruit flies are dipteran insects known to cause devastating losses of fruits and vegetables in the tropical and subtropical regions of the world (White and Elson-Harris, 1992). In Africa, the major fruit fly pests are members of the genera *Ceratitis* (De Meyer et al., 2002; De Meyer and Copeland, 2005) and *Dacus* (White, 2006). *Bactrocera invadens* (initially thought to be *Bactrocera dorsalis*), a highly invasive fruit fly species of Asian origin, was first reported in Kenya in 2003 (Lux et al., 2003). Since then, *B. invadens* has spread rapidly across the African continent and has been detected in 28 countries in eastern, western, central and southern Africa (Drew et al., 2005; Vayssières et al., 2005; Ekesi et al., 2006), and more recently, in Madagascar (Raolijaona et al., 2012). The pest is polyphagous with a wide range of cultivated and wild plant hosts (> 40) including important fruit and vegetable crops. It is now becoming a pest of major phytosanitary and economic importance in Africa, with considerable implications for agriculture and its export programmes (Muchemi et al., 2010). Worldwide, effective control of fruit flies has relied on the deployment of attractants for both detection and control. Fruit fly behaviours, such as host-searching, mate attraction and oviposition are mediated by semiochemicals (Fletcher and Prokopy, 1991; Jang and Light, 1996). Most adult fruit flies can detect volatiles of host fruits from some distance through olfaction and orient upwind towards the fruiting regions of a given tree host (Aluja and Prokopy, 1992). Three kinds of attractants have been proposed to catch fruit flies in traps: food lures, pheromones (sequestered by fruit flies from host plants) and sex pheromones. Food baits, such as hydrolyzed proteinaceous products, fermenting sugars and yeast have been the most usually employed in the field to catch tephritids. However, these lures are non-specific, have limited field life with low potency, attracting target flies only from short distances, and are difficult to handle (Siderhurst and Jang, 2006). Phenyl propanoid-based pheromones, such as methyl eugenol, cue-lure (4-(p-acetoxyphenyl)-2-butanone), and raspberry ketone (4-(p-hydroxyphenyl)-2-butanone), are very attractive to different species of Dacinae fruit flies (Fletcher, 1987; Metcalf, 1990; Chua et al., 1997; Vargas et al., 2010) and have been applied in traps to capture species of *Ceratitis, Dacus* and *Bactrocera* in the field. Recently, another phenyl propanoid, zingerone [4-(4-hydroxy-3-methoxyphenyl)-2-butanone], has been reported to be a potent male lure for a range of *Bactrocera* species (*B. carambolae, B. caudata, B. cucurbitae, B. tau, B. umbrosa, B. jarvisi* (Tan and Nishida, 2000; Harry, 2012). On-going research on fruitfly attractants has focused on improvements in attractants either through chemical structure-activity type screening (e.g. Nishida and Fukami, 1990; Nishida et al., 1992;...
In a survey undertaken at Nguruman in Kenya, Ekesi et al. (2006) found that *Mangifera indica* L. (Anacardiaceae) was the most preferred cultivated plant, while marula, *Sclerocarya birrea* (A.Rich) Hochst. (Anacardiaceae) and tropical almond, *Terminalia catappa* L. (Combretaceae), were most infested non-cultivated plants in the area (Mwatawala et al., 2006; Rwomushana et al., 2008). In the present study, the behavioural responses of male and female *B. invadens* to volatiles from fruits of the three plants at different maturity levels were compared in a dual choice olfactometer. The objective was to lay down the groundwork for follow up characterization of blends and constituents used by the fruit fly to locate its host fruits.

2. Materials and Methods

2.1 Insects and Study Area

Larvae of *B. invadens* were randomly collected from infested mango fruits that were picked from mango orchard at Nguruman (Long: 01° 48' 31 S, Lat: 36 ° 03' 34 E) and Embu (Long: 00° 29' 24 S, Lat: 37° 35' 31 E) in southwestern Kenya. The infested mangoes were then transferred into styrofoam containers (30 x 30 x 15 cm) with openings at the top that were covered by mosquito net materials to allow for aeration. The containers were kept in a rearing room in the insectary at the International Centre of Insect Physiology and Ecology (*icipe*), in Nairobi, Kenya. The larvae were left to develop in the infested native mango fruits and remained in the cage until they reached the fifth instar. They were then cleaned with water and transferred into clean sterile plastic bowls containing sterilized sand that mimicked the soil conditions in the field to facilitate their pupation. After one week, pupae were removed, cleaned with water, dried and kept in Petri-dishes in a Perplex cage (50 x 50 x 50 cm) until the emergence of adult flies. The adult fruit flies were fed on an artificial diet (sugar and enzymatic yeast hydrolysate ultrapure, 3:1; USB Corporation, Cleveland, Ohio, USA), and water on pumice granules. The rearing room was maintained at a temperature of 28 ± 2 °C, relative humidity of 60-65 % and 12L:12D photoperiod.

2.2 Fruits.

Three varieties of mango, *M. indica* fruits, namely apple, sensation and Kent, were collected from either Embu or Nguruman districts in Kenya. Fruits of marula, *S. birrea* and the Indian almond, *T. catappa* were collected from Nguruman. These served as sources of volatiles in the bioassays. Mango fruits were categorized into three maturity stages: fruitlets, mature unripe and mature ripe, while marula and tropical almond fruits were used only at mature unripe and ripe stages (fruitlets of these plants are small and were found dry rapidly before they reached the laboratory). All fruits were used for up to a maximum of three days from the day of collection from the field and were kept at ambient room temperature throughout the period.

2.3 Responses of *B. invadens* to fruit volatiles in a Dual Choice Olfactometer

Behavioural observations were made in a glass dual choice flatbed wind tunnel (30 x 30 x 100 cm) equipped with a 4 inch-extractor fan on top of the mid-section of the tunnel (Plate 2.1). Compressed medical air (BOC gases, Kenya) from a cylinder was passed through activated charcoal and then split into two streams to the opposite ends of the olfactometer. Teflon® tubings (5 mm diameter) were used as connectors. The extractor fan drew the air from the olfactometer at a flow rate of 15 ml/s. Comparative upwind flight behaviour of male and female *B. invadens* to the following choices were monitored:

(i) volatiles of each mango variety at each of 3 ripening stages compared with untreated control;
(ii) volatiles of *T. catappa* and *S. birrea* fruits at two ripening stages compared with untreated control;
(iii) volatiles of mature ripe mango of each variety with one another; and
(iv) volatiles of the most attractive mature mango (sensation) with mature *T. catappa* and *S. birrea* fruits, respectively.

The source of volatiles comprised of test fruit held in a 2 L flask that was connected to one end of the wind tunnel. The other end had a similar flask without the fruit for choices i and ii or had fruit(s) for choices iii and iv as explained above. The room temperature was maintained at 26 ± 2°C and relative humidity ranging between 59 and 65%.

12-15 day old flies were kept in Perplex® cages (25 x 25 x 25 cm) and allowed to acclimatize to the experimental room conditions for four hours prior to the tests. 10 males or females were then introduced into the wind tunnel at the central part of the working section through an aperture with a cover and observed for 10 minutes. The flies that flew upwind to at least 25 cm of either side (representing 50% of the distance from the point of release) were scored as responders. Flies that flew less distance were treated as ‘non-responders’ and excluded from statistical analyses. Each test with volatiles from a given fruit and maturity stage was replicated five times. After every five assays, the olfactometer was cleaned with acetone and the odour and control sides
were swapped with a fresh fruit for another set of replicates. All assays were conducted between 10:00 and 16:00 hrs local time.

2.4 Data Analyses

For each test the average number of flies that flew upwind in the two olfactometer arms were calculated and compared using Chi-square ($\chi^2$, $\alpha = 0.05$) (PROC FREQ, SAS Institute 1999-2000). In addition, attractancy indices were calculated using the following formula: Attractancy Index (AI) = ($Nt-Nc/Nt+Nc$) ×100%, where $Nt$ = number of flies that flew into the test side and $Nc$ = number of flies that flew into the control section. AI means of male and female flies associated with different fruits at different stages of maturity were subjected to Analysis of Variance (ANOVA) and compared by Student-Newman-Keuls (SNK) Test (PROC MEANS, SAS Institute 1999-2000).

3. Results

Responses of fruit flies to volatiles from mature unripe and mature ripe fruits of a given host were significantly different ($F_{(d,2.85)} = 8.66, n = 145, P = 0.003$, Student-Newman-Keuls Test) from those of volatiles from the fruitlets, but not between each other (Figures 1 and 2). There was no significant difference ($F_{(d,1.89)} = 2.59, n =131, P = 0.1098$, Student-Newman-Keuls Test) between the responses of male and female flies to the fruit volatiles.

Results further indicated that, in most cases both male and female B. invadens were attracted more significantly ($P < 0.05$) to the fruit volatiles than to the control, except in some cases where there was no significant different between control and tested volatiles. These include: (i) female flies responding to volatiles from fruitlets of mangoes of the apple variety ($\chi^2 = 2.6552, P = 0.7530$) and kent variety ($\chi^2 = 0.012, P = 0.9128$); (ii) male flies responding to volatiles from fruitlets of mangoes of the apple variety ($\chi^2 = 0.1006, P = 0.7511$), sensation variety ($\chi^2 = 0.1756, P = 0.6752$) and kent variety ($\chi^2 = 0.1045, P = 0.7465$). Volatiles from mature unripe and mature ripe fruits of the Indian almond, T. catappa were significantly more attractive ($P < 0.05$) to both male and female flies than to the control. However, mature unripe fruits of S. birrea were not significantly more attractive ($P > 0.05$) to either male or female flies compared to the control. In addition, volatiles from mature ripe fruits of this plant were significantly more attractive ($P < 0.05$) to both sexes than the control.

Dual choice comparisons between volatiles from different varieties of olfactometric tests showed no significant differences ($P > 0.05$) in the responses of the flies to apple and sensation volatiles. However, the volatiles of fruits from these two mango varieties attracted significantly more female flies than those from kent mangoes ($\chi^2 = 10.9286, P = 0.0274, \chi^2 = 3.6000, P = 0.0578$, respectively) (Table 2). There was no significant difference ($P > 0.05$) when volatiles from fruits of different host plants were compared (Table 2).

4. Discussion

The present study provides results of an evaluation of the attractiveness of odours from fruits of different levels of maturity from three hosts to both male and female fruit flies, B. invadens in a dual choice wind tunnel. Both female and male B. invadens showed higher responses to odours from mature unripe and ripe mangoes, M. indica, marula, S. birrea and ripe Indian almond, T. catappa fruits than to those from fruitlets. In agreement with results of earlier studies on other species of fruit flies that select fruits that are at least half-ripe for oviposition (Liquido et al, 1989). Cornelius et al. 2000 have demonstrated that adult female oriental fruit flies, Bactrocera dorsalis, are highly attracted to odours from soft and ripe fruits. Preference of flies for mature and ripe fruits could be due to the presence of certain groups of compounds that are produced at these levels of maturity that are detected by the antennal olfactory receptors of the flies, thus facilitating location of their hosts. Furthermore, the presence of these compounds in the fruit volatiles may associatively be an indication to the gravid flies of the soft texture of the mature fruit whose skin can easily be punctured with the ovipositor. The presence of a certain group of compounds may also signal the availability of enough resources for the survival of the larval stages of the insect up to the time of pupation. The major hypothesis of the evolution of oviposition behaviour in insects is that, the females choose host plant species that maximize larval survival and development. (Thompson and Pelmyr, 1991).

The ability of B. invadens flies to be attracted to the volatiles emitted by fruits of three different hosts, viz. mango, M. indica, Indian almond, T. catappa and marula, S. birrea demonstrate the polyphagous character of this insect. Oviposition behaviour plays a critical role in the survival of insects and it necessitates some of them to have a broad range of hosts. Selection of suitable oviposition substrates influences the potential survival and development of larvae, although in these flies, it also reflects on the extent of economic losses caused by oviposition punctures and larval feeding. With regard to differences in the responsiveness of flies to fruit volatiles from different varieties of mangoes, these could be due to quantitative and qualitative differences in the
composition of their volatiles. Pino and Mesa 2006 demonstrated quantitative and qualitative differences in the composition of volatiles among 20 different mango varieties. These compositional differences may be due to various factors, such as climate, soil content and other cultivation practices (Narain and Galvã 2004).

Attraction of both male and female flies to crude fruit volatiles may be indicative of the presence of a number of components that are attractive to both sexes. A number of laboratory-cultured male oriental fruit flies, B. dorsalis, have been observed on trees with fruits when released in the field at dusk (Prokopy et al 1996). Host plants have also been reported to influence the sex pheromone biology of phytophagous insects (Landolt 1997). For example, the search for mates by both sexes of Rhagoletis sp. is linked to the search for their host plants. Therefore, the cues used to locate a mate also play a role, either alone or with other cues (visual, tactile, and chemical) in the location of fruits on the host plant (Bush, 1969, 1974)

Host plants play an important role in the synthesis of sex pheromones of some phytophagous insects through the acquisition of bioactive chemicals and the necessary chemical precursors for the pheromones via consumption, absorption or inhalation of host plant materials (Landolt 1997) Many phytophagous insects aggregate at the primary feeding and oviposition sites and any other plant that is preferred by females (Landolt 1997). What guide them are the chemical stimuli emanating from these plant species (Landolt 1997). This ensures that there is high chance for mate location and mating and hence propagation of their generations. In other studies, observations have been made on Ceratitis capitata where both males and females were strongly attracted to citrus volatiles (Katsoyannos et al 1997). Shelly et al. 2001 showed that the medfly, C. capitata males exposed to oranges performed significantly more copulations than non-exposed males. Similarly, male medflies exposed to the bark and/or of fruits of guava tree, Psidium guajava L. had mating advantage over those that were deprived access to these substrates (Shelly and Villalobos, 2004).

The findings from these investigations indicate that, fruits of the three host release volatiles that have candidate attractants for both male and female B. invadens. This is a very significant finding since most attractants already in the market for fruit flies attract only males. One approach in fruit fly control programmes is the use of host-derived compounds.

References


Fitt G.P. (1984) Oviposition behaviour of two Tephritid fruit flies, Dacus tryoni and Dacus jarvis, as influenced by the presence of larvae in the host fruit, Oecologia, 62, 37–46


Acknowledgement

The research was financed by the Netherland Government in Cooperation with International Organisations (SII) through International Centre of Insects Physiology and Ecology (icipe) under African Regional Postgraduate Program in Insect Sciences (ARPPIS).

Figure 1. Attractancy Indices (A.I. ± SE %) of Bactrocera invadens females to Volatiles from Fruits of Three Mango, Mangifera indica Varieties at Different Stages of Maturity in a Dual Choice Wind Tunnel
Figure 2. Attractancy Indices (A.I. ± SE %) of *Bactrocera invadens* males to Volatiles from Fruits of Three Mango, *Mangifera indica* Varieties at Different Stages of Maturity in a Dual Choice Wind Tunnel

Table 1. Attractancy Indices (A.I. ± SE %) of *Bactrocera invadens* to Volatiles from Fruits of *Terminalia catappa* and *Sclerocarya birrea* at Different Stages of Maturity in a Dual Choice Wind Tunnel

<table>
<thead>
<tr>
<th>Host</th>
<th>Maturity of fruits</th>
<th>Attractancy Index (A.I. %)</th>
<th>Responders (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Females</td>
<td>Males</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>T. catappa</em></td>
<td>Mature ripe</td>
<td>40.10 ± 0.40</td>
<td>33.20 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>Mature unripe</td>
<td>14.67 ± 1.12</td>
<td>28.00 ± 1.38</td>
</tr>
<tr>
<td><em>S. birrea</em></td>
<td>Mature ripe</td>
<td>51.68 ± 0.51</td>
<td>42.29 ± 1.00</td>
</tr>
<tr>
<td></td>
<td>Mature unripe</td>
<td>40.76 ± 0.97</td>
<td>30.19 ± 0.74</td>
</tr>
</tbody>
</table>
Table 2. Attraction responses (± SE) of Female and Male *Bactrocera invadens* to Volatiles from Fruits of Different Hosts in a Dual Choice Wind Tunnel. *Same Letters in the Same Row Indicate Values that are not Significantly Different (Chi-square test, *P* < 0.05)

<table>
<thead>
<tr>
<th>Sex</th>
<th>Host pairs</th>
<th>Response (%)</th>
<th>Total Respondents (%)</th>
<th>Statistics*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(± SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>Apple</td>
<td>47.94 ± 9.12 a</td>
<td>72.00 ± 4.90</td>
<td>$\chi^2 = 2.2857, P = 0.1306$</td>
</tr>
<tr>
<td></td>
<td>Sensation</td>
<td>52.06 ± 9.12 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sensation</td>
<td>61.71 ± 8.00 a</td>
<td>56.00 ± 7.48</td>
<td>$\chi^2 = 10.9286, P = 0.0274$</td>
</tr>
<tr>
<td></td>
<td>Kent</td>
<td>38.29 ± 8.00 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>60.33 ± 8.33 a</td>
<td>56.00 ± 6.00</td>
<td>$\chi^2 = 3.6000, P = 0.0578$</td>
</tr>
<tr>
<td></td>
<td>Kent</td>
<td>39.67 ± 8.33 b</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>45.78 ± 6.61 a</td>
<td>58.00 ± 9.70</td>
<td>$\chi^2 = 0.3103, P = 0.5775$</td>
</tr>
<tr>
<td></td>
<td><em>T. catappa</em></td>
<td>54.22 ± 6.61 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>46.95 ± 6.67 a</td>
<td>58.00 ± 3.74</td>
<td>$\chi^2 = 0.0345, P = 0.1306$</td>
</tr>
<tr>
<td></td>
<td><em>S. birrea</em></td>
<td>53.05 ± 6.67 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. birrea</em></td>
<td>46.00 ± 2.69 a</td>
<td>58.00 ± 3.74</td>
<td>$\chi^2 = 0.5775, P = 0.5775$</td>
</tr>
<tr>
<td></td>
<td><em>T. catappa</em></td>
<td>54.00 ± 2.69 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>Apple</td>
<td>44.05 ± 7.65 a</td>
<td>58.57 ± 7.04</td>
<td>$\chi^2 = 0.2195, P = 0.6394$</td>
</tr>
<tr>
<td></td>
<td>Sensation</td>
<td>55.95 ± 7.65 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sensation</td>
<td>56.45 ± 9.18 a</td>
<td>68.00 ± 8.00</td>
<td>$\chi^2 = 0.1176, P = 0.7316$</td>
</tr>
<tr>
<td></td>
<td>Kent</td>
<td>43.55 ± 9.18a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>55.90 ± 4.21 a</td>
<td>58.00 ± 3.74</td>
<td>$\chi^2 = 0.3103, P = 0.5775$</td>
</tr>
<tr>
<td></td>
<td>Kent</td>
<td>44.10 ± 4.21a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>51.90 ± 5.58 a</td>
<td>70.00 ± 4.47</td>
<td>$\chi^2 = 0.0286, P = 0.8658$</td>
</tr>
<tr>
<td></td>
<td><em>T. catappa</em></td>
<td>48.10 ± 5.58 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Apple</td>
<td>57.52 ± 9.70 a</td>
<td>70.00 ± 6.32</td>
<td>$\chi^2 = 0.7143, P = 0.3980$</td>
</tr>
<tr>
<td></td>
<td><em>S. birrea</em></td>
<td>42.48 ± 9.70 a</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>S. birrea</em></td>
<td>54.00 ± 7.48 a</td>
<td>52.00 ± 2.00</td>
<td>$\chi^2 = 0.1538, P = 0.6949$</td>
</tr>
<tr>
<td></td>
<td><em>T. catappa</em></td>
<td>46.00 ± 7.48 a</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>