Journal of Applied Biosciences 49: 3425– 3434

ISSN 1997–5902

Susceptibility of four mango varieties to the Africa Invader fly, *Bactrocera invadens* Drew, Tsuruta and White (Diptera: Tephritidae) in Ghana

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Originally Submitted on 25th October 2011. Published online at www.m.elewa.org on January 29, 2012.

ABSTRACT

Objective: The susceptibility of four economically important mango varieties *(Kent, Keith, Palmer* and *Haden*) in Ghana to the attack by *Bactrocera invadens* was assessed through a series of laboratory-based choice and no-choice experiments, as well as some fruit quality parameters. The developmental periods of immature stages of flies in the varieties were also determined.

Methodology and results: Susceptibility was determined by counting and comparing the number of puparia recovered from the different varieties after exposure to the flies in cages. *Kent* was found to be the most susceptible, followed by *Palmer, Haden* and *Keith*. Flies took significantly longer periods to complete development on the least susceptible variety (*Keith*) (25.53 ± 2.3 days), than on the most susceptible variety (Kent) (19 ± 2.3 days). Significant differences ($P < 0.05$) were also observed in the peel thickness, firmness, Percent Titratable Acidity (% T.A.) and Total Soluble Solids (TSS) of the four varieties.

Conclusion and application: Keith was found to be the least susceptible variety to *B. invadens* followed by *Haden*, *Palmer* and *Kent* was the most susceptible variety. The differences observed in varietal susceptibility suggest that potentials exist for further genetic improvement to develop mango varieties that may be more tolerant to *B. invadens*, and could be incorporated into an integrated management strategy against the pest because of the added advantages of it being easy to use, economical and compatible with other methods of control.

Key words: Mango, *Bactrocera invadens*, fruit flies, fruit quality, susceptibility, developmental period.

INTRODUCTION

Fruit production is one the fastest growing agricultural sectors in Africa, providing both income and employment to growers and exporters (Lux et al., 2003a, Ekesi and Billah, 2006). The Natural Resource Institute (NRI) estimated that up to 45 million people in the African, Caribbean and Pacific (ACP) countries depend on horticultural goods for export to Europe alone (Braun, 2002). A diverse range of fruits including mango, citrus, apple, pineapple, guava, avocado and watermelon are among the most common fruits grown for domestic urban markets and for export to the Middle East and the European Union (Ekesi and Billah, 2006). Mango, a tropical fruit crop originates from South-

East Asia and constitutes about 50% of all tropical fruits produced worldwide (Stefan *et al*., 2003). World production of mango was estimated at 28.5 million tonnes (Mt) in 2005 (Evans, 2008) and by 30 million tonnes in 2006 (Mt) (Hanemann et al., 2008). It was estimated to reach 31 million tonnes by 2010, accounting for nearly 50% of world tropical fruit production (Sarris, 2003). Of the 28.5 million tonnes produced in 2005, Africa produced only 2.5 million tonnes, accounting for about 8.7% of fresh fruits and 11% of processed mango. Ghana's current production is said to have increased from 1,200 tonnes in 2007 to about 2,000 tonnes in 2008 (Quartey, 2008). It is targeted as the next non-traditional export crop expected to earn the highest foreign exchange for the country, and possibly replacing cocoa (Quartey, 2008).

The production of this crop is, however, threatened by the attack of many insect pests, including fruit flies, and especially *B. invadens,* which has been described as the most devastating fruit fly pest in Africa (French, 2005; Ekesi and Billah, 2006). Fruit flies attack both ripe and unripe fruits by laying eggs under the skin of the fruit. The eggs hatch into larvae which feed on the fruit tissue resulting in the rotting of the fruit and premature fruit drop (Afreh-Nuamah, 2007). Some common fruit fly genera are *Dacus* Fabricius*, Bactrocera* Macquart *Ceratitis* MacLeay *and Trirhithrum* Bezzi (Ekesi and Billah, 2006). *Bactrocera invadens* (originating from Asia) was first detected in Africa in Kenya, and has rapidly spread across the continent (Lux et al., 2003b). In Ghana, it was discovered in 2005 (Billah et al., 2006), where an assessment of the damage attributed to it on mango showed losses ranging from 60-85%, depending on variety and locale. Stricter maximum residue levels by the European Union (EU), the use of less persistent insecticides and the paradigm shift from chemical control to integrated pest management (IPM) has necessitated the move towards development of alternative control measures that will fit into an IPM programme. Modern crop protection practices create an unfavorable environment for damaging organisms through the use of pest resistant cultivars and farming practices which render the field environment less attractive to the pest (Kumar, 1984). Resistant varieties are less damaged or less infested by pests than other varieties (Kumar, 1984). Studies by Rwomushana et al. (2008) showed mango to be the most preferred host of *B. invadens*. However, the susceptibility of the different mango varieties grown in Ghana to *B. invadens* has not been studied, and this study was therefore undertaken to determine the susceptibility of four exotic and economically important mango varieties grown in Ghana to the Africa invader fly, *B*. *invadens*.

MATERIALS AND METHODS

Fruit collection and incubation: At least 50 mango fruits of different varieties (*Haden*, Hd; *Keith*, Kh; *Kent*, Kt and *Palmer*, Pm) were collected from three orchards (*Prudent Export* Farms at Dodowa, *Modest-Step* Farms at Somanya and the *University of Ghana* Farm in the Legon Botanical Gardens) for incubation at the laboratories of the West African sub-regional Centre of the African Regional Postgraduate Programme in Insect Science (ARPPIS) at the University of Ghana, Legon. Incubation was by way of placing the fruits on a thin layer of sand in cages to allow exiting larvae enter the sand to pupate. Sand for the pupariating medium was collected behind the ARPPIS building, washed to remove dust and debris, heat-sterilized at 100 °C for 12 hrs and sieved to remove larger particles. Processing and incubation of fruits followed the methodology described by Rwomushana et al. (2008) and Ekesi & Billah (2009). Fruits were placed on large plastic racks (45 x 29 x 9 cm) over a tray of moistened sand to collect larvae which exited fruits. The set-up (tray and rack) were placed in wooden cages and covered with wire gauze to prevent other insects from infesting the fruits. Puparia were collected every other day by removing the tray of sand under the rack for sieving. After 3 weeks of incubation, fruits were dissected to ensure all hidden larvae and/or puparia were recovered. Puparia were picked with forceps, counted, placed in Petri dishes lined with moist filter paper and held till fly emergence. Emerged flies were then transferred to holding cages, the top and bottom of which were covered with plastic containers and muslin cloth to ensure ventilation at the sides, and with a zip at

one side to allow access into the cages. Adult flies were provided with water-soaked cotton wool on Petri dishes and fed on a diet of pure baker's yeast and sugar (in a ratio of 1 to 3, vol/vol). Cages were regularly cleaned to maintain a hygienic environment.

Choice and No-Choice Experiments: These experiments were carried out in rectangular plastic cages of size 26 x 26 x 38 cm. Portions of the plastic containers were cut out and replaced with muslin cloth for ventilation and a sleeve for access into the cages (Plate 1). Fruits were washed and examined with a hand lens for visible signs of oviposition punctures and held in a refrigerator for 2 days. Experimental fruits were removed from the refrigerator and slowly allowed to warm up to room temperature by placing them in a ventilated plastic container in the laboratory for 2-3 hours. As an additional precaution, fruits were re-examined for visible oviposition punctures and a random sample from each consignment of fruits incubated to ensure there was no possibility of field infestation in the experimental fruits. Cages were washed, sun-dried and the test fruits placed in them for the experiments.

Combinations in choice experiments: Since the four varieties were available at slightly different periods, the experiments were carried out in a staggered fashion depending on the maturity stages of the varieties during the 12-week fruiting season. Individual fruits were washed, weighed and placed on 10 cm stages in the cages. Stages were improvised from 500 ml mineral water bottles that were cut to those heights and placed in the cages. Matured ripe fruits were used, with distances of 20 cm between them (in choice experiments). Table 1 shows the various combinations of choice and no-choice tests used. Forty (40) *B. invadens* adults (20 males and 20 females) of 7-14 days old were then introduced into the cages with the fruits. Flies were fed on 1:3 volume mixtures of hydrolyzed enzymatic yeast and sugar. Water was also provided on Petri dishes as soaked cotton wool. All treatments were replicated three times. Fruits and flies were left in the cages for two days. After the first day, positions of fruits were changed to ensure that flies did not develop learned behaviours of finding a particular variety in one area of the cage. After the 2-day exposure period, fruits were removed and individually incubated in separate plastic bowls, and the sand checked for puparia every three days. All experiments were carried out under laboratory temperature conditions of 27-31.6 °C and relative humidity values of 55-87%.

Plate 1: Set-up for choice and no-choice tests.

Table 1. Variety combinations of mango fruits for choice and no-choice tests.

Mango Variety Combinations			
No-Choice	**Two-Choice**	**Three-Choice**	**Four-Choice**
Haden (Hd) *Keith* (Kh) *Kent* (Kt) *Palmer* (Pm)	Hd-Kh Hd-Kt Hd-Pm Kh-Kt Kh-Pm Kt-Pm	Hd-Kh-Kt Hd-Kh-Pm Hd-Kt-Pm Kh-Kt-Pm	Hd-Kh-Kt-Pm

Assessment of Fruit Quality Parameters

Fruit Firmness and Peel Thickness: Firmness of the fruit peel of each of the varieties was determined using a Penetrometer (Fruit Pressure tester). This parameter was taken at the equatorial circumference of the ripe fruits. The procedure was repeated at five (5) different

spots on each fruit at the equatorial region for each variety. In determining thickness, the skin (peels) of five ripe fruits of each of the four varieties was removed and cleared of all pulp and measured with Vernier calipers. Five measurements were taken for each set of five fruits per variety and the mean value calculated. These were also replicated three times for each variety.

Percent Total Acidity (% T.A.) (Titratable Acidity): Titratable Acidity of the fruits was determined using procedures outlined by Ruck (1969) on the physical methods for analysis of fruit and vegetable products. Five fruits of each variety were used for the assay. The juice was squeezed from each set and bulked for any particular variety. Twenty five (25) ml of the juice was pipetted into a beaker and 250 ml of distilled water added and thoroughly stirred. Fifty (50) ml of the diluted juice was pipetted and titrated against 0.1M Sodium hydroxide (NaOH). Two drops of phenolphthalein was added to the solution as an indicator. The volume of the 0.1M NaOH required to neutralize the acid in the juice was recorded at the point when there was a colour change from yellow to pink. The total acidity of the juice was then calculated as below:

$$\%\text{Total acid} = \frac{1}{10} \times \frac{\text{equivalent weight of acid x normality of NAOH x titer}}{\text{weight of sample}}$$

Total Soluble Solids (TSS): The total soluble solids (brix) of the samples were determined using procedures outlined by Egan et al. (1985) on the chemical methods for the analysis and vegetable food products. Five fruits of each variety were used for the assay. The juices from each variety were squeezed and bulked together. Representative samples (about three drops) were taken and placed on an absolute dry refractometer prism (SHIBUYA Manufacturing Company, Japan) and read directly at 20 °C. These were replicated three times.

Data Analysis and Presentation: Puparia retrieved from incubated fruits were counted and held for fly emergence. Emerged adults were counted and sexed. The duration in days from egg to pupa, pupa to adult and total developmental period of immature stages in the different fruit varieties were observed and recorded. Proportion of emerged flies from total number of puparia was expressed as percentage adult emergence. Infestation by *B. invadens* in all the combinations to determine the susceptibility of the varieties followed the method of Vayssières et al. (2007) (the ratio of number of puparia per kilogram of fruit). The four-choice, three-choice and no-choice tests were analyzed by performing a one-way analysis of variance at 95% level of significance using GENSTAT Release Version 9.2. Least significant difference (LSD) was used to separate means. Data for the two-choice experiments were subjected to comparison using unpaired two sample t-test at 95% level of significance.

RESULTS

Four-Choice Test : Puparia recovery was significantly different ($P < 0.05$) among the four varieties. The highest number of puparia was recovered from *Kent*, followed by *Palmer, Haden*, and *Keith* (Figure 1). However, there was no significant difference ($P > 0.05$) in the number of puparia recovered from *Kent* and *Palmer*, as well as between *Keith* and *Haden*.

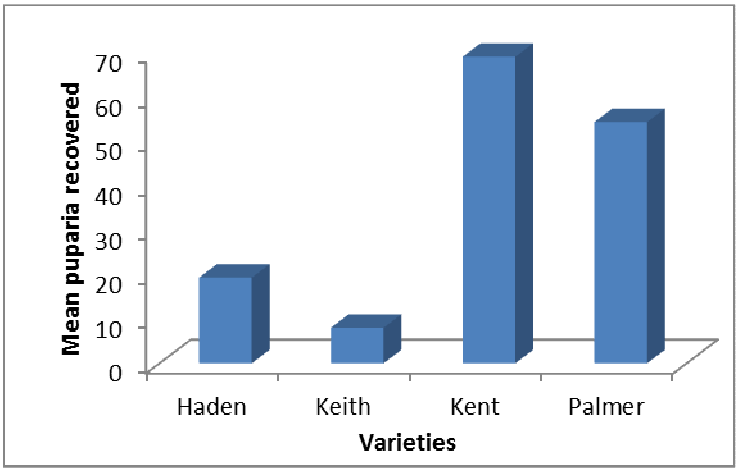

Figure 1: Mean puparia recovered from different mango varieties used in the four-choice experiment.

Three-Choice Tests: Four (4) possible combinations from the four (4) mango varieties were available under the three-choice tests (Hd-Kh-Kt, Hd-Kh-Pm, Hd-Kt-Pm and Kh-Kt-Pm). There were significant differences in the number of puparia recovered from the four varieties (Figure 2). In the Hd-Kt-Kh combination, there were significant differences ($P < 0.05$) in the mean puparia recovered, with the highest number from *Kent*, followed by *Haden* and *Keith* (Fig 2A). In the Hd-Kh-Pm combination, there was no significant difference ($P > 0.05$) in the pupal recovery. However, the highest number of puparia was recovered from *Palmer*, followed by *Haden* and *Keith* (Fig 2B). In the Hd-Kt-Pm combination, the highest puparia recovery was from *Kent*, followed by *Palmer* and *Haden* (Figure 2C). The Pm-Kt-Kh combination showed no significant differences ($P > 0.05$) in the number of puparia recovered, but the highest was from *Kent*, followed by *Palmer* and then *Keith* (Fig 2D).

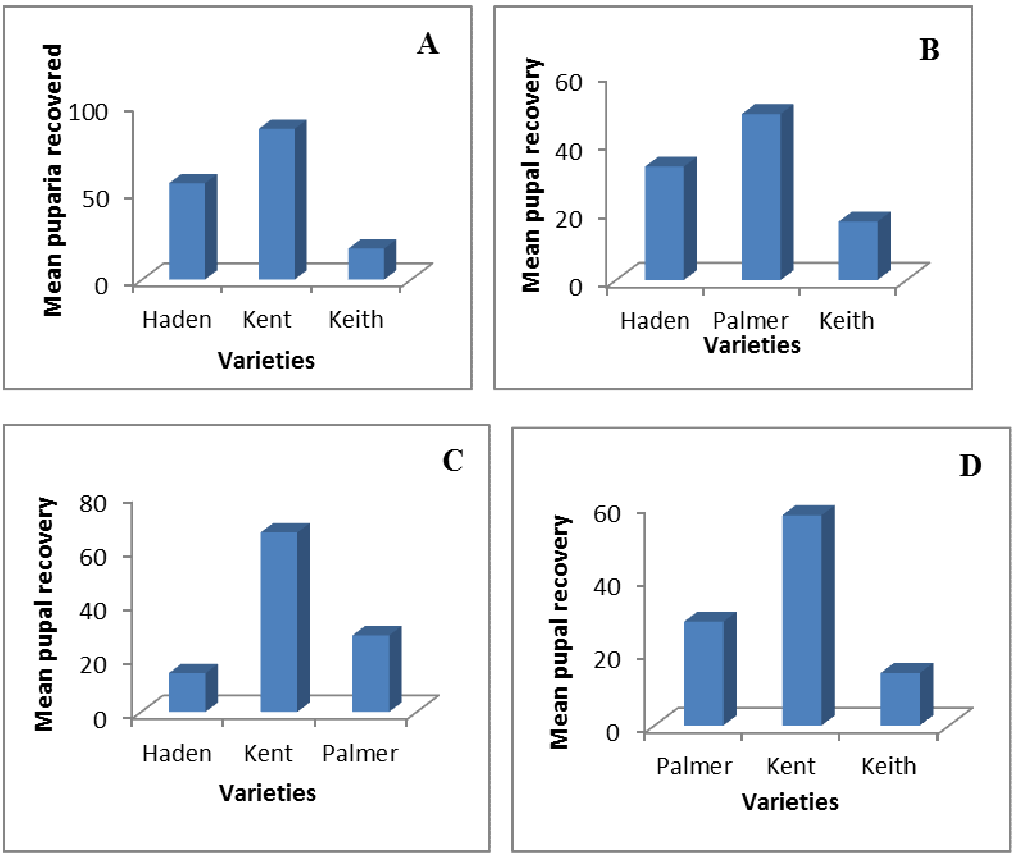

Figure 2: Mean puparia recovery from four mango varieties in the three-choice tests. **A** = Hd-Kh-Kt, **B** = Hd-Kh-Pm, **C** = Hd-Kt-Pm and **D** = Kh-Kt-Pm

Two-Choice Tests: Here six (6) different combinations were possible from the four (4) fruit varieties (Hd-Kh, Hd-Kt, Hd-Pm, Kh-Kt, Kh-Pm and Kt-Pm). The only combination with significant difference between varieties was from the *Keith-Palmer* test (Fig 3E). All other combinations showed no significant differences between the varieties (Figures 3 A, B, C, D and F).

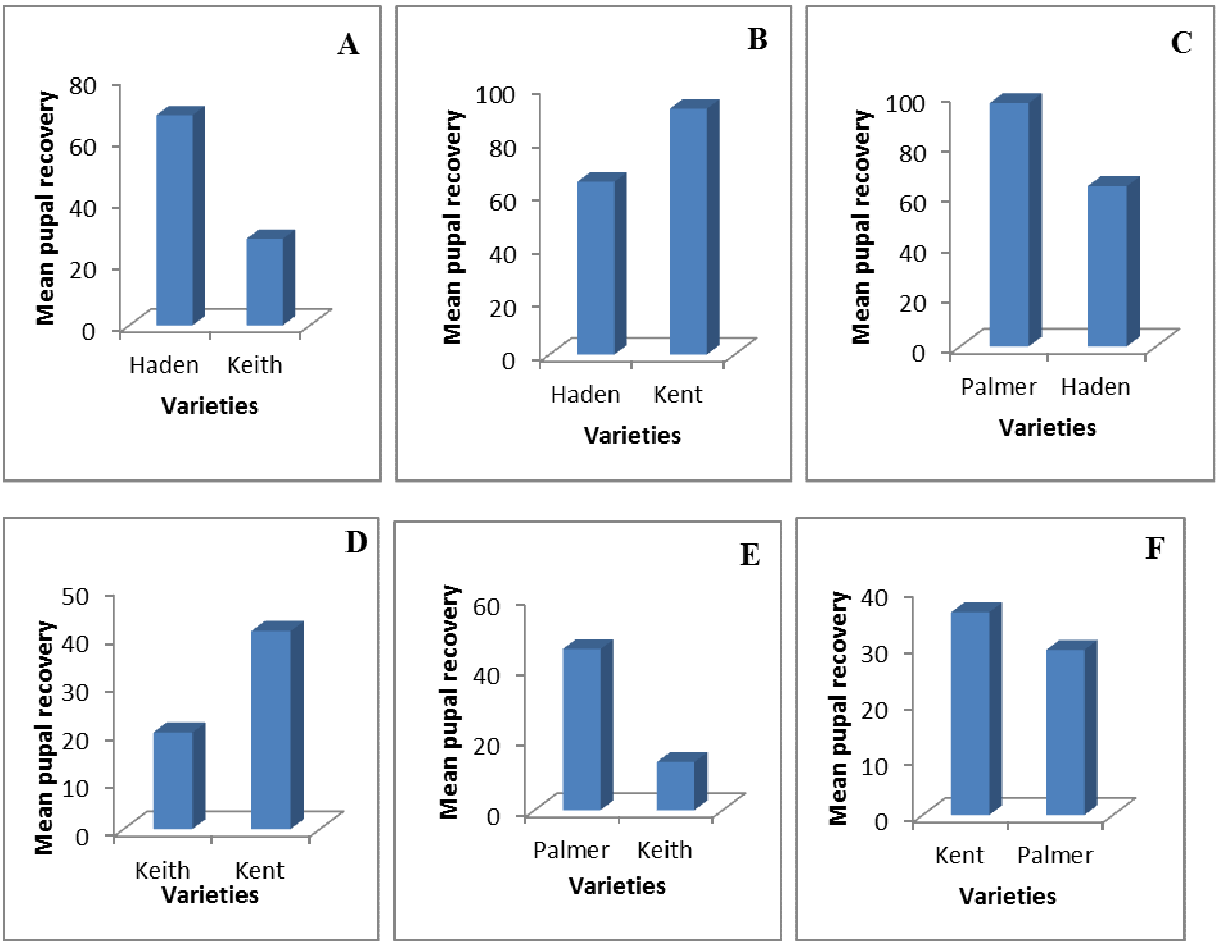

Fig 3: Mean puparia recovery from four mango varieties in the two-choice tests. **A** = Hd-Kh, **B** = Hd-Kt, **C** = Hd-Pm, **D** =Kh-Kt, **E** = Pm-Kh and **F** = Kt-Pm.

No-Choice Tests: These were conducted for each of the four (4) varieties under consideration. There were no statistical differences ($P > 0.05$) in mean puparia recovery. However, the highest number of puparia was recovered from *Kent*, followed by *Palmer*, *Haden* and *Keith* (Figure 4).

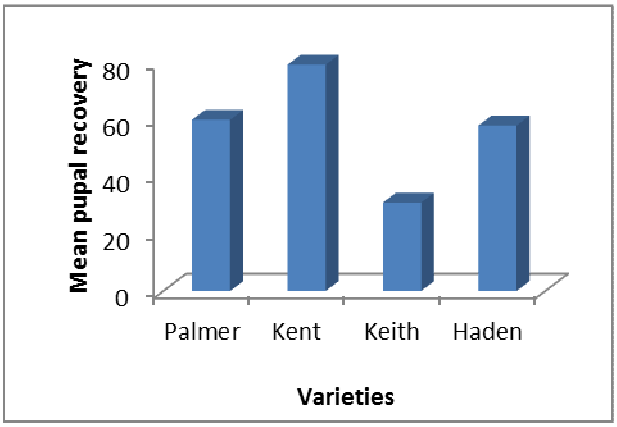

Fig 4: Mean puparia recovery from four mango varieties in the no-choice experiments.

Fruit infestation from the field: Table 2 shows the total number of puparia recovered from the different fruit varieties from the field and their subsequent infestation levels. The infestation indices were calculated and used for comparison because it gives a better measure of the susceptibility of fruits. *Kent* had the highest infestation index, followed by *Palmer*, *Haden* and *Keith*. In addition, all flies that emerged from the different varieties were *B. invadens.* No other fruit fly species was reared out from the different fruit varieties.

Table 2: Infestation indices of field-collected fruits of four mango varieties.

Mango Variety	No. fruits	Weight (kg)	No. Puparia	No. Flies	% Emergence	Infestation Index (Puparia/kg)
Haden	50	10.15	546	294	53.9	53.79
Keith	54	14.14	376	204	54.3	26.59
Kent	50	8.91	546	279	51.1	61.28
Palmer	69	14.74	854	532	62.3	57.94
LSD (P < 0.05)	**36.24**	**3.50**	**3.83**	**5.97**	**5.97**	**35.24**

Developmental Periods of Flies: There were significant differences in the duration of development from egg to puparium on the different mango varieties (P < 0.05) (Table 3). For *Kent*, mean egg to puparium developmental time was most rapid (in 11.71 days), followed by *Haden* and *Palmer* (both in 13.29 days) and *Keith* (in 17.01 days). There were no significant differences (P > 0.05) in the number of days taken for adults to emerge from puparia, and total time from eggs to adults in the different varieties (Table 3). Development, however, was most rapid on *Kent*, followed by *Haden, Palmer* and *Keith*.

Table 3: Mean developmental times of immature stages of flies reared from four mango varieties.

Mango Variety	*Mean Developmental Time (Days)		
	Egg to Puparium	Puparium to Adult	Total (Egg to Adult)
Haden	13.29	7.85	21.15
Keith	17.01	8.48	25.53
Kent	11.71	8.04	19.75
Palmer	13.29	8.25	21.54
LSD (P < 0.05)	**4.82**	**1.96**	**4.7**

*Means were calculated from the different fruit varieties in the different choice combinations.

Fruit Firmness and Peel Thickness: There were significant differences (P < 0.05) in the peel thickness and firmness among the varieties. *Keith* had the highest peel thickness and was the most firm, while *Kent* was the thinnest and least firm (Table 4).

Total Soluble Salts (TSS) and Total Acidity (% T.A.): There were significant differences (P < 0.05) in the % T.A. of the different fruit varieties. *Kent* had the highest mean % T.A. (0.53%), while *Palmer* had the lowest (0.21%). There was no significant difference in the TSS of the four varieties. However, *Palmer* had the highest value of 16.5, while Keith had the lowest value of 15 (Table 4).

Table 4: Some fruit quality parameters of *Haden, Keith, Kent* and *Palmer*.

Variety	TSS	% T.A.	Peel Thickness (cm)	Peel Firmness	Wt (kg)
Haden	16.2	0.27	0.15	3.1	0.42
Keith	15.0	0.30	0.17	4.9	0.70
Kent	15.8	0.53	0.13	2.3	0.57
Palmer	16.5	0.21	0.14	2.3	0.62
LSD (P < 0.05)	**1.07**	**0.07**	**0.02**	**0.66**	**0.04**

DISCUSSION

Peel thickness and firmness: The choice and no-choice experiments show *Kent* as the most susceptible variety, followed by *Palmer, Haden* and *Keith* as the least susceptible variety. Though there was no significant difference in peel thickness and firmness between the four varieties*, Keith* had the thickest skin and highest firmness value. It therefore comes as no surprise that *Keith* recorded the least number of puparia in the choice and no-choice experiments as well as in the fruit incubation from the field. Studies by Rossetto et al. (2006) and Rattanapur et al. (2009) showed that peel firmness and thickness greatly affected the oviposition preference of fruit flies, with female tephritids having oviposition preference for fruits with softer pericarp over those with hard pericarp (Balagawi *et al*., 2005). Rossetto et al. (2006) also showed that when eggs of *Ceratitis capitata* were artificially inserted directly into fruit pulp of a resistant variety, the resistance broke down – indicating that one of the main factors of resistance/susceptibility of mango to fruit flies was in the fruit peel. However, Christenson and Foote (1960) had shown earlier that the thickness of fruit skin was not a problem for the penetration of the ovipositor of fruit flies. *Kent, Palmer* and *Haden* had the smallest peel thickness and firmness, and if peel thickness and firmness were the only factors responsible for susceptibility of mango, the three (3) varieties (*Kent, Palmer* and *Haden*) would have recorded correspondingly higher values of puparia recovered and infestation indices to confirm the findings of Rossetto et al. (2006) that peel thickness is the main mechanism of resistance among mango varieties to fruit flies. However, this was not the case – indicating that there might be other factors that contribute to host acceptability and susceptibility.

Percent Total Acids (% T.A.) and Total Soluble Solids (TSS): TSS measures the sugar content of fruits, while % T.A. measures the total acid content. High acidity and low free sugar content in some mango varieties have been shown to negatively affect larval survival to pupation (Hennessey and Schnell, 2001). Ibrahim and Rahman (1982) found that when a food resource was too acidic, many larvae of *Bactrocera dorsalis* failed to pupate, and that even if they successfully pupated, the puparia were lighter and smaller in size. *Kent,* with the highest % T.A. level, was therefore expected to record the least number of puparia, but the reverse was the case. *Keith* recorded the least number of puparia but had the least mean TSS and lower % T.A. values than *Kent*. The high number of puparia from *Kent* could be as a result of a higher preference of *B. invadens* for that variety.

In the putative aboriginal home of *B. invadens* in Sri Lanka, there are a number of fruit flies belonging to the *Bactrocera dorsalis* complex that have mango as one of the most preferred fruits, thus leading to high levels of competition between those members of the complex. In most of the cases, *B. dorsalis sensu strictum* does appear as the most dominant species (Billah et al. – unpublished data). It may therefore stand to reason that the use of high acidic fruits (which do not favour optimum puparia formation by *B. dorsalis*), could be a character displacement or niche partitioning strategy by *B. invadens* to minimize competition between it and *B. dorsalis s.s*.

Developmental times: Consistent with the results of pupal recovery, *Kent* was the best variety for offspring survival, with a higher percentage of larval survival to pupation and a shorter developmental time as compared to *Palmer*, *Haden* and *Keith*. This agrees with the work of Rattanapur et al. (2009), who reported that tephritid flies complete their development faster in suitable hosts than in unsuitable hosts. The study shows that fruits vary in the resources they offer immature stages of insects with respect to quality of available nutrients, which particularly influence developmental time, adult eclosion rate and reproductive maturation time of adult flies (Kaspi et al., 2002). This implies that *Kent* may have the requisite resources which are required for the faster development of the flies. Work in Ghana by Ogaugwu (2007) on the biology of *B. invadens* showed that egg to puparium duration varied from 11 and 23 days, which is consistent with the mean values obtained for *Kent, Palmer* and *Haden*. *Keith* on the contrary, had a mean developmental time of 25 days. The developmental times in this study did not show any significant differences ($P > 0.05$) in the varieties. Adults emerged from puparia at a mean eclosion age of 8 days, confirming work by Ogaugwu (2007), who reported a puparium to adult duration of 7-9 days. This suggests that irrespective of how long a puparium takes to form in different varieties, the duration from puparium to adult is relatively stable or takes about the same time. Coincidentally, all adult flies reared from field collected fruits yielded only *B. invadens* species, which is a clear indication of the dominance of the Africa Invader fly and its gradual trend in displacing the indigenous species. This is in line with the observation of Ekesi et al. (2009), who documented how *Ceratitis cosyra* was displaced

by gradually displaced by *B. invadens* on mango and the mechanisms contributing to the displacement in an 8-year study in Kenya. *Ceratitis cosyra* has long been recognized as the most damaging tephritid fruit fly pest of mango in Africa including Kenya (Lux et al., 2003b).

Based on the results of the number of puparia recovered and infestation indices, it could be concluded that *Keith* was the least susceptible variety to *B. invadens* followed by *Haden*, *Palmer* and *Kent. Kent* was therefore the most susceptible variety. It is therefore suggested that for qualities that confer some level resistance to this devastating pest should be considered for incorporation in future breeding programmes and integrated pest management strategies. This is because the use of varietal resistance for the management of *B. invadens* would have a number of advantages as the method is easy to use, economical and moreover, it is compatible with other methods of control (Kumar, 1984).

ACKNOWLEDGEMENTS

Funding for the completion of this study was provided by the Deutcher Akademischer Austauschdienst (DAAD), and it is very much appreciated. This work formed part of a bigger project on ***Development of sustainable management strategies against the fruit flies for environmental and fruit safety,*** with initial funding from the University of Ghana Research and Conferences Committee. Results are part of Ambele F.C.'s M. Phil. thesis at the African Regional Postgraduate Programme in Insect Science (ARPPIS), University of Ghana, Legon.

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