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Diversity and Distribution of Fruit Flies (Diptera: Tephritidae) Across Agroecological Zones in Swaziland: On the Lookout for the Invasive Fruit Fly *Bactrocera invadens*

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Fruit flies are among the most economically important pests in crop production and the highly invasive *Bactrocera invadens* has rapidly spread across sub-Saharan Africa in recent years. In 2008, Swaziland initiated a national fruit fly surveillance programme to facilitate continued trade with export partners and to develop an early detection and monitoring system for invasive fruit fly species. Fruit fly traps were set up at major border posts, markets and agricultural estates where target host plants of *B. invadens* are grown. Traps baited with Methyl eugenol, Trimedlure and Cuelure were placed in agricultural estates while only traps with Methyl eugenol were placed at markets and border posts. Fourteen species, dominated by *Ceratitis capitata* (40.2%), *C. rosa* (22.7%) and *Dacus bivittatus* (32.7%) were collected from agricultural estates. There were significant differences in the number of flies trapped per day per trap between lures ($p>0.05$) and estates ($p=0.0204$). Only 9 species, dominated by *C. rosa*, (76.35%) were collected from border posts and markets. There were significant differences in the number of flies trapped between border gates ($p>0.05$) and none were trapped from the national marketing board. No species of phytosanitary concern were trapped during the survey. However, the country remains vulnerable to invasion by *B. invadens* due to its reported presence or increased sightings in neighbouring countries. This emphasises the need for continued surveillance to ensure early detection of invasive species, which would enhance the country's ability to influence the chances of invasive species establishment and spread.

Key words: Fruit flies, invasive species, *Bactrocera invadens*, early detection, monitoring

Introduction

Fruit flies (Diptera: Tephritidae) constitute a major crop production constraint in the horticultural industry and are among the most economically important pests of fruits worldwide (Billah *et al*., 2008). About 70 species of fruit flies are considered important agricultural pests due to the heavy losses they inflict on fruits and vegetables as a result of their feeding (Mwatawala *et al*., 2009). Due to the phytophagous habits of their larvae, both native and introduced tephritid fruit fly species inflict heavy economic losses on fruit and vegetable crops within the African continent (Ekesi *et al*., 2006; Vayssiéres *et al*., 2008). Economic effects of fruit fly pest species include direct loss of yield and increased control costs, loss of export markets and/or the high cost of constructing and maintaining fruit treatment and eradication facilities. Most fruit fly species are also potential invaders of other tropical and subtropical regions and are therefore considered as pests of quarantine importance. Consequently, in many countries, the exportation of most commercial fruits is severely restricted by quarantine laws to prevent the spread of fruit fly species (Copeland *et al*., 2006; Mwatawala *et al*., 2009). Despite having a number of fruit fly species indigenous to the

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continent, African countries are also vulnerable to the introduction of alien invasive fruit fly species and a number belonging to the genus *Bactrocera* have been recently introduced (Mwatawala *et al*., 2004).

There are only 11 native *Bactrocera* species in Africa though the genus forms a very large group in Asia, Australia and the Pacific with about 500 described species including numerous pests (Mwatawala *et al*., 2004; White, 2006). Four of these Asian species have invaded Africa as a result of humans moving fruit and vegetables between continents for trade or personal use. Two of these, *B. invadens* and *B. cucurbitae*, were introduced in recent years and have established in a number of African countries, e.g. *B. invadens* was detected in Kenya in early 2003 and was detected in 29 sub-Saharan countries within the next two years (Drew *et al*., 2005; Mwatawala *et al*., 2006). *B. invadens*' rapid spread has been attributed to its high competitive advantage over indigenous species. Contributing characteristics include that it is polyphagous, has a high reproductive capacity and mobility, and freed of natural enemy control. In Kenya, it was observed to have displaced *Ceratitis cosyra* within four years of detection and monitoring (Ekesi *et al*., 2009; Rwomushana *et al*., 2009). Swaziland is presently one of the few remaining sub-Saharan countries free of this aggressive pest but the risk of introduction remains high (Mwatawala *et al*., 2004; Barr *et al*., 2006; De Meyer *et al*., 2010).

While fruit flies cause serious crop losses and reduction in essential nutrients for consumers, major losses due to these pests are those related to the loss of export markets as happened in 2008, when South Africa banned all agricultural products in the host range of *B. invadens* from Swaziland and Mozambique. This had significant impact on regional trade since the South Africa is one of Swaziland's largest export markets. There was therefore an urgent need for a surveillance programme in order to ascertain the country's status with regard to this species. The project's primary objective was to develop a trapping and monitoring programme across the country's major agricultural systems in all regions for the detection of *B. invadens*. Additionally, this was to generate baseline data on which economically important fruit fly species are present in the country including their distribution across major agricultural production areas. This was intended to facilitate the formulation of an early detection and monitoring system for invasive species, primarily *B. invadens*, in order to safeguard the country's horticulture industry, trade and food security as well as to facilitate the formulation of a national action plan against invasive fruit fly species.

Materials and Methods

Swaziland (c.a. 17,200 km^2) is a landlocked country bordered by the Republic of South Africa and Mozambique. The country is divided into six agroecological zones based on altitude, rainfall and geography, i.e. high, upper and lower middleveld, western and eastern lowveld and Lubombo ridge (Sweet and Khumalo, 1994) and sampling sites were selected across all regions in the country (Fig. 1).

Sampling sites

Sampling sites were selected based on their susceptibility with regard to the high risk of introduction of fruit fly pests as a result of importation of host materials, e.g. ports of entry and market places as well as target crop production, i.e. agricultural estates. Chempac bucket traps were baited with one of three lures, i.e. Cuelure (CUE) Trimedlure (TM) or Methyl eugenol (ME) (Insect Science) plus an insecticide (Dichlorvos) block were placed at agricultural estates while only ME lures were used at ports of entry and markets (Table 1). One polymeric gel plug of each lure was individually placed in each bucket trap.

i. Main market places: Single Chempac bucket traps, each with an ME gel plug, were placed at four major market places in the country, i.e. Manzini, Mbabane, Mahlanya and the national agricultural marketing board in Encabeni.
ii. Border posts: Single Chempac bucket traps, each with an ME gel plug, were placed at six major overland ports of entry bordering the Republic of South Africa (Ngwenya, Matsamo, Mananga, Mahamba) and Mozambique (Lomahasha and Mhlumeni). Small-scale fruit and vegetable vendors were present at all borders.
iii. Agricultural estates: Several commercial agricultural estates growing *B. invadens* host fruits and vegetables were also targeted across the country. Sampling estates were at Tambuti, Tshaneni, Mpopotha, Mbabala, Ngonini, Nsoko and Sidvokodvo with their respective target plants as indicated in Table 1. Bucket traps were placed in trees within orchards, pack houses and fruit stores of the estates between 1.5 and 2 m above ground. The estates had varying numbers

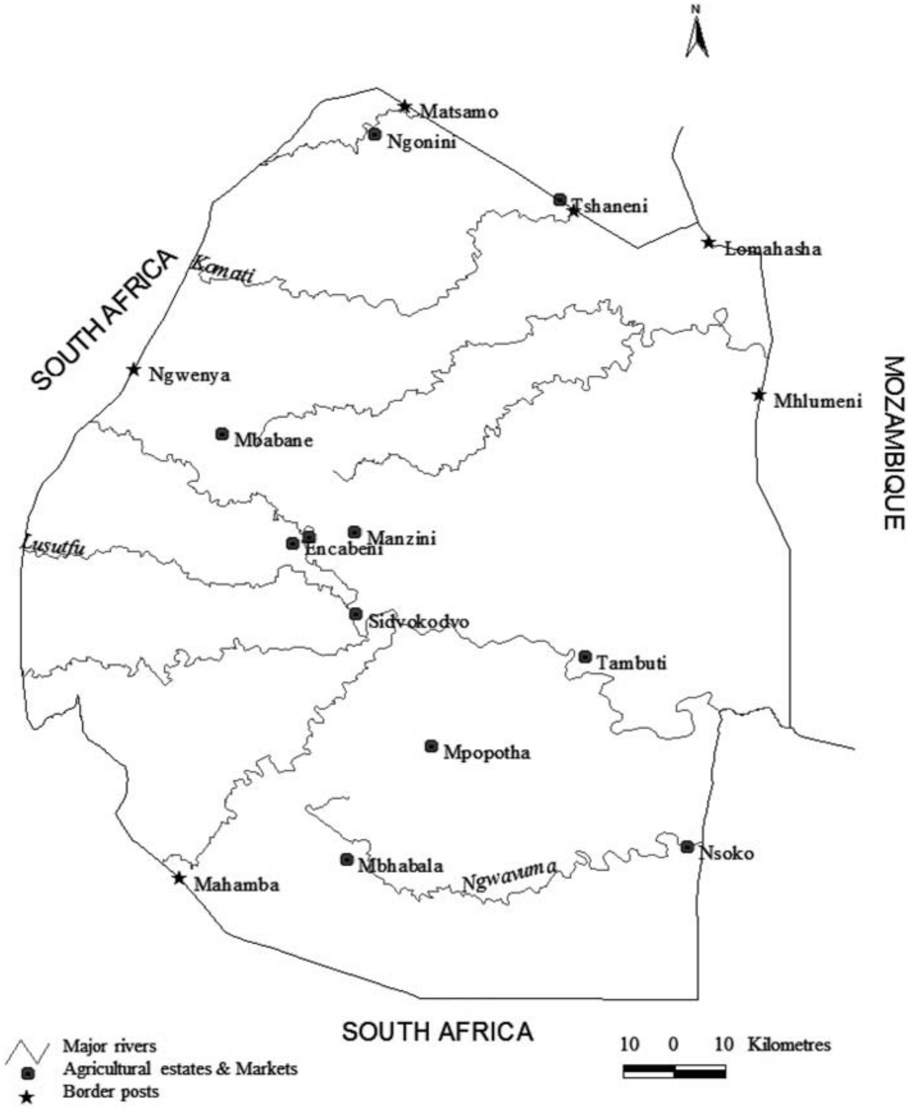

Fig. 1. Sampling sites across the country

of traps so for purposes of this assessment, only one group of traps with all three lures and consistently undisturbed throughout the 18 months were used in the analyses. Additionally, since the national monitoring programme is primarily intended to determine the presence or absence of *B. invadens*, most of the estates had higher numbers of traps with ME lures.

Traps were serviced fortnightly and rebaited monthly. Results presented are for an 18 month period from January 2010 to June 2011. Specimens collected were put in vials with alcohol and preliminary identification carried out locally then verified by external project partners in South Africa.

The results of each trap site were reported as the number of flies trapped per day (FTD) (IAEA 2003; Ekesi and Billah, 2007), calculated as:

$$\text{FTD} = \frac{\text{Total number of flies trapped}}{\text{(Total number of serviced traps * no. of days traps exposed in the field)}}.$$

Statistical tests were used to determine differences within traps in estates (*t* test), between estates, months of sampling, type of host plants grown and lures (ANOVA after log (x+1) transformation).

Results

Total number of flies in agricultural production areas

A total of 12,554 fruit flies representing 14 species

Table 1. Sampling sites, AEZ of each site, major host plants and lures used

Sampling site	Agroecological zone	Type	Host plants
Mahlanya	Upper middleveld	Market	Fruits, vegetables
Manzini	Upper middleveld	Market	Fruits, vegetables
Mbabane	Highveld	Market	Fruits, vegetables
Encabeni	Upper middleveld	Market	Fruits, vegetables
Ngwenya	Highveld	Border post	Fruits, vegetables
Matsamo	Highveld	Border post	Fruits, vegetables
Mananga	Lowveld	Border post	Fruits, vegetables
Mahamba	Highveld	Border post	Fruits, vegetables
Mhlumeni	Lubombo	Border post	Fruits, vegetables
Lomahasha	Lubombo	Border post	Fruits, vegetables
Ngonini	Upper middleveld	Agricultural estate	Citrus, banana
Tshaneni	Western lowveld	Agricultural estate	Citrus
Sidvokodvo	Lower middleveld	Agricultural estate	Baby vegetables
Mpopotha	Highveld	Agricultural estate	Banana
Mbhabala	Highveld	Agricultural estate	Avocado
Tambuti	Eastern lowveld	Agricultural estate	Citrus
Nsoko	Eastern lowveld	Agricultural estate	Citrus

were collected during the sampling period. Of these, no *B. invadens* flies were trapped from all of the estates. *C. capitata*, *C. rosa* and *D. bivittatus* were the most dominant species, making up 95.6% of the total catch (Table 2). *C. capitata* was the most dominant species at Tshaneni (97.5%) and no *D. bivittatus* flies were collected from the same estate. *D. eclipsis* was the only Dacine species collected from the Tshaneni estate and was 44% of the species collected from this estate. *D. bivittatus* was the most dominant species collected from Sidvokodvo representing 96.3% of the flies from this estate. Only 0.3% of *Ceratitis* species were collected from this estate. The highest population levels of *P. curta* were collected from Tambuti (97.8%) during the sampling period. Of all the estates sampled, only Tambuti was dominated by the non-economically important species, i.e. *P. curta*, which made up 34.1% of the total catch from this estate, while the 3 most dominant species made up only 32.2 % combined.

For comparison between of overall catches between lures, the total number of flies in ME traps was not significantly different from those in CUE traps in all estates ($p>0.05$). In 4 of the 7 estates, the total number of flies in ME traps were significantly lower than total number of flies in TM traps, i.e. Tshaneni ($p=0.0147$, $t=-2.71$), Mbabala ($p=0.0019$, $t=-3.67$), Nsoko ($p=0.0064$, $t=-3.11$) and Ngonini ($p=0.0191$, $t=-2.59$). In Tshaneni ($p=0.0146$, $t=2.72$) and Mbabala ($p=0.0019$, $t=3.67$), the total number of flies in TM traps were significantly higher than CUE traps, while the opposite was observed at Sidvokodvo ($p=0.0092$, $t=-2.94$) (Fig. 2).

Traps with TM lures were significantly different from those with ME with regard to the flies trapped per day and overall (Table 2). TM baited traps had high numbers of *C. capitata* and *C. rosa* per trap per day while CUE traps had the highest *D. bivittatus* catches. This would be expected based on the target species for these lures. ME traps had the lowest overall catches compared to the other two lures, primarily due to the absence of its primary target species (Manrakhan, 2007).

Trends in agricultural estates

There were significant differences in number of flies per trap per day between the estates ($p=0.0204$; df 6,371). Separation of means indicated that Sidvokodvo was significantly different from Tambuti (Tukey HSD, $p>0.05$) and this was due to the dominant species in either estate, where *P. curta* dominated in Tambuti and was absent at Sidvokodvo while *D. bivittatus* dominated at Sidvokodvo and very few collected from Tambuti. There were also significant differences with regard to the host plants grown in the estates ($p=$

Table 2. Fruit fly species trapped from agricultural estates per lure (arranged in order of dominance within each genus)

Locality	Ngonini			Tshaneni			Sidvokodvo			Mpopotha			Mbabala			Tambuti			Nsoko			Total			Total	% of
Lure *	M	C	T	M	C	T	M	C	T	M	C	T	M	C	T	M	C	T	M	C	T	M	C	T		total
Ceratitis capitata	7	0	230	2	0	3314	5	0	1	325	73	2	6	0	29	1	0	15	2	704	329	348	777	3920	5045	40.19
Dacus bivittatus	1	279	6	0	0	0	42	2336	194	16	11	421	4	111	208	19	52	0	14	391	1	96	3180	830	4106	32.71
C. rosa	12	4	74	3	0	63	1	1	0	244	256	5	419	11	1640	64	0	0	13	19	23	756	291	1805	2852	22.72
D. venetatus	1	28	5	0	0	0	0	5	0	3	2	38	4	43	1	2	0	0	0	0	0	10	78	44	132	1.05
D. ciliatus	0	0	0	0	0	0	63	1	6	0	0	0	0	48	0	0	4	0	0	0	0	63	53	6	122	0.97
D. punctatifrons	0	4	1	0	0	2	0	3	0	0	0	23	0	1	0	0	10	0	0	60	1	0	78	27	105	0.84
Perilampsis curta	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	89	0	0	0	0	0	91	0	0	91	0.72
D. eclipsis	2	1	0	0	0	11	0	2	0	0	1	14	1	0	0	0	2	0	0	1	0	3	7	25	35	0.28
D. famona	0	3	0	0	0	0	0	0	6	0	0	8	0	1	0	2	1	0	0	0	0	2	5	14	21	0.17
D. vertebratus	0	0	0	0	0	0	1	3	0	0	0	10	0	0	0	0	0	0	1	2	0	2	5	10	17	0.14
C. cosyra	0	1	1	0	0	0	0	1	0	8	0	1	2	1	1	0	0	0	0	0	0	10	3	3	16	0.13
Ceratitis sp.	1	0	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	4	6	0.05
C. bremii	2	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	4	1	0	5	0.04
D. siliqualactis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0.01
B. invadens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total N	26	321	317	8	0	3394	112	2352	207	598	344	522	436	216	1879	177	69	15	30	1177	354	1387	4479	6688	12554	100
Species diversity	8	9	7	5	0	6	6	9	5	7	7	10	7	8	6	7	6	2	5	7	5	13	13	12	15	

*M=Methyleugenol; C=Cuelure and T=Trimed lure

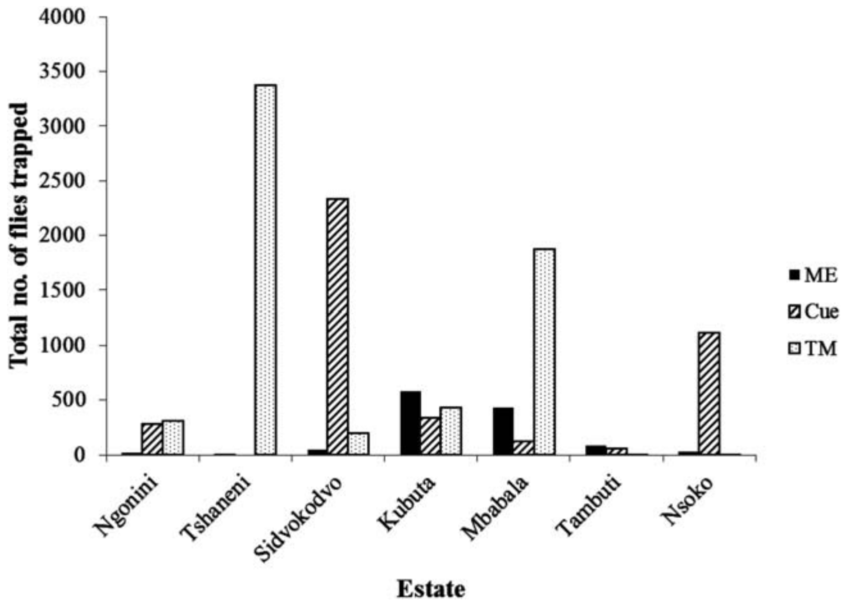

Fig. 2. Total number of fruit flies collected from agricultural estates per lure over the sampling period. ME=Methyleugenol, CUE=Cue lure, TM=Trimedlure

Table 3. Fruit fly infestations based on number of flies trapped per day in agricultural estates

Locality	Mean no. of flies/trap/day (range)	Prevalence
Mpopotha	1.88±0.48 (0–21.47)	Infested
Mbabala	3.26±0.78 (0–22.08)	Infested
Ngonini	0.83±0.26 (0–11.60)	Low prevalence
Nsoko	1.97±0.84 (0–44.73)	Infested
Sidvokodvo	3.60±1.30 (0–49.62)	Infested
Tambuti	0.14±0.06 (0– 3.21)	Low prevalence
Tshaneni	2.18±0.90 (0–39.82)	Infested

0.0037, df 3,374), with the baby vegetable estate (Sidvokodvo) being significantly different from the citrus estates. While Sidvokodvo had no species unique to it, it had the highest numbers of *D. bivittatus* (62.6 %) and *D. ciliatus* (57.4%) trapped in each trap per day and overall (Table 2).

Over the months, CUE traps showed the highest FTD in September 2010 and January 2011, which coincide with high numbers of the *Dacus* species, while TM traps had their highset FTD in February 2011, when the highest number of *Ceratitis* species were collected. Overall, fruit fly populations peaked in September 2010 and February 2011. Both peaks were due to the high number of *Ceratitis* species trapped. During February, when the highest fly populations were trapped, there are a number of feral hosts fruiting, e.g. marula, mangoes which may also have contributed to the attraction of these species. Based on the results of this study, fruit fly prevalence in the country's agricultural estates ranged from low to infested (Table 3) during the reported period.

Border gates and Markets

Only 241 fruit flies, representing 9 species were trapped from the border posts and markets, where only the ME traps were used (Table 4). These catches were dominated by 76.4% *C. rosa* (Fig. 3) with 69.5% of these from the Mbabane market. The next dominant species was *C. bremii* trapped only from the Matsamo border gate. Encabeni was significantly different from the Manzini market ($p=0.0010$, $t=-3.96$) and was

Table 4. Fruit flies trapped from border gates and markets

Location	Mahlanya	Manzini	Mbabane	Namboard	Ngwenya	Matsamo	Mananga	Lomahasha	Mhlumeni	Mahamba	Total No.	% of Total
Ceratitis rosa	9	21	128	0	11	7	1	3	1	3	184	76.35
C. bremii	0	0	0	0	0	29	0	0	0	0	29	12.03
C. capitata	3	0	1	0	1	0	0	0	0	1	6	2.49
C. cosyra	0	1	0	0	0	0	1	2	0	0	4	1.66
Dacus bivittatus	0	0	0	0	0	0	2	5	0	0	7	2.90
D. ciliatus	0	0	2	0	2	0	0	0	0	0	4	1.66
D. vertebratus	0	0	0	0	2	0	0	0	0	0	2	0.83
D. punctatifrons	0	0	0	0	0	0	0	0	0	0	0	0.00
Perilampsis curta	0	0	0	0	0	1	1	2	0	0	4	1.66
Unid Tephritidae	0	0	0	0	0	0	0	1	0	0	1	0.41
Bactrocera invadens	0	0	0	0	0	0	0	0	0	0	0	0.00
Total number	12	22	131	0	16	37	5	13	1	4	241	100.00
Species diversity	2	2	3	0	4	3	4	5	1	2	9	

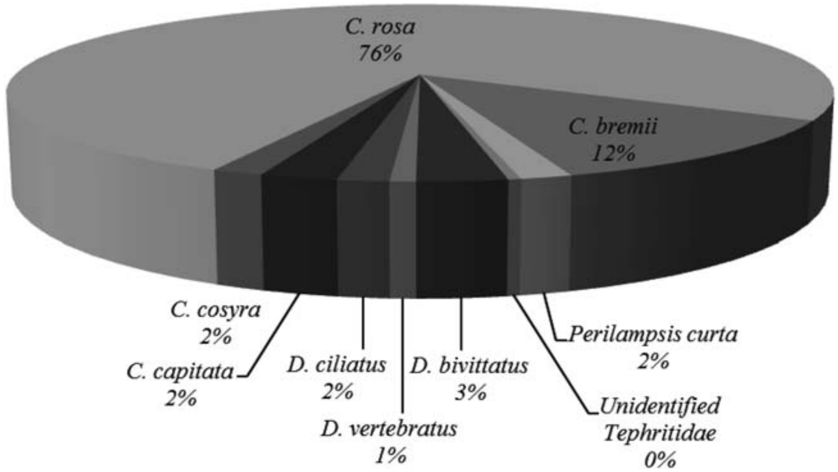

Fig. 3. Fruit fly species collected from markets and border gates during the sampling period

totally free of fruit flies during the sampling period. For the border gates, Matsamo had a significantly higher number of flies trapped compared to Ngwenya, Mahamba, Mananga, Mhlumeni and Lomahasha, while Ngwenya had a significantly higher number than Mananga, Mhlumeni, and Mahamba ($p > 0.05$), and Lomahasha had significantly more than Mahamba ($p > 0.05$). The average number of flies trapped per day was 0.09, indicating a low prevalence of fruit flies at the country borders and markets.

Discussion

No invasive fruit fly species of phytosanitary concern were trapped during the sampling period. All species collected during the sampling period are indigenous to the region and are primarily of economic importance due to the damage they cause on agricultural produce. The three most dominant species are of Afro-tropical origin, although some like *C. capitata* may occur as pests in other tropical and subtropical areas of the world (De Meyer *et al*., 2002). Stringent measures for their control are thus carried out in all agricultural estates due to high restrictions imposed by international markets (NPPO, pers. comm).

The results observed suggest that differences in species diversity between estates may be attributed to the dominant host plants grown within the respective estates. Sidvokodvo, which was significantly different from citrus estates, is primarily a cucurbit grower and the dominant species in this estate, i.e. *D. bivittatus* and *D. ciliatus* are known to utilise cucurbits as their primary host plants (Annecke and Moran, 1982; White, 2006). *C. capitata* dominated in citrus estates, e.g. Tshaneni and Nsoko while *C. rosa* was the dominant species in the avocado estate (Mbabala). Similar species occurrence was observed in avocado orchards in South Africa where *C. rosa* was observed to be a species of concern in avocado orchards compared to *C. capitata*, which primarily uses citrus as its host (Grové *et al*., 1998).

Of the 3 types of lures used, those with ME traps had the least number of fruit flies and this was in line with its target species. This lure primarily targets males of *Bactrocera* species, including *B. invadens* (Manrakhan, 2007) and none of these species occur in Swaziland. However, the trapping of non-target species such as *C. capitata* and *P. curta* in ME traps indicates that incidental catches do occur. This was also corroborated by the species caught in ME traps at borders and markets in the survey. Results by Thomas (2003) and Wih and Billah (2012) also showed similar outcomes. The two other lures used in the survey were dominated by their target species, i.e. *Ceratitis* species for TM and *D. bivittatus* for CUE (Manrakhan, 2007).

Despite there being no active control measures in markets, these had very few fruit flies trapped despite the large amount of fruit and vegetable produce traded in these areas. This may be indicative of the efficacy of phytosanitary control measures from the source, i.e. agricultural production estates. As Swaziland is signatory to the International Plant Protection Convention (IPPC, 2012), these measures have to be in line with international standards to facilitate trade. The national marketing board was virtually pest free throughout the study period. This has important implications with regard to the movement of fruit fly pest species through trade and movement of people within and between countries (Maynard and Nowell, 2009) because this is the primary area for movement of host plant produce. The country's partnership between the private sector and National Plant Protection Organisation (NPPO) for coordinated response in establishment of the national fruit fly surveillance programme will contribute towards appropriate planning of a contingency action plan in the event of alien species introduction. This is all the more urgent with the increased frequency of reports of *B. invadens* trappings in South Africa, where 10 reports of *B. invadens* were made between May 2010 and September 2012 (IPPC, 2012). Early detection of any invasive species is important since this will influence the establishment and spread of the pest (Holcombe and Stohlgren, 2009).

In addition to its high reproductive capacity and mobility, *B. invadens* is known to be polyphagous, reported to have more than 30 host plants (Ekesi *et al*., 2009). This characteristic contributes to one of the country's primary areas of concerns since a number of *B. invadens* host plants are widely distributed in the country and feral, e.g. guava, marula, mangoes (Loffler and Loffler, 2005). Its polyphagy suggests that this pest may be capable of sustaining its populations through reproduction on the wide range of commercial and feral host plants throughout the year (Rwomushana *et al*., 2008). While the organised agricultural production areas have effective control strategies within and near their estates, there are no control programmes in place for the feral hosts. This emphasizes the need for a coordinated plan which targets likely pathways of introduction of the pest so as to minimise introduction into the country.

Currently, Swaziland is thus an endangered area with regard to *B. invadens* and needs to maintain its *B. invadens* free status to avoid economic losses and associated trade repercussions (Schrader and Unger, 2003). Current phytosanitary measures carried out locally and neighbouring countries have so far been effective in preventing its introduction into the country (IPPC, 2012). Based on the survey, the country has not been invaded by *B. invadens*, with only the indigenous species being of economic concern.

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