Original article

Monitoring and managing *Ceratitis* spp. complex of sweet orange varieties using locally made protein bait of brewery waste

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Abstract — Introduction. Fruit flies contribute significantly to yield losses experienced by citrus farmers in Nigeria. The majority of farmers have meager resources and limited knowledge of appropriate citrus pest management strategies. The best alternative for them is the application of control methods that are environmentally friendly and affordable. Our work was therefore designed to develop baits from a cheap source for monitoring fruit fly populations and controlling them while minimizing environmental hazards. **Materials and methods**. Protein bait prepared locally from brewery yeast waste by the process of heat autolysis was compared with the imported protein hydrolysate bait in McPhail traps. These traps were hung on the trees of four sweet orange varieties during the major citrus fruiting seasons of 2003 and 2004. **Results**. Species of *Ceratitis* dominated in the fruit fly samples collected. Both the imported and locally made baits caught significantly higher numbers of fruit flies than the non-baited control. There was no significant difference between the populations of fruit flies caught by the two baits during the studies. The decreasing order of varieties according to trapped fruit fly numbers for both the imported and locally made baits were: Washington navel > Parson Brown > Valencia late > Agege-1. The fruit fly numbers observed on Washington navel were significantly higher than those of Agege-1 and Valencia late. **Discussion**. Our results show the effectiveness of the locally made protein bait in fruit fly monitoring and management. Varietal differences also played a part in determining the extent of fruit fly attacks on sweet oranges.

Nigeria / *Citrus sinensis* / *Ceratitis* / agricultural warning services / traps / attractants / breweries / waste utilization / hydrolyzed proteins

Surveillance et gestion du complexe de *Ceratitis* spp. sur variétés d'orange douce en utilisant un appât local fait de protéines issues de résidus de brasserie.

Résumé — Introduction. Les mouches des fruits sont significativement responsables des pertes de rendement subies par les planteurs d'agrumes au Nigéria. La majorité de ces agriculteurs ont de faibles ressources et une connaissance limitée des stratégies appropriées à la gestion des parasites des agrumes. La meilleure solution pour eux serait l'application de méthodes de contrôle favorables à l'environnement et abordables financièrement. Nos travaux ont donc été définis pour développer des appâts à partir d'une source bon marché, afin de surveiller les populations de mouches des fruits et de les contrôler en réduisant au minimum les risques environnementaux. **Matériel et méthodes**. Un appât protéiné préparé localement par un processus d'autolyse à chaud à partir de résidus de levure de brasserie a été comparé, dans des pièges McPhail, à un appât d'hydrolysat de protéine importé. Les pièges ont été accrochés sur les arbres de quatre variétés d'orange douce pendant les principales saisons de production des agrumes en 2003 et 2004. **Résultats**. Les espèces du genre *Ceratitis* ont dominé dans les échantillons de mouches des fruits collectés. Les appâts importés et de fabrication locale ont permis la capture d'un nombre de mouches des fruits significativement plus élevé que dans les pièges de référence sans appâts. Pendant nos études, il n'y a eu aucune différence significative entre les populations de mouches des fruits capturées par l'un ou l'autre appât. Les variétés classées par ordre décroissant en fonction du nombre de mouches des fruits attrapées par les deux types d'appâts ont été telles que : Washington navel > Parson Brown > Valencia late > Agege-1. Le nombre de mouches des fruits observées sur Washington navel a été significativement plus élevé que celui obtenu sur Agege-1 et Valencia late. **Discussion**. Nos résultats montrent l'efficacité de l'appât protéiné de fabrication locale pour la surveillance et la gestion des mouches des fruits. Les différences variétales ont également été déterminantes vis-à-vis de l'ampleur des attaques de mouches des fruits sur les oranges douces.

Nigéria / *Citrus sinensis* / *Ceratitis* / avertissement agricole / piège / attractif / brasserie / utilisation des déchets / hydrolysat de protéines

1. Introduction

Citrus production in Nigeria is faced with the problems of fruit fly attack [1, 2]. Fruit flies cause fruit damage and premature fruit drop. The extent of fruit fly damage is believed to be associated with the degree of ripeness of the fruits [3]. Observations have shown that fruit fly damage occurs at all times when fruits are set in most parts of Nigeria. Late harvest allows some fruits to over-ripen, thereby increasing their sugar content [3, 4] and consequently becoming more vulnerable to fruit fly attacks [5]. Most often, damage can be so severe that 70% of set fruits are lost [6].

The majority of citrus producers in Nigeria are incapable of solving the perennial problem of fruit fly damage. Only large-scale farmers can sometimes afford the use of insecticides. Small-holders cannot afford these insecticides and in most cases do not apply any other form of control. As a result, large proportions of annual yields are lost due to fruit fly damage. Sometimes, farmers embark on controlling fruit flies by applying inappropriate insecticides or wrong dosages, ending up not achieving their desired goals. Excessive use of insecticides is presently being discouraged due to their adverse impact on the environment and human health. Selective and environmentally safe pesticides, and appropriate protein sources [7–9] are used as baits in traps or as cover sprays to reduce fruit fly populations below the economic threshold. Cultural practices such as removal of dropped fruits and early harvest, and minimal application of insecticidal mixtures have been reported to reduce fruit fly attack on sweet oranges in Nigeria [5]. These practices were therefore recommended in situations where small farmers can afford the cost of minimal insecticide applications. Alternatively, protein hydrolysate or para-pheromones (synthetic lures) are used in baits to attract fruit flies away from fruits and kill them. The use of protein baits in any form has never been evaluated for fruit fly monitoring or control in Nigeria.

It is opined that control methods that attract the flies away from the fruits, thus avoiding direct insecticide contact with the fruits during spraying, are better options for farmers. The use of commercial protein hydrolysates in monitoring and reducing the population of fruit flies has been effectively used in many countries [10, 11]. However, the importation of these products increases their prices, making them unaffordable to small-scale farmers. Therefore, the ability of Nigerian farmers themselves to locally produce protein baits will reduce purchasing costs and sustain fruit fly control.

Our study was therefore aimed at developing fruit fly protein bait from locally available brewery wastes by heat autolysis using a method that can be easily adopted by farmers. The specific objective was to compare imported commercially produced protein hydrolysate bait with locally made protein bait of brewery yeast waste for their efficacy in monitoring and managing fruit flies in small-scale sweet orange production.

2. Materials and methods

2.1. Preparation of crude protein bait

Bulked brewery yeast waste (yeast slurry) of Star™ and Gulder™ lager beer was obtained courtesy of the manufacturers, the Nigerian Brewery Ltd. The brewery waste consisted of *Saccharomyces cerevisae* (Meyer ex Hansen) at a pH of 4.5–6.0 and solid content of 15–20%. The brewery waste was poured into a 3-L aluminum pot immersed in a 5-L open vessel containing water. The vessel was heated on a hot plate and left to boil for 15 h, thus reducing the volume of the brewery waste by approximately 50% and increasing the solid content to 30–35%. This process also eliminated alcohol to make the product more attractive to the flies. The process caused some degree of yeast cell autolysis and resulted in amber-colored thick slurry with a strong yeast-like odor (about 7.5% protein). This was carried out repeatedly for the quantity of crude protein bait material needed. The pot was then allowed to cool down to room temperature. The method used in the present study was adopted from the work of Gopaul and Price [10] but modified according to local farmers' conditions in Nigeria.

2.2. Field evaluation of the baits

The trial was superimposed on a portion of an orchard planted to the sweet orange varieties Agege-1, Parson Brown, Valencia late and Washington navel, and it was laid out in a randomized complete block design at the National Horticultural Research Institute of Ibadan (Nigeria). The study was initiated when the fruits reached 5 cm in diameter during the 2003 and 2004 major fruiting seasons (August to December). Trees selected for the trial were spaced 21 m apart. These trees were labeled for continuous monitoring. A tree constituted a sampling unit for all parameters studied. The factors investigated included the number of trapped fruit flies on the four sweet orange varieties and the two types of protein bait and non-baited control. Three trees were sampled per variety and per treatment, thus giving a [4 × 3] factorial trial, replicated three times and arranged in a randomized complete block design [12].

The two baits, *i.e.*, protein hydrolysate (Era® bait pellets dissolved in lukewarm water at 50 °C) and autolysed brewery waste, each at a volume of 300 mL, were poured into McPhail traps and hung on the earmarked trees 1.8 m from the ground. Control traps with only water were also set up. After every 7 d of exposure, the fruit flies in each trap were emptied into a plastic container, sorted out and categorized by species and sex. The baits were replaced after each removal of trapped fruit flies, and the position of the traps changed in a clockwise direction in each block, thus eliminating any bias due to tree position on the number of flies captured. Fruit fly identifications were done in the laboratory using identification keys compiled by White and Elson-Harris [13]. Unidentified fruit fly specimens were shipped to nephritid experts abroad for confirmation.

The number of fruits attacked on the trees (*i.e.,* fruits with oviposition damage, eggs and larvae) was assessed once before harvest by taking two fruits in each of five points along the circumference of the upper and lower portions of the sampled trees; thus, a total of 20 fruits were sampled per tree. The fruit samples were stored in polythene bags and examined for damage in the laboratory. To ascertain that the fruit flies caught by the baits were actually those attacking sweet oranges, attacked fruits of each sample unit were placed in cages of (0.4 × 0.6 × 0.6) m dimensions. Each cage was covered at the side by fine wire mesh, and on the top by a perspex glass, while the base was layered with soil to facilitate the pupation of fruit flies. The emerged fruit flies were collected after 3 d for identification. Fruit fly number was bulked for *Ceratitis* species since they constituted over 98% of the trapped population. This was also confirmed by *in vivo* rearing of larva in infested fruits to adults in cages.

2.3. Statistical analysis

Data on number of fruits attacked by fruit flies were transformed using square root transformation $(X + 0.5)^{0.5}$ and subjected to Analysis of Variance (ANOVA) using SAS software [14]. Similarly, ANOVA was separately computed on each set of data using PROC SORT [14] to cover (i) comparison of the number of fruit flies captured by the types of protein baits, (ii) varietal effect on the number of trapped fruit flies, and (iii) the interactive effect of bait and sweet orange variety on the number of captured fruit flies. Means of significantly different treatments were separated using Duncan's Multiple Range Test [12]. Simple linear correlation analyses were conducted to determine the relationship between fruit fly numbers and those of attacked fruits in 2003 and 2004 [14]. All tests were judged significant at $p = 0.05$.

3. Results

Eight species were identified among the fruit flies captured (*table I*). They included three species of *Ceratitis*: *C.* (*Pardalaspis*) *ditissima* (Munro), *C.* (*Pterandrus*) *penicillata* (Bigot) and *C. capitata* (Wiedmann). These three species constituted more than 98% of the collections on each sampling date. The other species captured were *Bactrocera* (*Zeugodacus*) *curcubitae* (Coquillett), *B. invadens* (Drew, Tsuruta and White),

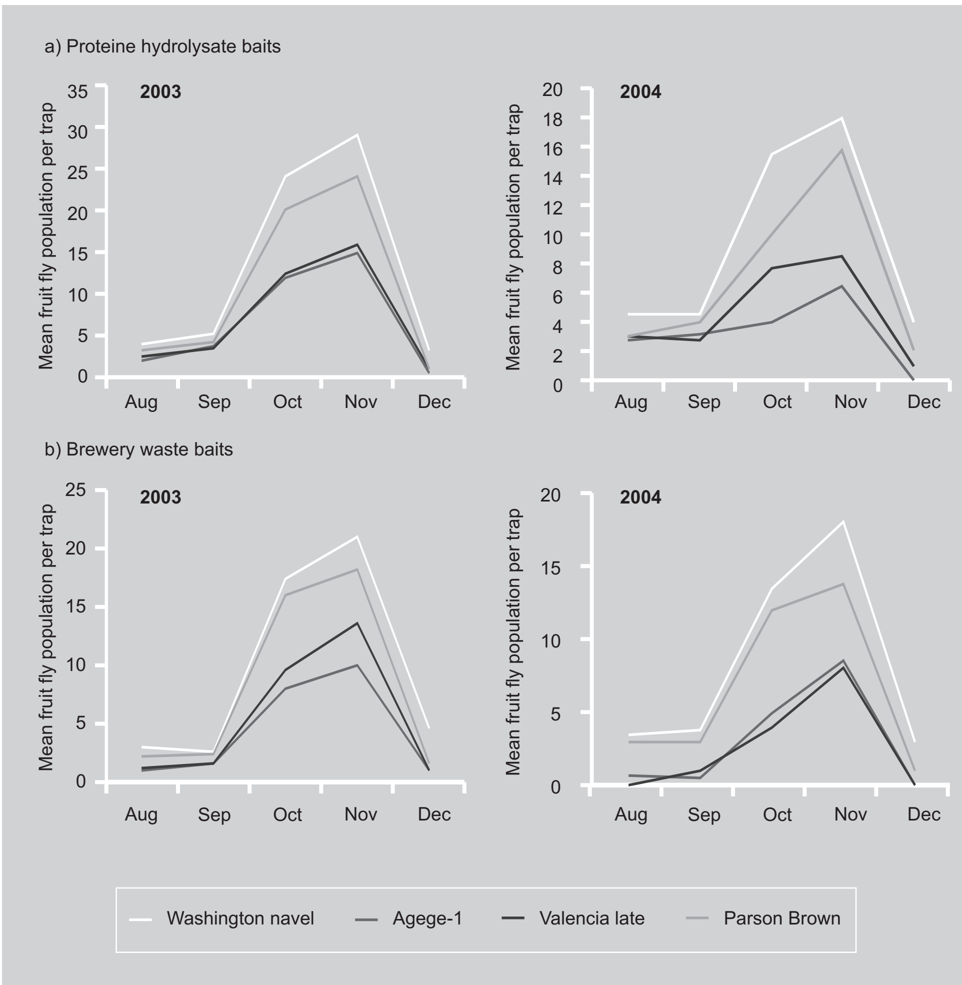
Table I.
Major fruit fly species of *Ceratitis* found in traps baited with protein hydrolysate and autolysed brewery waste in sweet orange orchards located in Ibadan, Nigeria.

Number of fruit flies	Bait type	*C. capitata*	*C.* (*Pardalaspis*) *ditissima*	*C.* (*Pterandrus*) *penicillata*
Mean number per trap[1]	Protein hydrolysate	6.2 ± 1.1	12.2 ± 1.4	10.1 ± 1.5
	Brewery waste	5.0 ± 0.9	10.4 ± 1.3	8.3 ± 1.6
Mean % of females in trap[2]	Protein hydrolysate	65 a	76 a	86 a
	Brewery waste	76 a	58 a	64 a

[1] Mean number of individual fruit flies caught was pooled for 2 years; data for non-baited traps were not presented due to 0 catch. Mean number of fruit fly species was not significantly ($P > 0.05$) different between traps.

[2] Mean percentages of fruit flies in the same row followed by the same letters were not significantly ($P > 0.05$) different in the same trap by Duncan's Multiple Range Test.

Figure 1.
Population dynamics of fruit flies caught in 2003 and 2004 trials in Ibadan (Nigeria) by protein hydrolysate and brewery waste baits hung on trees of four sweet orange varieties .



Dacus bivittatus (Bigot), *D. (Didacus) ciliatus* Loew, and *Celidodacus obnubilus* (Karsch.); they were only observed in 2004 from the tail end of the trial (late November to early December). *Dacus* and *Bactrocera* species were observed at such a low number that they were not included in the results. During our studies, higher percentages of female flies belonging to the various species were caught in the traps compared with males (*table I*).

The number of fruit flies was low in August in both 2003 and 2004, increased from September, and peaked in November before harvest. The number dropped drastically in December when most of the fruits had been harvested (*figure 1*). Both the imported and locally made baits attracted significantly (F = 16.57; df = 22; $P < 0.001$) higher mean numbers of fruit flies in the 2 years of the trials compared with the control traps without bait, which had no fruit flies throughout the trials in 2003 and 2004. Although a higher mean number of fruit flies was attracted to the protein hydrolysate bait (9.8 ± 1.9) than to the brewery waste bait (7.3 ± 1.5) in 2003, these baits were not significantly ($P > 0.05$) different from each other. Similar results were obtained in 2004, with records of 6.0 ± 1.3 and 5.1 ± 1.4, respectively, for the two types of baits. There was no significant difference between the two types of protein bait when they were compared on each of the sweet orange varieties considered (*figure 2*).

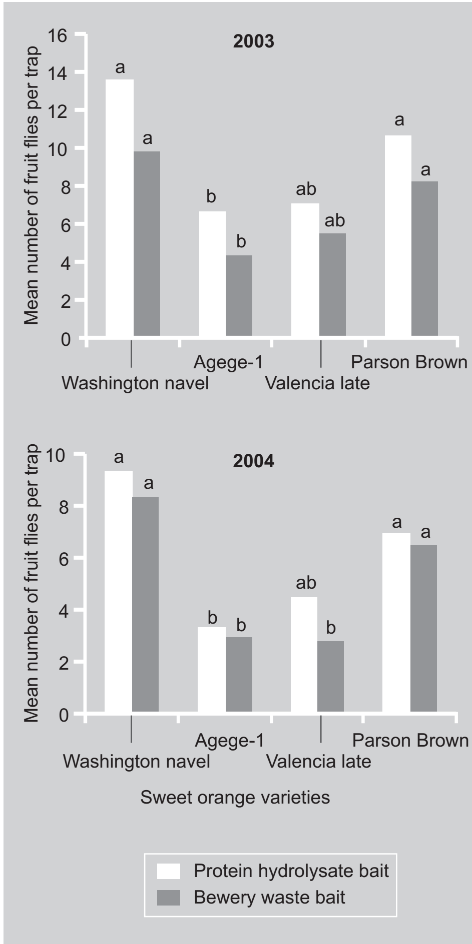


Figure 2.
Number of fruit flies caught in 2003 and 2004 in McPhail traps baited with protein hydrolysate and brewery waste on four varieties of sweet oranges in Ibadan (Nigeria). Fruit fly numbers in the two traps followed by the same letters are not significantly ($P > 0.05$) different within each variety. No fruit fly was captured in the non-baited traps in either 2003 or 2004.

Analysis of variance showed that the number of fruit flies caught in the traps was significantly (F = 6.32; df = 22; $P < 0.01$) affected by the sweet orange varieties. Agege-1 was least attacked in most cases, followed by Valencia late, while Washington navel was the most attacked. Agege-1 recorded a significantly ($P < 0.01$) lower mean number of fruit flies than Washington navel and Parson Brown in 2003 and 2004. There was no significant difference between the numbers of fruit flies captured on Valencia late and Agege-1 (*figure 3*). The numbers of fruit flies attracted to the four sweet orange varieties irrespective of the type of bait were in the following decreasing order: Washington navel > Parson Brown > Valencia late > Agege-1 in 2003 and 2004. However, ANOVA showed that there was no significant (F = 0.93; df = 22; $P > 0.05$) interaction between sweet orange varieties and the type of bait on the number of fruit fly captured.

Sweet orange variety significantly affected the number of fruits attacked (F = 4.06; df = 22; $P < 0.05$). In 2003 and 2004, Washington navel recorded a significantly ($P < 0.05$) higher mean number of attacked fruits per tree than the other varieties (*i.e.,* a minimum of 10.4 fruits per tree) except when compared with Parson Brown, which suffered a minimum of eight attacked fruits per tree. The mean number of fruits attacked per tree was significantly lower in Agege-1 and Valencia late, with minimums of 2.6 and 3.4 fruits, respectively, compared with other varieties in 2003 and 2004 (*table II*). There

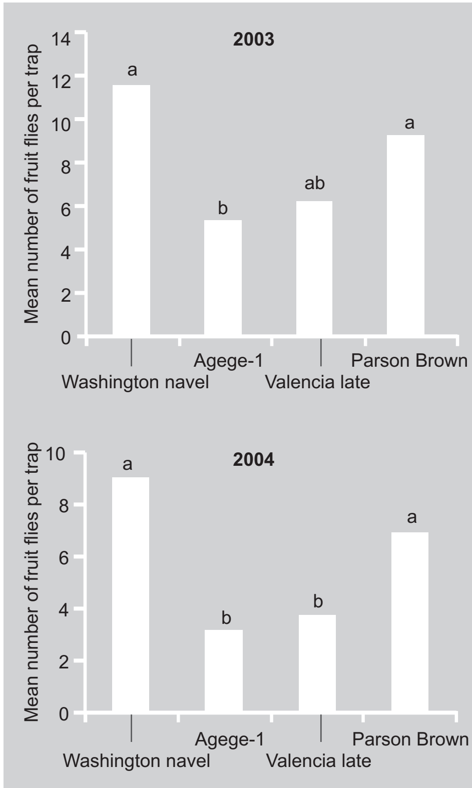


Figure 3.
Number of fruit flies caught in 2003 and 2004 on four sweet orange varieties in Ibadan (Nigeria). Fruit fly numbers followed by the same letters are not significantly ($P > 0.05$) different between sweet orange varieties.

was no significant difference between the numbers of attacked fruits in all the sweet orange varieties with foreign or locally made protein baits. However, the numbers of attacked fruits in the trees with baits were significantly lower than those of trees without baits (*figure 4*). The number of attacked fruits on the various sweet orange varieties generally followed the same trend as the number of fruit flies observed in the traps placed on them. A positive correlation was observed between the number of attacked fruits and the number of fruit flies caught in the traps in 2003 ($r = 0.85$; $n = 8$; $P < 0.01$) and 2004 ($r = 0.92$; $n = 8$; $P < 0.01$).

4. Discussion

Observations on *Ceratitis* spp. population dynamics during the trials showed that populations increased during fruit maturity and peaked at ripening. These results suggest that fruit fly control by the use of baited traps must be initiated at least a month prior to fruit maturity rather than during the ripening stage when they tend to attract more fruit flies [4, 15]. The numbers of *Dacus* spp., *B. invadens* (a new invasive species) and *B. curcubitae* were so negligible that they were not considered in the population studies. However, the presence of the invasive species *B. invadens* calls for nationwide monitoring due to its polyphagous nature, which may adversely affect the fruit industry [16–18].

Sweet orange varieties also influenced the level of fruit fly attack, as affinity was shown by *Ceratitis* towards some sweet orange varieties more than others. Similar results were obtained when Umeh *et al.* [2] assessed the susceptibility of twelve varieties of sweet oranges to *C. capitata* attack. They observed that some varieties were less attacked than others. The phenomenon was evidenced by the positive correlation

Table II.
The effect of variety on the number of fruits attacked by fruit flies in four sweet orange varieties (Ibadan, Nigeria).

Sweet orange variety	Mean number of attacked fruits per tree[1]	
	2003	2004
Agege-1	2.6 ± 1.4 b	2.8 ± 1.1 b
Parson Brown	9.0 ± 2.3 a	8.0 ± 2.5 a
Valencia late	4.0 ± 0.6 b	3.4 ± 1.6 b
Washington navel	10.7 ± 2.1 a	10.4 ± 2.3 a

[1] Mean number from 20-fruit sample. Means in the same column followed by the same letters are not significantly ($P > 0.05$) different by Duncan's Multiple Range Test.

between the number of attacked fruit and the number of fruit flies that were caught in the traps. The numbers of both the fruit flies and the attacked fruits were higher in Washington navel and Parson Brown throughout the trials. This implies that the variety of sweet orange on which the trap is set may affect the number of fruit flies that visit it. These varietal differences may be linked to fruit characteristics such as reduction of the acidity of fruit rind and fruit juice [3, 15]. It is therefore important for farmers in fruit fly-endemic areas to choose varieties less susceptible to fruit flies and to initiate fruit fly management at the appropriate time to reduce losses.

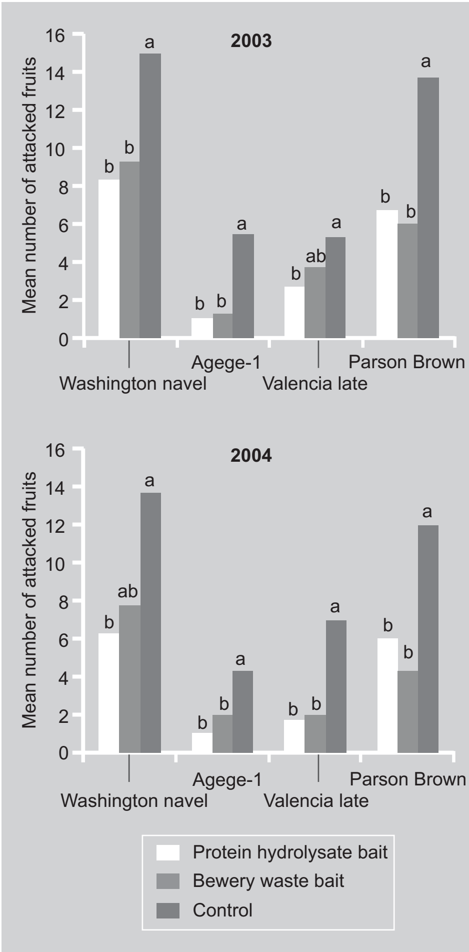


Figure 4.
Number of fruits attacked by fruit flies in 2003 and 2004 with two types of protein bait and control on four sweet orange varieties in Ibadan (Nigeria). Mean numbers of attacked fruits followed by the same letters are not significantly ($P > 0.05$) different within sweet orange varieties.

The effectiveness of locally made protein bait of brewery waste in attracting fruit flies for trapping and consequent reduction in the number of damaged fruits was evidenced by the significantly higher fruit attack observed on trees without bait (water control) in all the varieties assessed. The attraction of more female than male fruit flies by the locally made bait implies that there will be a reduction in the number of females laying eggs that would have become destructive larvae. The level of fruit fly control achieved by the locally made bait as demonstrated in the present trials will be most applicable to small orchards. However, the fruit fly control level can be increased by augmenting the number of traps per tree for large orchards in order to achieve a meaningful control. Unlike the pheromone baits that are specific to some genera or species, protein baits are attractive to almost all the species of destructive fruit flies in an orchard. Although the locally made bait is believed to contain less protein than the imported one due to its method of preparation, it will be of immense benefit to low-income citrus farmers because of its cost effectiveness. Preparations of local protein bait can be further simplified at small farmers' level by using metal containers or cooking pots, while brewery waste is readily available in all parts of Nigeria due to high distribution of breweries in the country. However, the addition of papain (a proteolytic enzyme usually derived form papaya) to the brewery yeast waste will enhance further proteolitic release of yeast [10, 18] and thus increase its attractiveness to fruit flies. Further studies have been designed to compare the attractiveness of brewery waste with or without papain in other vulnerable crops such as mango and guava. The results will be published in subsequent reports.

The results obtained in the present study emphasize the effectiveness of locally made bait as a tool for monitoring and mass-trapping fruit flies to reduce damage. The use of this form of protein bait will ensure reduced costs and maintain the sustainability of fruit fly control by low-income farmers. The results also confirmed that more female fruit flies are trapped than the males, thus leading to reduced egg laying and consequent fruit damage. The problem of procurement of McPhail traps by small-holders (due to importation) can be solved by using local traps made from discarded plastic water containers that can be easily sourced free of charge.

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Vigilancia y gestión del complejo de *Ceratitis* spp. en las variedades de naranjo dulce empleando un cebo local hecho de proteínas procedentes de residuos de cervecería.

Resumen — Introducción. Las moscas de las frutas son significativamente responsables de las pérdidas de rendimiento sufridas por los plantadores de cítricos en Nigeria. La mayoría de estos agricultores tienen escasos recursos y un conocimiento limitado de las estrategias apropiadas para la gestión de los parásitos de los cítricos. Para ellos la mejor solución sería la aplicación de métodos de control favorables con el medioambiente y abordables financieramente. Por ello nuestros estudios se definieron con el fin de desarrollar cebos a partir de una fuente económica, y poder vigilar las poblaciones de moscas así como de controlarlas reduciendo al mismo tiempo los riesgos medioambientales. **Material y métodos**. Se comparó un cebo proteínado preparado localmente mediante un proceso de autolisis en caliente a partir de residuos de levadura de cerveza, en trampas McPhail, con un cebo de hydrolysate de proteína importado. Se suspendieron las trampas en cuatro variedades de naranjo dulce durante las estaciones principales de producción de los cítricos en 2003 y 2004. **Resultados**. Las especies del tipo *Ceratitis* dominaron en las muestras de moscas de las frutas recogidas. Los cebos importados y de fabricación local permitieron la captura de un número de moscas de las frutas significativamente más elevado que la que se hizo en las trampas de referencia sin cebos. Durante nuestros experimentos no apareció ninguna diferencia significativa entre las poblaciones de moscas de las frutas capturadas por un cebo u otro. El orden decreciente de las variedades en función del número de moscas de las frutas atrapadas por los dos tipos de cebos fue de la siguiente manera: Washington navel > Parson Brown > Valencia late > Agege-1. El número de moscas de las frutas observado en Washington navel fue significativamente más elevado que aquel obtenido en Agege-1 y Valencia late. **Discusión**. Nuestros resultados muestran la eficacia del cebo proteínado de fabricación local para la vigilancia y la gestión de las moscas de las frutas. Las diferencias de variedades fueron también determinantes respecto a la amplitud de los ataques de moscas de las frutas a las naranjas dulces.

Nigeria / *Citrus sinensis* / *Ceratitis* / avisos agrícolas / trampas / atrayentes / cervecerías / aprovechamiento de desechos / proteínas hidrolizadas