Title

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Journal
Journal of Applied Entomology, 141(10)

ISSN
0044-2240

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Publication Date
2017-12-01

DOI
10.1111/jen.12419

Received: 5 August 2016 | Accepted: 7 June 2017
DOI: 10.1111/jen.12419

ORIGINAL CONTRIBUTION

WILEY JOURNAL OF APPLIED ENTOMOLOGY

Assessment of a liquid larval diet for rearing *Dacus* species and *Bactrocera dorsalis* (Diptera: Tephritidae)

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Funding information
IITA; BMZ - 'German Federal Ministry for Economic Development Cooperation'

Abstract

Species of the genus *Dacus* are important insect pests of fruits and vegetables. Two *Dacus* species, *Dacus punctatifrons* Karsch and *Dacus vertebratus* Bezzi, as well as *Bactrocera dorsalis* (Hendel), were reared on a liquid artificial diet, a carrot (*Daucus carota* L.)-based solid artificial diet, and a natural fruit host to assess the suitability of the liquid diet for small-scale rearing of these species. Egg hatch, pupal production, adult emergence and F_1 productivity were recorded to evaluate performance of the three species on each diet. Egg hatch on the three diets was more than 50% for *D. punctatifrons* and *B. dorsalis*, but for *D. vertebratus*, egg hatch was less than 40% when they were introduced to the liquid artificial diet. Pupal production for both *Dacus* species was very low or nil on the liquid artificial diet and the carrot-based artificial diet, respectively. Adult emergence was low for *D. punctatifrons* and nil for *D. vertebratus* on the liquid artificial diet. This study showed that the two *Dacus* species did not develop well on either the liquid or solid carrot-based artificial diet whereas *B. dorsalis* performed well on the liquid diet. Cucumber, the natural host of both *Dacus* species, was better for small-scale rearing of these species than the liquid and carrot-based artificial diets. Nutrients found in cucumber need to be identified to formulate alternative rearing media for *Dacus* species that are economical and easy to use.

KEYWORDS
artificial carrot-based diet, liquid artificial diet, oriental fruit fly, small-scale rearing

1 | INTRODUCTION

Fruit flies (Diptera: Tephritidae) represent one of the most important worldwide constraints to horticultural production (Ekesi & Billah, 2007). Pest fruit flies infest many commercially grown fruit and vegetable species including mango (*Mangifera indica* L.), citrus (*Citrus* spp.), tomato (*Solanum lycopersicum* L.), capsicum (*Capsicum annuum* L.) and cucurbits (e.g. *Citrullus lanatus* [Thunb.]; *Cucumus sativus* L.), with developing larvae causing fruit drop and direct fruit damage that makes infested fruit inedible (Clarke et al., 2005; Gnanvossou et al., 2008; White & Elson-Harris, 1992). There are several fruit flies that are economically important horticultural pests in African countries (White & Elson-Harris, 1992). This includes *Bactrocera dorsalis* (Hendel), *Dacus punctatifrons* Karsch and *Dacus vertebratus* Bezzi, which are major insect pests of many cultivated and wild cucurbits as well as a few species in the Solanaceae (e.g. tomatoes) (Caroll et al., 2002).

The development of fruit fly pest control methods is facilitated by the availability of efficient laboratory rearing techniques. This allows studies on their biology and ecology (Ekesi & Mohamed, 2011). However, laboratory colonization and adaptation of fruit flies (Diptera: Tephritidae) can be a long and difficult process (Parker, 2005; Rössler, 1975). Furthermore, scaling up of laboratory rearing to mass production of insects also remains one of the most important components of fruit fly management programmes because mass rearing is required to produce the large numbers required to study and implement control tactics such as classical biological control and the sterile insect technique (SIT). Mass rearing should balance insect quality, rearing performance and low costs. However, artificial larval and adult diets, as well as rearing conditions, influence the quality of the insects produced by mass rearing (Ekesi & Mohamed, 2011) and are subject to intensive research.

Larvae of tephritid fruit flies are reared on several media. These include artificial rearing diets based on carrot, *Daucus carota* L. (Christenson, Maeda, & Holloway, 1956) and the liquid artificial diet developed by Chang, Caceres, and Ja (2004). The advantages of the latter over solid artificial diets include simplified spent diet management because of higher consumption of liquid diets, reduced labour and a reduction in the volume of waste that needs to be disposed (Chang et al., 2004). The melon fly, *Zeugodacus cucurbitae* (Coquillett), formerly *Bactrocera cucurbitae* (Coquillett) (Virgilio, Jordaens, Verwimp, White, & De Meyer, 2015), was the first fruit fly species successfully reared using a liquid artificial diet at a small scale (Chang et al., 2004; Schroeder, Miyabara, & Chambers, 1971). *Zeugodacus cucurbitae* reared on this diet successfully develop, mate and reproduce (Chang et al., 2004). However, larval rearing resulted in approximately 20% fewer pupae with mass 10% less than those reared from the control diet (the ARS mill feed diet), which suggested the need for further improvement. Later, *B. dorsalis* (Chang, Vargas, Jang, Caceres, & Cho, 2006) and *Ceratitis capitata* Wiedemann (Chang, Caceres, & Ekesi, 2007) were successfully reared with the liquid larval diet. More recently, liquid artificial diet has been used to rear a number of tephritid fruit fly species including *Anastrepha fraterculus* Wiedemann (Vera et al., 2014) and also other Diptera including *Musca domestica* L. (Chang, 2008). Only one attempt has been made to rear a member of the genus *Dacus* (*Dacus ciliatus* Loew) using the liquid diet (Chang, 2008). According to feedback by Dr David Nestel described by Chang (2008), *D. ciliatus* was not able to survive on a liquid diet. No other study has been conducted on the performance of other *Dacus* species maintained by small-scale rearing on artificial solid or liquid diets.

This study was conducted as part of the worldwide transfer of the liquid diet technology. Specifically, we studied laboratory performance and quality control parameters of *D. punctatifrons*, and *D. vertebratus* (tested species) and *B. dorsalis* (used as a control species with previously known performance on the same liquid diet) reared at a small scale on liquid artificial diet, carrot-based artificial diet and natural fruit substrates. The rationale for using the carrot-based artificial diet in these tests was its availability as a mass-rearing medium for *B. dorsalis* at the IITA fruit fly rearing facilities. This diet was used either alone or in combination with fruit substrates, with good results for rearing *B. dorsalis* (Ekesi & Mohamed, 2011; Ekesi, Nderitu, & Chang, 2007).

2 | MATERIALS AND METHODS

2.1 | General experimental conditions

This study was conducted at the Biological Control Laboratory of the International Institute of Tropical Agriculture (IITA- Benin); approximately 15 km north-west of Cotonou (06°28N; 002°21E; 15 m above sea level), Abomey-Calavi, Benin. All procedures were completed at 25 ± 2°C, and 60% ± 10% relative humidity (RH), and a photoperiod of 12:12 (L:D) hr. Temperature and RH were recorded with an indoor HOBO data logger (HOBO Pro V2, NH, USA).

2.2 | Insects

Colonies of *D. punctatifrons*, *D. vertebratus* and *B. dorsalis*, were initiated in the laboratory with 500 pupae obtained from stock colonies maintained in the laboratory with periodic restocking of wild types from the same locations for 1–2 years. The first two species were reared on cucumber, *C. sativus*, while *B. dorsalis* was reared on mango. Upon emergence, adult flies were maintained in transparent Plexiglas cages (25 × 25 × 25 cm) with water and food (sugar and hydrolysate yeast powder in a ratio of 3:1) until maturation (2 weeks after emergence). Cucumber was provided to 100 males and 100 females of 14-day-old adults of *D. punctatifrons* and *D. vertebratus* and mango for *B. dorsalis* in rearing cages for 48 hr. The infested fruits were transferred individually into incubation units made of cylindrical transparent (1.5 L) plastic containers with sterilized (heated at 72°C for 72 hr), humidified beach sand for larval development and pupation. The beach sand was kept moist to prevent pupal desiccation. For 4 weeks, the sand was checked every 2 days, starting from day 5, by washing the sand through a sieve and retrieving pupae with a pair of soft tweezers. The sand was replaced if needed. Fruit fly puparia placed in clean plastic Petri dishes (90 mm of diameter, with a fine mesh screen) for further use.

2.3 | Larval rearing media

Three larval rearing media were tested in this study: (i) liquid artificial diet developed by Chang et al. (2004); (ii) a carrot paste-based diet; and (iii) fruits. The composition of the liquid diet is provided in Table 1. The carrot paste-based diet that was developed by Christenson et al. (1956) is also currently used in the insectary at IITA-Benin. Initial pH for both diets was set at 3.5 (Table 1). In addition to these two artificial rearing media, cucumber and mango were tested as natural fruit substrates for *Dacus* species and *B. dorsalis*, respectively. Fruits and diet ingredients were weighed using a balance (Salter 125 Mechanical Food Scale, Tonebridge, England).

TABLE 1 Composition of liquid and solid carrot-based diets for rearing larvae of two *Dacus* species and *Bactrocera dorsalis*

Ingredients	Liquid diet (%)	Carrot-based solid diet (%)
Potassium sorbate	–	0.4
Sodium benzoate	0.09	–
Nipagen	0.09	–
Sugar	5.96	16.2
Brewer's yeast	11.52	8.1
Citric acid	1.26	0.6
Water	81.08	–
Dehydrated carrot paste	–	74.7

–, Absent in the diet.

2.4 | Experimental procedures

Freshly laid eggs (< 1 hr old) were obtained from cohorts of 300 males and 300 females of *D. punctatifrons*, *D. vertebratus* and *B. dorsalis*. The cultures of each species had been maintained for about 20 generations in the laboratory at IITA-Benin. Eggs were collected using the modified funnel method described by Vargas (1989). Four replicates of each diet were assessed in this study.

2.5 | Fruit flies reared on liquid artificial diet

The liquid artificial diet developed by Chang et al. (2004) was tested. This represented the first time that this diet had been used in West Africa for *Dacus* species. The diet was prepared as described by Chang et al. (2004) and Ekesi et al. (2007).

2.6 | Fruit flies reared on carrot-based artificial diet

To prepare this medium, fresh carrot was blended for 5 min in a 1-L electronic blender. After blending, the resulting paste was hard-pressed to remove the water. This paste was combined with other ingredients listed in Table 1. Rearing of flies was carried out using the 'diet ball method' (Lux, Ekesi, & Zenz, 2005) consisting of 200 g of diet infested with 0.2 ml of eggs evenly spread on the diet ball. Each diet ball was placed in a 350 cm^3 plastic container with a layer of sterilized beach sand at the bottom as pupation medium. After infestation, the container was transferred in incubation units made of 1.5-L plastic containers. Newly formed host puparia were placed into plastic vials (8 cm diam., 5 cm deep) until adult flies emerged.

2.7 | Fruit flies reared on fruits

We used cucumber for *D. punctatifrons* and *D. vertebratus*, and mango for *B. dorsalis*. One kilogram of each of these fruits was thoroughly washed with soapy water and rinsed clean before infesting them with 0.2 ml of eggs. Eggs were evenly distributed in needle punctures made on each fruit. The infested fruits were transferred individually into incubation units made of cylindrical transparent (1.5 L) plastic containers with sterilized humidified beach sand for larvae to enter the pupal stage. Collection of first puparia started 10 days after fruit infestation. Newly formed puparia were placed into plastic vials (8 cm diam., 5 cm deep) until adult flies emerged.

2.8 | Assessment of quality control parameters

To assess the relative suitability of the liquid and carrot-based diets and fruits for rearing of the two *Dacus* species and *B. dorsalis*, several parameters were measured: egg hatch, percentage pupal recovery, adult emergence and F_1 productivity.

To determine egg hatch on each diet, a random sample of 100 eggs collected 1 day after oviposition was carefully deposited on each of the three rearing media with a fine camel hair brush. Four replicates were sampled for each diet. The number of unhatched eggs was recorded 4 days later, and the difference was expressed as a percentage.

Pupal recovery was calculated following the protocol used by Chang et al. (2006) with a few modifications. Total mass of pupae collected from sand was weighed, and pupae were weighed in batches of 40 for eight sets to obtain the mean mass. Total number of pupae yielded was generated by dividing total pupal mass by mean mass from eight sets of 40 pupae by 40. Pupal production as a percentage was calculated as 100 times total pupal yield divided by total eggs times parental percentage of egg hatch. Parental percentage of egg hatch was calculated as follows: after rinsing the eggs using distilled water, four sets of 100 eggs each were seeded on a green blotting paper. The numbers of eggs that did not hatch from each 100 eggs after 4 days were counted and recorded (Chang et al., 2006).

For the assessment of adult emergence, puparia collected from second-day pupal collections (or the largest daily collection) were randomly selected, from which four lots of 100 puparia were set and placed in a Petri dish covered in a transparent Plexiglas cage (15 × 15 × 15 cm) without food or water until all emerged flies had died (usually 4 days after adult emergence). Adult fly emergence was calculated as the total initial number of puparia (100) minus the average percentage of unemerged puparia (Chang et al., 2004, 2006, 2009).

To estimate the impact of larval media on F_1 productivity, a piece of either cucumber or mango weighing 40 g was offered daily to the flies (10 females and five males) in a transparent Plexiglas cage (10 × 10 × 10 cm) for seven consecutive days. The fruit was removed after 24 hr and transferred to incubation units (described above) for collection of pupae. The number of puparia retrieved was used for analysis.

2.9 | Statistical analysis

The nonparametric Kruskal–Wallis test was used to test the effect of each diet on egg hatch, pupal recovery, adult emergence and F1 productivity. Treatment groups were compared with the Wilcoxon each pair test. All analyses were performed using JMP 8.0.0 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

3.1 | Egg hatch

Egg hatch was significantly different among diets for *Dacus* species (Table 2). There was no difference in egg hatch among diets for *B. dorsalis*. The best egg hatch for all three species was obtained on fruit. Egg hatch was very low for *D. vertebratus* on the liquid artificial diet.

3.2 | Pupal production

Pupal production was significantly different among diets for the three species (Table 2). High pupal production was obtained from fruit for *Dacus* species and on the liquid artificial diet for *B. dorsalis*. No pupae were recovered from the artificial carrot-based diet for either *Dacus* species. Pupal production of *D. vertebratus* from the liquid artificial diet was low.

TABLE 2 Quality control parameters (Mean ± *SE*) of *Bactrocera dorsalis*, *Dacus punctatifrons* and *Dacus vertebratus* reared on artificial liquid diet, carrot-based diet and fruit

Species	Diet	Eggs hatch (%)	Pupal production (%)	Emergence (%)	F_1 productivity[a]
B. dorsalis	Carrot diet	75.3 ± 4.6	8.3 ± 3.3c	36.5 ± 3.5b	3.1 ± 0.1b
	Fruit	86.6 ± 6.2	37.1 ± 2.5b	37.5 ± 4.0b	2.1 ± 0.3b
	Liquid diet	83.5 ± 3.3	74.4 ± 2.5a	61.0 ± 4.5a	5.6 ± 0.3a
	Statistics	$\chi^2 = 2.3$, $df = 2$, $p = .3162$	$\chi^2 = 9.8$, $df = 2$, $p = .0073$	$\chi^2 = 7.4$, $df = 2$, $p = .0245$	$\chi^2 = 9.3$, $df = 2$, $p = .0097$
D. punctatifrons	Carrot diet	61.5 ± 6.9b	0 ± 0	0 ± 0	0 ± 0
	Fruit	90.3 ± 4.1a	54.3 ± 5.5a	61.3 ± 3.0a	6.5 ± 0.3
	Liquid diet	83.0 ± 3.0ab	32.8 ± 2.5b	11.3 ± 3.0b	5.6 ± 0.3
	Statistics	$\chi^2 = 6.9$, $df = 2$, $p = .0311$	$\chi^2 = 5.3$, $df = 1$, $p = .0209$	$\chi^2 = 5.3$, $df = 1$, $p = .0209$	$\chi^2 = 1.3$, $df = 1$, $p = .2482$
D. vertebratus	Carrot diet	79.3 ± 1.9a	0 ± 0	0 ± 0	0 ± 0
	Fruit	85.0 ± 6.5a	47.6 ± 2.6a	50.3 ± 5.1	3.4 ± 0.4
	Liquid diet	32.8 ± 2.3b	4.6 ± 1.5b	0 ± 0	0 ± 0
	Statistics	$\chi^2 = 7.7$, $df = 2$, $p = .0218$	$\chi^2 = 5.3$, $df = 1$, $p = .0209$	$\chi^2 = 6.1$, $df = 2$, $p = .0139$	

Means values ± *SE* in the same column for each level followed by the same letters are not significantly different (Wilcoxon each pair test).
[a]Expressed as number of puparia per female.

3.3 | Adult emergence

Adult emergence was significantly different among diets for all three species (Table 2). Absence of pupal production by *Dacus* species on carrot-based artificial diet did not allow for testing emergence of these species reared on this diet. For *B. dorsalis*, adult emergence from the carrot-based artificial diet was not significantly different from emergence of this species on fruit. However, the highest levels of *B. dorsalis* adult emergence were from the liquid artificial diet. *Dacus punctatifrons* emergence was highest when reared from fruit, in contrast to very low emergence when reared on the liquid artificial diet. No emergence was recorded for *D. vertebratus* when reared on the liquid artificial diet; adults only emerged when reared on fruit.

3.4 | Productivity of F_1 adult females

Productivity of F_1 females, expressed as number of pupae produced, was significantly different among diets for *B. dorsalis*. Even productivity of *B. dorsalis* females obtained from the liquid artificial diet was low. However, productivity of females reared from the liquid artificial diet was still higher than productivity of those obtained from fruit and the carrot-based artificial diet (Table 2). Productivity of *D. punctatifrons* obtained from fruit and the liquid artificial diet did not differ significantly.

4 | DISCUSSION

The principal objective of this study was to assess the performance of *D. punctatifrons* and *D. vertebratus*, representing two major cucurbit-infesting species in sub-Saharan Africa, to develop and survive in the liquid diet developed by Chang et al. (2004, 2006, 2007); Chang (2008). *Dacus punctatifrons* performed better than *D. vertebratus* on the liquid artificial diet for all tested quality control parameters. Species already evaluated include *A. fraterculus*, *Anastrepha ludens* (Loew), *Anastrepha obliqua* (Macquart), *Anastrepha serpentina* (Wiedemann), *Anastrepha striata* Schiner, *Bactrocera correcta* (Bezzi), *B. dorsalis*, *Bactrocera latifrons*, *Bactrocera oleae* (Rossi), *Bactrocera passiflorae* (Froggatt), *Bactrocera pyrifoliae* Drew & Hancock, *Bactrocera tryoni* (Froggatt), *Bactrocera xanthodes* (Brou), *Bactrocera zonata* (Saunders), *C. capitata*, *Ceratitis cosyra* (Walker), *D. bivittatus*, *D. ciliatus*, *M. domestica*, *Ophyra aenescens* (Wiedemann) and *Z. cucurbitae*. These studies were conducted in 14 countries with either successful or negative results (Chang, 2008). The 10 successful evaluations proved that larvae of *A. fraterculus*, *B. correcta*. *B. dorsalis*, *B. passiflorae*, *B. zonata*, *C. capitata* and *Z. cucurbitae* (all Diptera: Tephritidae) can develop successfully when reared on the liquid diet developed by Chang et al. (2004). However, it is important to mention that many of these studies used the liquid diet technology (use of sponge cloths) but not necessarily the same formulation. For instance, for *A. fraterculus* (Vera et al., 2014), the authors used a diet based on sugar, brewers' yeast and wheat germ at the same proportions, which was likely much more nutritious than the one used here.

Egg hatch was relatively high for *D. punctatifrons* and *B. dorsalis*. This suggests that egg hatch of these species was not affected by the quality of the rearing media. The same conclusions were made by Chang (2009) who demonstrated that there were no significant differences in egg hatch regardless of the type of diet for rearing *B. dorsalis*, *C. capitata* or *Z. curcurbitae*. In contrast to *D. punctatifrons*, hatch of *D. vertebratus* eggs on the liquid artificial diet was low, which suggests that the liquid artificial diet is not appropriate for *D. vertebratus* egg hatch. Larval survival on the liquid artificial diet for both *Dacus* species, indicated by pupal production, and adult emergence was poor and significantly lower than that from the control diet of cucumber. This result was different from those reported by Mittler and Tsitsipis (1973) *B. oleae*, who found a 10-fold increase in pupal yield from 1 g of liquid diet in comparison with a solid diet. In *Z. cucurbitae*, Chang et al. (2004) found that pupal recovery was lower on liquid artificial diet (65.2%) compared with the control diet (83.8%), but adult emergence from these pupae did not differ.

No pupae of either *Dacus* species were reared from the carrot-based artificial diet, and pupal production of *B. dorsalis* on the carrot-based diet was low. These observations are similar to those for *B. dorsalis* and *B. latifrons* from other studies (Ekesi et al., 2007; Vargas & Mitchell, 1987). In the case of the two *Dacus* species, it is highly likely that the nutritional content of the carrot-based diet did not match that of their native hosts in the plant family Cucurbitaceae. Due to this, an artificial diet formulated with cucurbit fruit species may lead to better results. Another potential explanation for low pupal production on the carrot-based diet in the three tested species is the carrot-based artificial diet contained too little water. According to Fay (1989), the first two larval instars of tephritid fruit flies require 85%–90% of water for their normal development. The carrot-based diet processing method involved the removal of both the flesh and all water content of the carrot, which may have consistently led to a medium that was too dry for these flies, as well as a lower nutrient content in comparison with the cucumber.

This study demonstrates that the tested liquid diet formulation is not suitable for rearing *D. punctatifrons and D. vertebratus*. Our results are similar to previous observations on the cucumber fly, *D. ciliatus* (Chang, 2008). However, it is important to continue to evaluate different larval diet formulations that are economical and easy to use while also enhancing larval survival and adult emergence. Further studies should identify nutrients found in cucurbits that were not available in the tested artificial diets to permit the formulation and testing of alternatives. In the interim, the use of cucurbits instead of carrot for rearing *D. punctatifrons* and *D. vertebratus* will improve the number available for research.

ACKNOWLEDGEMENTS

We thank Jeannette Winsou and Carine Songbe for providing insect rearing and test materials. Three anonymous reviewers provided constructive comments on earlier versions of the manuscript. Chris Weldon (University of Pretoria) provided advice on the structure of the manuscript and provided English language editing. Financial support by IITA core donors and the German Federal Ministry of Economic Cooperation (BMZ) is gratefully acknowledged.

AUTHOR CONTRIBUTION

FMA: Study conception and design, Data collection, Data analysis and interpretation, Drafting of manuscript; AHB-G: Study conception and design, Data analysis and interpretation, Critical revision; DG: Study conception and design, Data analysis and interpretation, Critical revision; RH: Study conception and design, Data analysis and interpretation, Drafting of manuscript, Critical revision; CLC: Critical revision.

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How to cite this article: Anato FM, Bokonon-Ganta AH, Gnanvossou D, Hanna R, Chang CL. Assessment of a liquid larval diet for rearing *Dacus* species and *Bactrocera dorsalis* (Diptera: Tephritidae). *J Appl Entomol*. 2017;00:1–6. https://doi.org/10.1111/jen.12419