

## Adaptation to and Small-Scale Rearing of Invasive Fruit Fly *Bactrocera invadens* (Diptera: Tephritidae) on Artificial Diet

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**ABSTRACT** Larval rearing of *Bactrocera invadens* Drew, Tsuruta & White (Diptera: Tephritidae) on artificial diet is described. The adaptation process for this insect, when moved from whole mango, *Mangifera indica* L., fruit rearing to artificial diet based on wheat bran, took between three and five generations to reach the plateau of quality control parameters observed for rearing the insect on whole mango fruit. Small-scale rearing on wheat, *Triticum aestivum* L., or carrot, *Daucus corata*-based diet revealed significantly higher pupal recovery for flies reared on the wheat-based artificial diet (68.8%) compared with the carrot-based diet (58.2%). Weekly production of puparia was 3,966.8 on wheat- and 3,012.1 on carrot-based diet. Other quality control parameters, including pupal weight, adult emergence, flight ability, fecundity, and fertility did not differ significantly between the two artificial rearing media tested.

**KEY WORDS** *Bactrocera invadens*, adaptation, artificial diet, rearing

In March 2003, an invasive fruit fly species was detected in coastal parts of Kenya and has been described as *Bactrocera invadens* Drew, Tsuruta & White (Diptera: Tephritidae) (Drew et al. 2005). This fly is thought to belong to the *Bactrocera dorsalis* Hendel complex of tropical fruit flies and was detected in Sri Lanka soon after it was reported from Kenya (Drew et al. 2005). With adult traits that can include high mobility and dispersive powers, high reproductive rates, and extreme polyphagy, members of the *B. dorsalis* complex of fruit flies are generally recognized as being among the most destructive insects of fruits and vegetables worldwide, and they rank high on quarantine lists around the world (White and Elson-Harris 1992, Clarke et al. 2005). This is true for *B. invadens*, which within a span of 3 yr after its first report in Kenya has spread to 22 African countries, including Comoros Island, attacking >30 plant species (Ekesi et al. 2006, Ekesi and Billah 2006, Mwatawala et al. 2006). Although the insect attacks several host plants, its primary host seems to be mango, *Mangifera indica* L. (Ekesi et al. 2006), and limited numbers of the insect were reared on whole mango fruit at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya soon after its detection in 2003. After about five generations of rearing on whole mango fruit, we started a series of experiments to gradually rear the larvae of *B. invadens* on artificial diet. This was necessary to provide large numbers of quality flies for evaluation of attractants, entomopathogens, and post-

harvest treatment, and for basic biological studies in the laboratory and greenhouse.

Laboratory colonization and mass production of fruit flies on artificial diet may require several generations for the insects to adapt to the artificial diet (Kamikado et al. 1987, Souza et al. 1988, Economopoulos 1992). In some cases, attempts to raise a colony from wild populations on artificial diet have failed (Rössler 1975), but, if successful, long-term rearing on artificial diet may improve insect performance, e.g., through reduction in the preoviposition period and an increase in egg production (Vargas and Carey 1989). Here, we assessed the adaptation process of *B. invadens* to artificial diet when the flies were moved from rearing on whole mango fruit to a wheat, *Triticum aestivum* L.,-based artificial diet. We also tested the laboratory performance and quality control parameters of the insect under a small-scale rearing procedure when maintained on wheat- or carrot, *Daucus corata*-based artificial diets.

### Materials and Methods

**Insects.** The adults of *B. invadens* used in the study originated from rotten mangoes collected at a local market in Nairobi, Kenya. The insects that were used for adaptation studies had been maintained on whole mango fruit for five generations. The insects used for the small-scale rearing were maintained on a carrot-based diet by using the "diet ball methodology" (Lux et al. 2005) for six generations. This methodology involves wrapping the artificial diet into an 8-cm-diameter ball with a food grade ClingFilm (Fay Cling-Film Wrap, Nairobi). After wrapping, the balls were surface sterilized with 0.03% sodium hypochloride and

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Table 1. Artificial larval diet ingredients used in the study

Ingredient	Wheat	Carrot
Torula yeast, g	3.55	
Brewer's yeast, g		8.10
Methyl p-hydroxybenzoate, g	0.11	0.20
Sodium benzoate, g	0.11	
Sugar, g	7.83	16.20
Citric acid, g		0.60
Mill feed, g	31.20	
Carrot, g		24.20
Water, ml	57.20	50.70

Sources of ingredients (from top to bottom, excluding water): ISCA Technologies, Riverside, CA; East African Breweries Limited, Nairobi, Kenya; Kobian Kenya Limited, Nairobi, Kenya; J. T. Baker, Phillipsburg, NJ; Mumias Kenya Limited, Bungoma, Kenya; Chemoquip Limited, Nairobi, Kenya; Unga Limited, Nairobi, Kenya; and obtained as fresh fruit from hawkers market, Nairobi, Kenya, and then crushed, dried, and blended into a powder.

exposed directly to adult flies in Plexiglas cages (50 by 50 by 50 cm.). The diet balls were pierced with an entomological pin (size 3) to facilitate direct oviposition by flies into the balls. Each cage containing the diet balls had  $\approx 5,000$  flies. The diet ball oviposition devices were normally exposed to the flies for a period of 48 h after which they were removed and transferred to 2-liter rectangular plastic containers provided with presterilized beach sand (99% sand, 0.5% silt, and 0.5% clay, pH 5.2), obtained from the shores of Lake Victoria, western Kenya, and sifted through a 16-mesh screen). After 7 d, the diet balls were split open to facilitate exit of larvae into sand to pupate. The method does not involve direct seeding of eggs onto the artificial diet.

**Adaptation Studies.** Eggs of *B. invadens* were collected using a mango dome (that had the pulp and the seed removed) that was placed into fly stock colonies for 1 h. Eggs were carefully removed from the underside of the dome with a fine camel's-hair brush and placed on a 9-cm-diameter moist blotting paper. After 36 h, 100 newly emerged larvae from the above-described lots of eggs were gently introduced with a fine camel's-hair brush onto the surface of 100 g of wheat-based diet (Table 1) in open 150-ml plastic cups or into artificially drilled holes on whole mango fruit. Each hole was perforated with an entomological pin and measured  $\approx 1$  mm in diameter and 1 cm in depth. After inoculation with larvae, the fruit were placed in 200-ml plastic cups over a 1-cm-deep layer of sand, and the cups were covered with muslin cloth. Mature larvae exited the fruit and pupariated in the sand. Each of the artificial diet cups was maintained in larger 250-ml plastic cups that contained a layer of heat-sterilized sand on the bottom as pupariation medium. Mature larvae left the artificial cups and entered the larger cups to pupariate.

An experiment consisted of four cups of diet containing 100 larvae, with each group of four cups replicated three times or four fruit with the same number of larvae and replications. At every generation tested, records were kept on the following: 1) adult survival expressed as percentage insect-days of 20 d (total

possible days), 2) larval stage duration, 3) percentage of puparia recovered from diet or fruit, 4) weight of puparia, 5) adult emergence and flight ability, and 6) fecundity and fertility over a 10-d period. Adult survival was tested in 15- by 15- by 15-cm Plexiglas cages. Percentage of pupal recovery was calculated based on the initial number of larvae introduced into each container of rearing medium. Pupal weight was based on four lots of 20 puparia from each replicate. Adult emergence was based on four lots of 20 puparia from each replicate that were placed in screened 12-cm-diameter plastic containers and observed for 21 d. The flight ability test was conducted with four lots of 20 puparia from each replicate by using the method of Boller et al. (1981) in a 20-cm-high plastic tube coated with talc in a 9-cm-diameter petri dish. Fecundity and fertility were based on daily egg collections from 10 pairs of flies held after a preoviposition period of 7 d. Eggs were collected using a mango dome, and hatch rate was assessed after 72 h. In all experiments, adults were fed on a diet consisting of 3 parts sugar and 1 part enzymatic yeast hydrolysate ultrapure (USB Corporation, Cleveland, OH), and water on pumice granules. All experiments were carried out in a room maintained at  $28 \pm 1^\circ\text{C}$ ,  $50 \pm 8\%$  RH, and a photoperiod of 12:12 (L:D) h.

**Small-Scale Rearing.** In this experiment, eggs (0.5 ml,  $\approx 6,000$  eggs) collected within 1 h after oviposition were seeded using a 1-ml transfer pipette onto a 5- by 5-cm strip of moist black cloth. The cloth was placed on the center top of 500 g of wheat- or carrot-based diet (Table 1) in 3- by 10- by 15-cm plastic trays. The experimental conditions were similar to the adaptation experiment. Upon hatching from the egg, larvae fed ad libitum, and mature larvae were allowed to freely leave the rearing tray for pupation into larger plastic trays (6 by 20 by 30 cm) that contained a 1-cm-deep layer of moist (5–8% water) sand. After 7 d in the sand, puparia were separated from the pupation medium by gentle sifting. The following data were obtained: 1) percentage of pupal recovery calculated based on the number of eggs seeded onto the diet, 2) pupal weight (milligrams) based on four lots of 50 puparia from each tray, 3) adult emergence based on four lots of 100 puparia from each tray, 4) flight ability based on four lots of 100 puparia tested according to Boller et al. (1981), 5) fecundity, and 6) fertility were determined for 20 flies over 10 d as described above.

**Statistical Analysis.** Data for developmental time, pupal weight, and fecundity were transformed to natural logarithms before analyses. Percentage of adult survival, pupal recovery, and egg hatch were transformed by arcsine before analysis. A factorial analysis of variance (ANOVA) was applied to examine differences between media and generations (Winer et al. 1991). Differences in quality control parameters between the artificial diet and mango for each generation and rearing media were determined by ANOVA, and means were compared using the *t* statistic. All analyses were performed using the SAS package (SAS Institute 2001).

Table 2. Mean  $\pm$  SE adult survival and larval duration of adult *B. invadens* reared on wheat-based artificial diet and whole mango fruit

Generation	% adult survival		Larval developmental period (d)	
	Wheat-based artificial diet	Whole mango	Wheat-based artificial diet	Whole mango
P	52.2 $\pm$ 6.5a	95.4 $\pm$ 2.2b	11.6 $\pm$ 1.3a	11.3 $\pm$ 1.2a
1	54.3 $\pm$ 5.2a	80.3 $\pm$ 4.6b	11.4 $\pm$ 1.6a	10.2 $\pm$ 0.8a
2	69.6 $\pm$ 2.2a	78.6 $\pm$ 2.2b	10.4 $\pm$ 0.7a	10.8 $\pm$ 2.1a
3	88.4 $\pm$ 4.3a	92.5 $\pm$ 1.3a	11.2 $\pm$ 1.2a	11.1 $\pm$ 1.5a
4	80.6 $\pm$ 3.6a	82.4 $\pm$ 3.8a	7.8 $\pm$ 1.3a	10.5 $\pm$ 1.1b
5	92.3 $\pm$ 1.1a	86.6 $\pm$ 4.0a	8.2 $\pm$ 0.8a	10.2 $\pm$ 1.2b

For each quality parameter, means within a row followed by the same letter do not differ significantly by *t*-test ( $P = 0.05$ ).

### Results

There were significant differences between the two rearing media tested for six quality control parameters: adult survival ( $F = 18.56$ ;  $df = 1, 24$ ;  $P = 0.0241$ ), larval development ( $F = 8.12$ ;  $df = 1, 24$ ;  $P = 0.0038$ ), percentage of pupal recovery ( $F = 9.18$ ;  $df = 1, 24$ ;  $P = 0.0082$ ), pupal weight ( $F = 22.14$ ;  $df = 1, 24$ ;  $P = 0.0001$ ), fecundity ( $F = 24.19$ ;  $df = 1, 24$ ;  $P = 0.0001$ ), and fertility ( $F = 11.14$ ;  $df = 1, 24$ ;  $P = 0.0108$ ). Significant differences also were observed for the same quality parameters over the five generations of *B. invadens*: adult survival ( $F = 16.15$ ;  $df = 5, 24$ ;  $P = 0.0012$ ), larval development ( $F = 10.13$ ;  $df = 5, 24$ ;  $P = 0.0028$ ), percentage of pupal recovery ( $F = 11.60$ ;  $df = 5, 24$ ;  $P = 0.00140$ ), pupal weight ( $F = 13.10$ ;  $df = 5, 24$ ;  $P = 0.0029$ ), fecundity ( $F = 15.10$ ;  $df = 5, 24$ ;  $P = 0.0033$ ), and fertility ( $F = 9.18$ ;  $df = 5, 24$ ;  $P = 0.0016$ ). However, no significant differences were observed in adult emergence or flight ability due to media (adult emergence:  $F = 1.92$ ;  $df = 1, 24$ ;  $P = 0.0874$ ; and flight ability:  $F = 1.16$ ;  $df = 1, 24$ ;  $P = 0.0658$ ) and across generations (adult emergence:  $F = 2.15$ ;  $df = 5, 24$ ;  $P = 0.0765$ ; and flight ability:  $F = 1.84$ ;  $df = 5, 24$ ;  $P = 0.1076$ ).

During the first three generations, survival of *B. invadens* was significantly lower on artificial diet compared with flies reared on whole mango fruit (Table 2). Thereafter, no significant difference was observed between the media in the other generations. No significant difference was detected in larval development between the rearing media from the parent generation

to generation  $F_3$ . However, in generation  $F_4$  and  $F_5$ , development took significantly fewer days (7.8–8.2 d) on the artificial diet compared with insects reared on whole mango (10.2–10.5 d) (Table 2).

Pupal recovery was significantly less in *B. invadens* reared on artificial diet between generations P and  $F_2$  (Table 3). From generation  $F_3$  onward, the rate of pupal recovery was not significantly different between the two rearing media. At generations  $F_4$  to  $F_5$ , pupal weight was higher on artificial diet compared with rearing on mango (Table 3).

Adult emergence and flight ability were not affected by the rearing media throughout the five generations tested (Tables 3 and 4). Over the generations, emergence ranged from 72 to 83% on artificial diet and from 73 to 83% on mango. Flight ability varied from 75 to 85% on artificial diet and from 72 to 82% on mango.

Fecundity over a 10-d period was found to be significantly lower (77–97 eggs) on artificial diet during generations P to  $F_2$  compared with mango (101–124 eggs) (Table 4). However, by generations  $F_3$  to  $F_5$ , flies emerging from the artificial diet produced significantly more eggs (122–282 eggs) than insects reared on mango (109–117 eggs). During the first three generations, fertility was significantly reduced (34–61%) on artificial diet compared with mango (70–84%). However, from generation  $F_3$  onward, the rate of egg hatch did not differ significantly between the two rearing media (Table 4).

Small-scale rearing test carried out using wheat- or carrot-based diets showed that pupal recovery from the wheat-based diet was significantly higher (68.8%) than that from the carrot-based diet (58.2%) (Table 5). Other quality control parameters did not differ significantly between these two rearing media. This was consistent for weekly small-scale production parameters generated for the two diets (Table 6).

### Discussion

Conventional knowledge in fruit fly mass rearing demands that artificially reared flies produced for research should possess qualities and behavior nearly like insects in the wild (Calkins et al. 1994). However, laboratory colonization and adaptation of fruit flies can be a long and difficult process (Rössler 1975, Leppla et al. 1983, Souza et al. 1988, Parker 2005).

Table 3. Mean  $\pm$  SE pupal recovery, pupal weight, and adult emergence of *B. invadens* reared on wheat-based artificial diet and whole mango fruit

Generation	% pupal recovery		Pupal wt (mg)		% adult emergence	
	Wheat-based artificial diet	Whole mango	Wheat-based artificial diet	Whole mango	Wheat-based artificial diet	Whole mango
P	22.5 $\pm$ 2.0a	60.3 $\pm$ 6.4b	8.8 $\pm$ 1.7a	9.7 $\pm$ 2.1a	78.5 $\pm$ 6.5a	81.0 $\pm$ 2.4a
1	24.4 $\pm$ 1.8a	54.6 $\pm$ 1.3b	9.6 $\pm$ 1.1a	9.3 $\pm$ 1.1a	78.3 $\pm$ 2.2a	82.6 $\pm$ 6.2a
2	37.8 $\pm$ 2.5a	63.2 $\pm$ 3.1b	10.5 $\pm$ 1.5a	10.8 $\pm$ 0.8a	72.0 $\pm$ 6.3a	72.5 $\pm$ 12.1a
3	40.2 $\pm$ 1.6a	52.0 $\pm$ 4.0a	10.8 $\pm$ 2.8a	11.0 $\pm$ 1.2a	78.8 $\pm$ 10.5a	81.8 $\pm$ 1.5a
4	58.3 $\pm$ 5.2a	61.4 $\pm$ 8.2a	13.8 $\pm$ 1.1a	11.2 $\pm$ 2.1b	82.8 $\pm$ 4.1a	78.6 $\pm$ 8.4a
5	62.4 $\pm$ 3.2a	60.5 $\pm$ 1.4a	14.2 $\pm$ 1.4a	10.6 $\pm$ 0.8b	78.5 $\pm$ 2.2a	82.6 $\pm$ 3.2a

For each quality parameter, means within a row followed by the same letter do not differ significantly by *t*-test ( $P = 0.05$ ).

Table 4. Mean  $\pm$  SE flight ability, fecundity, and fertility of *B. invadens* reared on wheat-based artificial diet and whole mango fruit

Generation	% flight ability		Fecundity (10 d)		Fertility, % egg hatch	
	Wheat-based artificial diet	Whole mango	Wheat-based artificial diet	Whole mango	Wheat-based artificial diet	Whole mango
P	80.4 $\pm$ 6.5a	82.0 $\pm$ 4.6a	88.4 $\pm$ 26.2b	101.3 $\pm$ 10.4a	34.4 $\pm$ 12.4b	70.2 $\pm$ 1.2a
1	82.2 $\pm$ 5.2a	80.4 $\pm$ 4.3a	76.5 $\pm$ 15.4b	116.2 $\pm$ 7.8a	60.5 $\pm$ 7.7b	84.4 $\pm$ 10.6a
2	84.5 $\pm$ 1.1a	78.8 $\pm$ 14.2a	96.8 $\pm$ 8.2b	124.2 $\pm$ 20.6a	60.0 $\pm$ 2.0b	78.8 $\pm$ 5.5a
3	75.4 $\pm$ 2.4a	72.2 $\pm$ 7.0a	122.2 $\pm$ 34.3a	108.5 $\pm$ 33.2b	85.4 $\pm$ 1.4a	80.4 $\pm$ 2.2a
4	80.1 $\pm$ 5.1a	75.8 $\pm$ 4.6a	265.0 $\pm$ 22.2a	117.4 $\pm$ 13.4b	82.4 $\pm$ 1.6a	80.5 $\pm$ 4.2a
5	80.4 $\pm$ 0.8a	78.3 $\pm$ 5.2a	82.1 $\pm$ 24.5a	114.8 $\pm$ 24.5b	88.5 $\pm$ 2.0a	82.2 $\pm$ 1.5a

For each quality parameter, means within a row followed by the same letter do not differ significantly by *t*-test ( $P = 0.05$ ).

Here, we observed reduced survival of adult *B. invadens* reared on artificial diets for two generations compared with flies reared on whole mango fruit. In *Ceratitis capitata* (Wiedemann), Leppla et al. (1983) reported that adult survival was reduced during the first three generations of colonization on artificial diet when a colony kept on host fruit for the first five generations was switched to artificial diet with a continuous decline in the following four generations, but recovering to normal levels thereafter. Economopoulos (1992) also observed a reduction in adult survival for three consecutive generations after colonization of both sexes of newly colonized wild *C. capitata* with recovery to normal levels occurring in the fourth generation. Although this effect was shorter in our study, our findings are in general agreement with these previous studies. The reduced survival can be largely attributed to the reduced ability of flies to adapt to artificial diet and other rearing conditions, such as crowding.

Life history traits, including larval developmental period, are important criteria in the evaluation of diet and the rearing process for fruit flies, and nutritional content of a diet can considerably affect developmental time, growth, and survival of fruit fly larvae (Krainacker et al. 1987, Vargas et al. 1994). In our study, we observed a decrease in the length of larval developmental time in flies reared on artificial diet by generation  $F_4$  and a corresponding increase in pupal weight at the same generation. Kaspi et al. (2002) demonstrated that the higher the protein level in larval diet, the shorter the developmental time and the larger the size of individuals produced. It is probable that the amount of protein in the artificial diet used in our study was higher than the quantity in the variety of

mango used, which could explain the shorter developmental time and higher pupal weight of *B. invadens*.

Fecundity and fertility also were affected during the adaptation process. It took four generations for the insects maintained on artificial diet to reach or surpass the level of fecundity recorded for flies reared on whole mango fruit. Economopoulos (1992) showed that even after nine generations, fecundity of wild *C. capitata* did not match the levels of laboratory-adapted flies. In our study, the prolonged period of adaptation could be associated with poor mating, which we observed in flies maintained on artificial diet at the earlier stage of colonization. Previous studies with other fruit flies have demonstrated the importance of wheat and wheat-based products in fecundity and eclosion of larvae from eggs (Zumreoglu et al. 1979, Vargas et al. 1994, Chang et al. 2006). By generation  $F_4$ , we observed increased mating activity in the cages (data not shown), suggesting that the increase in mating from generation  $F_4$  and the high level of wheat in the diet may have contributed to high fecundity in *B. invadens*.

In general, it took three to four generations for *B. invadens* to adapt to artificial rearing medium. The rate of adaptation in laboratory rearing of insect depends on both the insect's ability and the quality of the rearing effort. Insects will adapt to artificial rearing more rapidly if insect rearing personnel are able to maximize the survival of each stage. Souza et al. (1988) observed that at least 10 generations were needed for adaptation of *C. capitata* to artificial diet. In the olive fruit fly, *Bactrocera oleae* (Gmelin), about three to four generations were required to adapt (Tsitsipis 1983), whereas in *B. cucurbitae* Coquillett, it took 14 generations to reach a permanent plateau (Kamikado et al. 1987). The period of adaptation for *B. invadens* reared on wheat-based artificial diet is within the range reported for other fruit flies.

Table 5. Effects of two artificial larval diets on pupal recovery, pupal weight, adult emergence, flier, fecundity, and egg hatch of *B. invadens*

Parameter	Wheat	Carrot
Pupal recovery, %	68.8 $\pm$ 3.1a	58.2 $\pm$ 2.6b
Pupal wt, mg	14.1 $\pm$ 1.4a	13.8 $\pm$ 1.2a
Adult emergence, %	82.0 $\pm$ 3.2a	88.4 $\pm$ 2.0a
Flier, %	80.4 $\pm$ 3.6a	82.6 $\pm$ 4.2a
Fecundity, 10 d	312.6 $\pm$ 33.7a	292.6 $\pm$ 41.8a
Egg hatch, %	86.4 $\pm$ 2.3a	82.3 $\pm$ 2.0a

Means within a row followed by the same letter do not differ significantly by *t*-test ( $P = 0.05$ ).

Table 6. Mean  $\pm$  SE weekly production ( $n = 28$ ) of *B. invadens* per tray on carrot and wheat-based artificial diets<sup>a</sup>

Parameter	Wheat	Carrot
Puparia	3,966.8 $\pm$ 287.2a	3,012.1 $\pm$ 605.4b
Pupal recovery, %	66.2 $\pm$ 6.5a	55.3 $\pm$ 7.2b
Pupal wt, mg	13.7 $\pm$ 1.2a	13.7 $\pm$ 2.2a
Adult emergence, %	81.4 $\pm$ 1.2a	84.0 $\pm$ 2.2a

Means within a row followed by the same letter do not differ significantly by *t*-test ( $P = 0.05$ ).

<sup>a</sup> Each tray (3 by 10 by 15 cm) contained 500 g of diet.

The small-scale production experiment indicated high recovery of puparia from both media tested, but percentage of recovery was higher on the wheat-based diet compared with the carrot-based diet. Other quality control parameters were not statistically different between the two diets. The wheat-based diet is currently used worldwide for mass rearing of different species of fruit flies, and data generated for *B. invadens* on wheat compares favorably with reported data for other species of *Bactrocera* (Vargas and Mitchell 1987; Vargas et al. 1993; Chang et al. 2004, 2006). In mass rearing of *Bactrocera latifrons* (Hendel), Vargas and Mitchell (1987) found carrot to be less nutritious than wheat on the basis of female reproductive parameters. In the current study, apart from pupal recovery, all quality control parameters on both diets were uniform and our experiments document, for the first time, a novel mass-rearing procedure for *B. invadens* by egg seeding. At the ICIPE rearing facility, *B. invadens* is currently being maintained on a carrot-based artificial diet and a number of factors favor the use of carrot over wheat. First, carrots are usually available year-round, although prices can be exorbitant during the off-season period. Second, inconsistency in the quality of wheat-based bulking agent associated with chemical pesticide residues have been observed. In Kenya, carrots are not treated with pesticides; thus, are free of residues. Third, ingredients in the wheat-based diet are more expensive than those found in the carrot-based diet.

We have demonstrated that it takes between three to five generations for *B. invadens* to adapt to artificial rearing and that mass rearing on both wheat- and carrot-based diets on a small-scale is feasible. Soon after detection, limited understanding of factors associated with eclosion of larvae from eggs, hindered production by egg seeding. However, we now demonstrated that production can be affected by direct egg seeding on artificial diet, and that, by using this method, healthy cultures are being maintained on the carrot-based diet. Continued efforts will be needed to improve efficiency and production output. Furthermore, improvements in egg collection techniques are essential and are being pursued.

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