Fruit fly liquid larval diet technology transfer and update

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fruit flies, liquid diet, technology transfer

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Abstract

Since October 2006, the US Department of Agriculture–Agricultural Research Service (USDA–ARS) has been implementing a fruit fly liquid larval diet technology transfer, which has proceeded according to the following steps: (1) recruitment of interested groups through request; (2) establishment of the Material Transfer Agreement with agricultural research service; (3) fruit fly liquid larval diet starter kit sent to the requestor for preliminary evaluation; (4) problem-solving through email or onsite demonstration; (5) assessment on feedback from the participants to decide whether to continue the project. Up to date, the project has involved 35 participants from 29 countries and 26 species of fruit flies. Fourteen participants have concluded their evaluation of the process, and 11 of these 14, have deemed it to be successful. One participant has decided to implement the project on a larger scale.

The 14 participants were, Argentina (*Ceratitis capitata* and *Anastrepha fraterculus*), Bangladesh (*Bactrocera cucurbitae, C. capitata,* and *Bactrocera dorsalis*), China (Fujia province) (*B. dorsalis*), Italy (*C. capitata*), Fiji (*Bactrocera passiflorae*), Kenya (*Bactrocera invadens, Ceratitis cosyra*), Mauritius (*Bactrocera zonata* and *B. cucurbitae*), Mexico (*Anastrepha* species), Philippines (*Bactrocera philippinese*), Thailand (*Bactrocera correcta*), Austria (*C. capitata,* Vienna 8 and *A. fraterculus*), Israel (*Dacus ciliatus* and *C. capitata*), South Africa (*C. capitata,* Vienna 8) and Australia (*C. capitata*).

The Stellenbosch medfly mass-rearing facility in South Africa and the CDFA in Hawaii were two mass-scale rearing facilities that allowed us to demonstrate onsite rearing in a larger scale. Demonstrations were performed in CDFA in 2007, and in Stellenbosch, South Africa in 2008; both were found to be successful. The Stellenbosch medfly mass-rearing facility in South Africa decided to adopt the technology and is currently evaluating the quality control of the flies that were reared as larvae on a liquid diet.

Introduction

Fruit flies are one of the greatest impediments to fresh produce exports worldwide. There are some 100 pest species of fruit flies worldwide, the majority of which thrive in the Asia–Pacific Region. Crop losses from 40% to 100% are experienced everywhere from villages to large area farming systems. A significant result of this loss is the lower dietary nutrition levels, reduced incomes for farmers, and heavy use of pesticides in many countries. Crop losses caused by fruit flies have become a major concern, as evidenced by recent UN/FAO data, which indicates that by 2020 there must be a 70% increase in world food production in order to prevent major famine (Kuching 2005; http://www.icmpff.org).

A group of scientists in Hawaii, from the Agricultural Research Service (ARS), Animal and Plant Health Inspection Service (APHIS) of the US Department of Agriculture (USDA), Hawaii Department of Agriculture (HDOA) and the University of Hawaii at Manoa (UH manoa) has implemented an Area Wide

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Pest Management (AWPM) programme to suppress the fruit fly population in Hawaii since 2000. The technologies developed within this programme have been transferred to many parts of the world that share Hawaii's fruit fly problems (Chang et al. 2007).

One of the most important components of the AWPM programme is a well-established rearing technology to mass produce fruit flies, in order to support the sterile insect technique (SIT) programme. USDA–ARS scientists have recently developed a liquid larval rearing diet, using sponge cloth to replace biological bulking agent used in solid diets such as wheat products, to rear medfly, oriental fruit fly and melon fruit fly larvae, and to reduce or eliminate the spent diet management problem (Chang et al. 2004).

The technology transfer components necessary to propagate this programme include, development of diet formulation for each species, identification of tray sizes/types/stacks, quantification of egg density/tray/area/diet (Chang et al. 2006), clarification of environmental conditions for rearing (i.e. temperature/humidity/light), mating competitiveness in field (Chang & Don 2008, unpublished, in Journal review), selection of main protein source (Chang 2008, in press), and quality control/life history of insects reared from this technology. The latter has been evaluated based on the following parameters: pupal recovery, larval developmental period, pupal weight, adult emergence, adult flier, mating, egg production, and egg hatch (FAO/IAEA/USDA, 2003).

Fruit flies reared on a liquid diet have been identified, evaluated and proved to have competitive mating performance in comparison with the conventional control diet (Chang et al. 2006, 2007). The components that exhibit the optimal performance of fruit flies raised on a liquid diet are expected to be recommended before this technology could be physically transferred or implemented to mass rearing facilities worldwide. This study reports on the recommended components for optimal performance and production for SIT as well as procedures for implementation of this technology under mass rearing and the current status of technology transfer worldwide.

Materials and Methods

The fruit fly liquid larval diet, developed by Chang et al. (2004, 2006, 2007), for the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann), the oriental fruit fly, *Bactrocera dorsalis* (Hendel), and the melon fruit fly, *Bactrocera cucurbitae* (Coquillett), is ready to be transferred to interested groups. Much of the liquid diet procedures have been published (Chang et al. 2004, 2006, 2007). We are not going to have any description here. However, before the technology transfer took place, evaluation on quality control (e.g. life history performance) using the recommended technology transfer components for upscale rearing (delivery system, diet formulation, tray types/sizes/stack, types of tray holder, rearing environmental conditions, field mating competitiveness, types of yeast, etc.) was carried out based on the following parameters: pupal recovery (%), larval developmental duration (d), pupal weight (mg), adult emergence (%), adult fliers (%), egg production (no. eggs/female/d), egg hatch (%), and mating (5). The most effective components were identified and recommended to interested parties. Evaluation was made on all these parameters was compared with those obtained from rearing in traditional mill feed control diet.

Procedures for fruit fly liquid diet technology transfer

According to the ARS Office of Technology Transfers, there are some regulations necessary to follow for an appropriate technology transfer. The procedures for technology transfer are listed as follows.

Recruitment of interested groups through request

Publications and presentations were made at various meetings to announce and deliver information about this new technology. The interested groups then communicated with the inventor to establish a Material Transfer Agreement.

Establishment of Material Transfer Agreement

A three-page agreement, as shown in fig. 1, was signed by the inventor, approved by inventor's supervisors, and sent to the office of technology transfer (OTT) for approval. Upon approval, the OTT sent the agreement to the interested groups for their acceptance signature. The OTT informed the inventor to send the starter kit to the requester as soon as the acceptance signature was received. The fruit fly liquid larval diet starter kit was sent to each interested party at ARS' expense (fig. 2). These interested groups, or requesters, officially became participants in the study.

Fruit fly liquid larval diet starter kit

The purpose of developing the starter kit was to allow the interested groups to have a hands-on experience to find out whether the diet was workable for the species of fruit fly they were working with.

MATERIAL TRANSFER AGREEMENT

PARTIES:

ARS: Chiou Ling Chang
USDA, ARS, Honolulu
Tropical Pests Research Unit
2727 Woodlawn Drive
Honolulu, Hawaii 96822

Recipient: Dr. Miguel C. Zapater
Profesor Adjunto
Universidad de Buenos aires
Facultad de agronomia
Catedra de genetica
Av. San Martin 4453
1417 Buenos Aires
Tel: (011) 4784-4499/(011) 4524-8067
Fax: (011) 4784-4499
E-Mail: mmzapater@arnet.com.ar

PURPOSE:

To provide Recipient with **Fruit fly liquid larval diet starter kit** and associated know how, hereinafter collectively referred to as the Material. The Material is released to Recipient under the following conditions:

1.The Material and associated know-how shall only be used for **testing and using the liquid larval diet technology for mass rearing of house fly, *Musco domestica.***

2. Recipient shall not transfer the Material, in whole or in part, to a third party without express written consent of ARS. Any third party requesting a sample shall be

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13. This Material Transfer Agreement shall be construed in accordance with United States of America Federal Law as Interpreted by the Federal Courts in the District of Columbia. This Material Transfer Agreement shall become effective upon date of final signature and shall continue in effect for a period of one year.

ACCEPTED FOR THE AGRICULTURAL RESEARCH SERVICE

chiou ling Chang 10/31/06
Signature (ARS Scientist) Date

Chiou Ling Chang Research Biologist
Typed Name Title

Eric Jang 11/2/06
Signature (ARS Research Leader) Date

Eric B. Jang Research Leader
Typed Name Title

ACCEPTED FOR RECIPIENT:

Signature Date

Miguel Zapater Professor
Typed Name Title

APPROVED:

David Nicolson 12/1/06
Signature Date
David Nicolson Technology Transfer Coordinator
Typed Name Title
MTA/OUT: August 2004 3

3. The Material shall remain the property of ARS and shall not be used for commercial or profit making purposes without an appropriate license or other permission from ARS.
4. Recipient shall keep ARS informed of the results obtained through your use of the Material and shall provide ARS with any manuscript that describes the work with the Material prior to submission for publication and acknowledge ARS's contribution to the work reported.
5. Recipient shall not in any way state or imply that this Agreement or the results of this Agreement is an endorsement of its organizational units, employees, products, or services.
6. Recipient shall comply with all laws, regulations, and/or guidelines applying to the use of the Material and to assume sole responsibility for any claims or liabilities which may arise as a result of the recipient's use of the Material. Both parties acknowledge and agree to comply with all applicable laws and regulations of the Animal Plant Health and Inspection Service, the Center for Disease Control, and/or Export Control Administration pertaining to possession or transference of technical information, biological materials, pathogens, toxins, genetic elements, genetically engineered microorganisms, vaccines, and the like.
7. ARS GIVES NO WARRANTIES OR GUARANTEES, EXPRESSED OR IMPLIED, FOR THE MATERIAL, INCLUDING MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE.
8. Upon completion of the activities performed using the Material, the Material shall be returned, destroyed or otherwise disposed of as instructed by ARS.
9. Recipient shall meet with U.S. Department of Agriculture representatives to determine inventorship if an invention should arise from work with the Material.
10. Recipient shall not disclose Material marked "Confidential" or "Proprietary" to any third party without written permission from ARS.
11. Material shall be excluded from the confidentiality requirements of this Agreement if: (1) Recipient had possession of the Material prior to disclosure; (2) the Material is generally available to the public at the time of disclosure; (3) the information becomes generally available to the public through no fault of Recipient after disclosure; or (4) after disclosure, Recipient receives the Material from a third party having the right to the Material and who does not impose a confidentiality obligation upon Recipient.
12. If the parties hereto decide, at some future date, to engage in a cooperative research project or program using the Material, a formal Cooperative Research and Development Agreement, or other research Agreement, must be negotiated and entered into between the parties. Such an Agreement shall supersede this Material Transfer Agreement.

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Fig. 1 Sample of Material Transfer Agreement.

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Fig. 2 Fruit fly liquid larval diet starter kit.

The starter kit (fig. 2) is composed of everything one would need to start a liquid diet evaluation of fruit flies. It includes four sets of weighed diet ingredients (sugar, yeast, nipagen, sodium benzoate, streptomycin, citric acid and wheat germ oil; see table 1 for the formulation), step-by-step instructions, diet containers and sponge cloth. The recipe in the starter kit is based on the formulation developed by Chang et al. (2006) is same for three species of fruit fly in Hawaii. The participants were expected to evaluate the applicability of this starter kit to their working species of insect at their earliest convenience.

Problem solving

Participants were invited to communicate with the inventor through phone calls or emails, in case problems were encountered during the process of evaluating the liquid diet starter kit. The inventor analysed the problems with the participants and helped them to fix the problems until the evaluations' conclusion. Onsite demonstration was also recommended, where funding is available.

Table 1 Liquid diet formulation for three species of fruit flies

Ingredients	Gram (g)	Percentage (%)
Yeast (LBI2240 and FNI LS65 3 : 1)	204.0	14.61
Sugar	121.8	8.72
Nipagen	2.0	0.14
Sodium benzoate	2.0	0.14
Streptomycin	1.5	0.11
Citric acid (adjust to pH3.5)	65.0	4.66
Water (ml)	1000.0	71.62
Wheat germ oil (ml)	10.0	1% of water volume

Assessment on feedback from liquid diet participants

The inventor sent emails to participants for feedback and updates every 6 months to find out whether the liquid diet was workable and what each participant group planned for their use of the technology in

their facility. Those facilities that found the technology to be highly workable and decided to implement it as a result, were invited to request further onsite demonstration or consultation with the inventor as necessary.

Results and Discussion

Quality control and recommended technology transfer components for up-scaling of mass rearing

Diet formulation

A formulation list (shown in table 1) was recommended for three fruit fly species: *C. capitata, B. dorsalis,* and *B. cucurbitae*. The starter kit diet formulation was based on the recipe listed in table 1 for all interested participants. The results from each participant were not expected to be optimal, given the minor or major discrepancies among different genus or species. Adjustments are made according to these discrepancies in order to achieve maximum performance. Therefore, the evaluation was claimed to be a success if fruit fly larvae developed/grew on the liquid diet. That is, these insects can be reared in a liquid diet. Those insects developed but could not complete development and/or failed to pupae, it was categorized in nutrient issue. The adjustments on formulation were made such as adding wheat germ oil as an enhancer (Chang and Vargas 2007).

Tray sizes/types/stacks

The measurements of ideal trays are recommended between 50 cm and 60 cm in length, 30 cm and 36 cm in width and no deeper than 2.5 cm in depth. The aspect of depth is especially important for the Mediterranean fruit fly. If the tray is deeper than 2.5 cm, larvae will have a hard time jumping out of the tray to perform pupation.

The material utilized for trays depends on each facility's budget and terms of use. They can be made by anything from Rubbermaid (plastic) to fibreglass. Fibreglass is highly recommended because of its durability (fig. 3). The trays stack up when up-scaled rearing is applied. The pupal recovery from 20-tray stacks was reduced by 50% from one-tray stack setting (fig. 4).

Tray holder

Up to date, the best tray holder is that as shown in fig. 5. The space between two trays, however, should be adjusted based on the insect species being worked on.

Fig. 3 Recommended tray types, size (in cm), egg volume, diet volume and fly. Species for liquid larval diet large scale rearing.

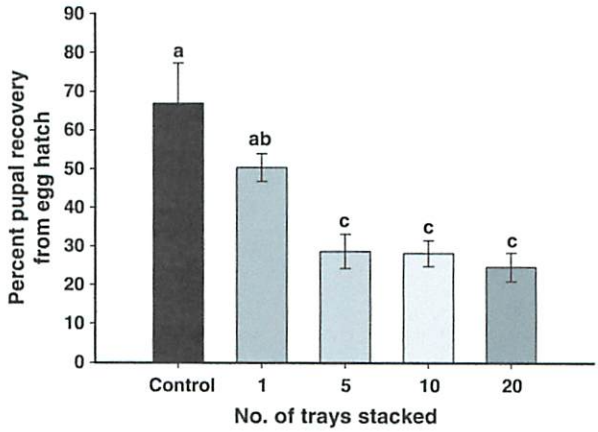

Fig. 4 Comparison of per cent pupal recoveries from 1, 5, 10 and 20 stacked trays to the conventional control.

Environmental conditions

The best rearing conditions are between temperatures of 25°C and 30°C (fig. 6), 80% humidity, and 12D : 12L (fig. 7) (Chang et al., unpublished).

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Fig. 5 Three types of recommended tray holders.

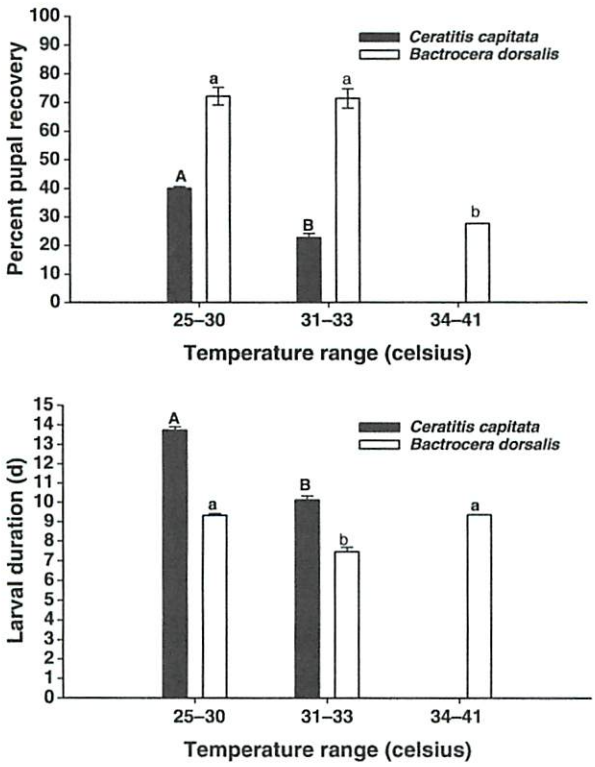

Fig. 6 Effect of temperature on pupal recovery (%) and larval duration (d), of *Ceratitis capitata* and *Bactrocera dorsalis*. No data were presented in 34–41°C, because they were not able to survive.

Egg density/tray/area/diet

Ideal egg seeding density is 2.5 ml/tray, or 30 eggs/gram of diet, or 23 eggs/cm^3.

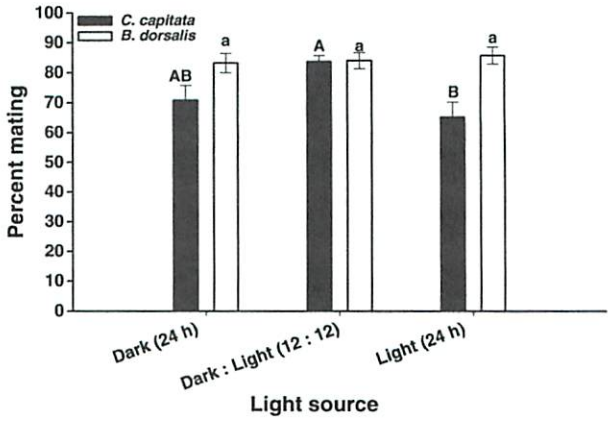

Fig. 7 Effect of light on per cent mating of *Ceratitis capitata* and *Bactrocera dorsalis*.

Mating competitiveness

Adults reared from larvae in a liquid diet have been evaluated in both laboratory indoor acrylic cages and field cages, and have proved that they can mate as well as those reared on a conventional diet (table 2) (Chang and McInnis, unpublished).

Yeast selection

Brewer's yeast LBI2240 and FNILS65 (3 : 1, abbreviated as 224065W) from Lallemand bio-ingredients, Canada, or Torula yeast from Borregaard yeast specialties, Riedholz, Switzerland, or local yeast that are suitable for mass production use are highly recommended (table 3).

Table 2 Results of mating competitiveness test in both indoor acrylic cages and field cages

Target strains	Competing strains	Mating proportions RSI	Target	Competing	Significance (P-value)
Indoor acrylic cage tests					
NIRR-BD-MF	IRR-BD-MF	0.62	0.38a	0.62a	0.1322
NIRR-BD-MF	IRR-BD-LD	0.70	0.30b	0.70a	0.0162*
IRR-BD-MF	IRR-BD-LD	0.70	0.62a	0.70a	0.5216
NIRR-BD-MF	IRR DTWP-MF	0.35	0.65a	0.35b	0.0002*
NIRR-BD-MF	IRR DTWP-LD	0.43	0.56a	0.43b	<0.0001*
IRR DTWP-MF	IRR DTWP-LD	0.43	0.35a	0.43	0.0563
NIRR Wildish	IRR DTWP-MF	0.38	0.62a	0.38b	0.0180*
NIRR Wildish	IRR DTWP-LD	0.38	0.62a	0.38b	0.0353*
IRR DTWP-MF	IRR DTWP-LD	0.38	0.38a	0.38a	0.9419
Field cage tests					
IRR DTWP-MF	IRR DTWP-LD	40	0.38a	0.40a	0.9188

NIRR, non-irradiated; BD, *Bactrocera dorsalis*; MF, mill feed diet; LD, liquid diet;
IRR, irradiated; DTWP, pupal color sexing strain of BD; RSI, Relative Sterility Index was used as an indicator of mating capability.

Table 3 Two recommended yeasts for liquid larval diet and the comparison of the life history performance of adult oriental fruit flies that reared as larvae in the liquid diet and control mill feed diet

Parameters*	224065W	65GW	Mill feed†	Proc ANOVA (d.f. = 2,8)
Pupal recovery (%)	70.15a	63.31a	81.49a	F = 2.39; P = 0.1721
Pupal recovery/control (%)	86.08a	77.69a	100.00a	F = 2.39; P = 0.1721
Larval duration (d)	9.95a	10.67a	8.43b	F = 17.69; P = 0.0030*
Pupal weight (mg)	11.20a	9.52b	10.23ab	F = 5.76; P < 0.0402
Adult emergence (%)	97.75ab	96.75b	98.83a	F = 4.69; P = 0.0594
Adult fliers (%)	98.80a	98.25a	90.90a	F = 0.87; P = 0.4673
Mating (%)	72.45a	81.05a	58.36abc	F = 1.75; P = 0.2518
Eggs/female/day	21.20ab	14.13ab	22.63ab	F = 1.23; P = 0.3565
Egg hatch (%)	72.89a	75.11a	73.24a	F = 0.11; P = 0.8998

†control diet.

Quality control

Life history of medfly from adults reared as larvae on a liquid diet is listed in table 3.

Status of worldwide fruit fly liquid larval diet technology transfer

Countries that participated in evaluating the starter kit include Argentina, Austria, Australia (southern and western), Bangladesh, Belgium, Brazil, Canada, China, Fiji, France, Greece, Guatemala, Israel, Italy, Kenya, Korea, Mauritius, Mexico, the Netherlands, Okinawa, the Philippines, Samoa, Slovakia, South Africa, Taiwan, Thailand, Tunisia, the UK, the USA (Michigan) and Vietnam. Insects involved in starter kit evaluation were *Anastrepha fraterculus, A. serpentine, A. ludens, A. oblique, A. striata, B. dorsalis, B. cucurbitae, Bactrocera oleae, B. invadens, B. philippinensis, B. correcta, B. pyrifoliae, B. passiflorae, B. xanthodes, B. zonata, B. latifrons, B. tryoni, C. capitata, Ceratitis cosyra, Dacus bivittatus, D. ciliatus, Musca domestica, Ophyra aenescens*. To date, 14 countries have concluded their evaluation of the starter kit, and 10 of these 14 conclusive evaluations were claimed to be workable. The countries that found the liquid diet to be workable include Argentina, Bangladesh, China, Fiji, Italy, Kenya, Mauritius, the Philippines, Thailand and South Africa. However, only nine of these 10 countries have demonstrated successful evaluations: Argentina, Bangladesh, Fiji, Italy, Kenya, Mauritius, the Philippines, Thailand and South Africa. These nine countries, therefore, are all that remain to continue the work on this technology.

The medfly mass-rearing facility in Stellenbosch, South Africa, has decided to adopt this technology in their programme, and continue to test the quality of liquid-diet reared fruit flies for the goal of future implementation. The 10 successful evaluations proved that larvae of *A. fraterculus, B. correcta. B. dorsalis, B. cucurbitae, B. passiflorae, B. philippinensis, B. invadens, B. zonata,* and *C. capitata* are able to develop successfully when reared on a liquid diet. The olive fruit fly, *B. oleae,* on the other hand, is not able to grow on the liquid diet – as has been further evaluated and confirmed by Drs C. L. Chang and Carlos Caceres in Seibersdorf, Vienna, Austria, due to the specific character of their larval stage. The cucumber fly, *D. ciliatus,* is another species that was found to be not able to survive on a liquid diet, according to feedback by Dr David Nestel. *Ceratitis capitata,* from Slovakia, was another case, where the larva was not able to develop on a liquid diet, because the third instars stay in the bottom of tray and cannot jump out. My experience told me that the last two unsuccessful cases were because the delivery system did not set up correctly. Further evaluations are undergone.

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Fruit fly liquid diet technology transfer in Stellenbosch mass-rearing facility

On 29 January 2008, Dr Jorge Hendrichs of IAEA considered Stellenbosch medfly mass rearing facility in South Africa to be one of the most ideal liquid larval rearing diet demonstration sites, and contracted Dr Chiou Ling Chang, a scientist of USDA–ARS, to begin the transfer of the liquid diet technology to other sites. The medfly mass rearing facility in Stellenbosch is one of the smallest facilities in the world successfully producing the sterile medfly, *C. capitata*, for an active SIT pilot project. The facility is part of a project assessing the feasibility of SIT for controlling the Western Cape Province medfly. The facility has an approximate capacity of 13 million sterile males a week.

The major problem with the Stellenbosch medfly rearing facility is the consistency of the bulking agent (bran). The quality of bran, which is received from two different companies in Stellenbosch depending on the season, is very inconsistent. The bran is frequently contaminated by pesticides, and has caused crashes of multiple colonies. Because of the metabolic heat produced by 3rd instar larvae when bran is used as a bulking agent, the trays have to be moved to different rooms with various environmental rearing conditions (e.g. humidity and temperature). Therefore, at least three humidity/temperature-controlled rearing spaces are required when bran is used, resulting in an even more limited amount of space for breeding. Furthermore, the management of the spent bran diet is very labour intensive. Each tray has to be scraped of diet completely before it is cleaned, which results in 30 h of extra labour every week. The bio-security of spent diet is another serious concern. With the management of spent diet in Stellenbosch facility, they could end up releasing more non-sterile flies into the environment. Additionally, the facility has been spending $80 000/year to purchase bran for producing 13 million pupae per week (personal communication with Stellenbosch medfly mass rearing facility manager, Mr Luciano Arnald in January 2008).

With all these disadvantages faced by the Stellenbosch facility, it would be greatly beneficial to find an alternative way of rearing flies that eliminates the use of bran as a bulking agent, or replacing bran with another biological or inert bulking agent – such as sponge cloth.

The liquid larval diet technology invented by USDA–ARS could be totally consumed by flies, if the proportion of diet volume to egg density were correct. That is, with the liquid diet, there is less amount of spent diet. Furthermore, spent liquid diet is totally water soluble, and can be simply rinsed off with a water gun. Liquid-diet reared fruit fly larvae can develop in a humidity/temperature-controlled room, without the necessity of moving trays around, which allows more space to be used for rearing. Additionally, flies can be reared in smaller and shallower trays than those currently used, and each tray can still generate an equal amount of pupal production. The weight of each larvae tray can therefore be reduced from 12 lbs to 2.2 lbs. Therefore, the liquid diet technology not only solves the contamination problem posed by using bran as a bulking agent, but also saves $80 000/year for bran purchase, saves at least 30 h/week of time and labour, and increases the amount of space available for spent diet management.

The most convenient inert bulking agent, to our knowledge so far, is the sponge cloth from Kalle, Gmbh. The sponge cloth is mainly composed of natural cellulose and fibre. It is light weight with high water absorbance, and is reusable, recyclable, biodegradable and also environment friendly.

Even with all these benefits from using the liquid diet, authorities from the SIT Africa and rearing facility were sceptical about implementing the liquid diet technology before the inventor demonstrated the technology's effectiveness in the Stellenbosch facility. The authorities were impressed with the diet's performance, and have therefore decided to adopt and pursue this technology in their expanded larval rearing area. However, in an effort to ensure the quality of adult flies whose larvae was reared on a liquid diet, the authorities have decided to run some quality control tests before putting the technology into practice.

The Inventor was accompanied by the advisor and the rearing manager of SIT Africa to meet with Dr Joohan van Zyl, Research & Technology Manager of ARC infruitec-Nietvoorbij, to discuss the potential of implementing the liquid diet technology in the near future. There were some questions and concerns brought up in the meeting. Following are our responses to all these questions:

(1) Can the current large trays be used for rearing flies on a liquid diet?

It is not recommended to continue to use the currently used large trays for liquid diet rearing, because they are difficult to handle and take up more space. In addition, many of the old trays need to be replaced anyway, because most are stained and cracked. Therefore, because the smaller trays can do the same

job as the larger trays, there is no reason to use the bigger trays just because we already have them.

(2) Can ARS yeast be replaced with the brewer's yeast manufactured in Stellenbosch (or other local yeast) for liquid diet and still perform as well?

Yes, this can be done. However, the formulation needs to be adjusted for optimal performance. This work has been preliminarily performed by the inventor over the course of the study. The results cannot be carried out until after the experts leave Stellenbosch. Additionally, it would be better to run some serious tests in Stellenbosch to ensure the correct quantity to be used.

(3) Can liquid diet save cost?

Yes, because of the lack of cost for bran. Sponge cloth is cheaper to purchase and is reusable for many times until it degrades. Additionally, the diet saves labour and space.

(4) How is the quality of adult flies that were reared as larvae in a liquid diet?

They are as good as conventionally reared insects (table 3) (Chang et al. 2004, 2006, 2007).

(5) How is the survival rate of adult flies?

Survival, and other parameters, is as good as the control (table 3) (Chang et al. 2004, 2006, 2007).

(6) What are the benefits of the liquid diet?

In addition to the ease of cleaning up spent diet, there is no worry about pesticide contamination on the liquid diet as there is with bran. Furthermore, there is no need to spray water daily throughout the larval developmental period, as long as the relative humidity in the rearing room is maintained at a minimum level of 80–90%. Plus, the trays do not have to be moved to other rooms for temperature adjustments – with the liquid diet, all you need to do is keep the insects at 25°C until pupation.

(7) What are the negative concerns about the liquid diet?

The only negative concern is that the larvae may die if the diet dries out, which may occur if the relative humidity is too low in the rearing room.

(8) Is there any additional equipment needed to convert to this new process?

A large capacity of washers is pertinent for cleaning the sponge cloth, and well-designed tray holders are required to hold the trays appropriately.

(9) Wheat germ oil is a rare and expensive item. Is there an effective substitute?

Yes. Any oil should serve the purpose, as long as it contains saturated and unsaturated fatty acids. However, tests should be performed before using a different oil, to ensure the quality of flies is maintained.

Problems encountered during the mission and the solutions

(1) There were not enough trays, both in types and overall, available for the inventor to prepare for liquid diet. In addition, some trays were sent from the inventor's lab before the inventor's arrival, and we went to local plastic product market and found some potential trays of the appropriate size and type, based on the inventor's model. However, the store did not carry enough in stock for us to purchase all that we needed. Therefore, we had to demonstrate the effectiveness of the liquid diet using different sizes, types and materials of trays. We seeded the same amount of eggs in all trays with different amount of diet. This way will be able to show how important the diet volume for larval development in a liquid diet.

(2) There were not pertinent materials and equipment available to perform the demonstration. It was difficult to run some tests in the rearing facility because there is not an adequate supply for the inventor to perform the test in the designed room. However, with the help from staff, we managed to collect the necessary material and equipment we needed from all over the facility.

(3) The relative humidity in the designated room was not high enough for the inventor to perform the mission. Therefore, some liquid diet dried out on the first day after diet preparation was performed. A second preparation had to be made by adding water to the tray to keep the diet moist.

(4) There were not enough bubbled eggs available for inventor to perform egg seeding on the prepared liquid diet for 3 days. Therefore, the diet sat for 3 days without eggs. As a result, we practice to ensure there are eggs available before we set up the diet.

Conclusion

The liquid diet technology transfer was successfully initiated worldwide through starter kit scaled rearing and thanks to the Stellenbosch medfly mass-rearing facility's up-scaled rearing. Stellenbosch proved that the technology is promising and workable, although there may be some changes or revisions required if further implementation is going to take place. With this technology on-hand, it will not only save a lot of expense in the rearing of fruit flies and other insects, but may also be used for further advancing nutritional or pesticidal research.

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