A fully defined artificial diet for Myzus persicae – the detailed technical manual

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A fully defined artificial diet for *Myzus persicae* – the detailed technical manual

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Abstract

We have been able to maintain a continuous culture of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) on a fully defined artificial diet at Reading, UK, for over 30 years, without any return to plants. Following multiple requests from aphid researchers, here we provide the details of our culturing method. Detailed instructions are given for rearing *M. persicae* on the diet. An improved recipe is included as well as the construction and changing of diet sachets.

Introduction

Aphids (Hemiptera: Aphididae) are a major group of sap-sucking insects of which some species can cause severe harm to cultivated plants and economic loss in agriculture (Blackman & Eastop, 2000). Early in the 20th century, Hamilton (1930) discovered that aphids would feed on liquids through a layer of onion skin. Parafilm, a 50:50 blend of waxes and polyolefins, provides a more convenient penetrable and self-sealing membrane. Although already patented by 1934, it was first used with aphids in the 1960s by Dadd & Mittler (1966) in California, USA, when they published a recipe for an artificial diet suitable for rearing *Myzus persicae* (Sulzer).

The technique has since been modified in many ways, although only slight changes have been made to the original 1966 Dadd & Mittler recipe. The success of these modifications is reflected by our ability to maintain a continuous culture of *M. persicae* on the diet at Reading for over 30 years (Figure 1). Following multiple requests from aphid researchers, here we provide the details of our culturing method.

Materials and methods

The diet

Analar sucrose (15 g) is dissolved in 100 ml ultrapure water. The diet ingredients are then added and completely dissolved one at a time in the order given in Table 1. It is helpful to number the jars of ingredients on the lid in the order in which they are to be dissolved. We use a beaker on a magnetic stirrer and take a whole day to make the diet, returning to the beaker at intervals while doing other work. Small, plastic tubes (25–30 ml, Figure 2A) are filled with the diet and deep frozen until needed. Such small volumes allow for short defrosting times when making the diet sachets. When frozen, the diet remains in good condition for 6 months or even longer before deterioration is signalled by a change to a darker colour.

Diet sachets

The diet is enclosed in sachets made from two layers of stretched Parafilm 'M'. To ensure sterile conditions while making the sachets, we conduct the work in a laminar flow cabinet with built-in UV lighting and a front blackout curtain to prevent damage to eyes. However, more cheaply though less conveniently, a sterile glove box with a desk lamp fitted with a UV bulb could be used.

The required number of frozen tubes of diet are thawed at room temperature for approximately 1 h. Each 25 ml of diet will make about 40 sachets. Cut a 5-cm width of Parafilm 'M' across into at least twice as many 3- to 4-cm-wide strips as the number of sachets to be made, although it is worth cutting 5% extra pieces, as some may tear later when being stretched.

A piece of Parafilm is stretched in both directions with a steady, even pull and placed lightly on a Perspex cylinder (25 mm long and 25 mm internal diameter) cut from clear Perspex tubing. A golf ball is placed on the membrane

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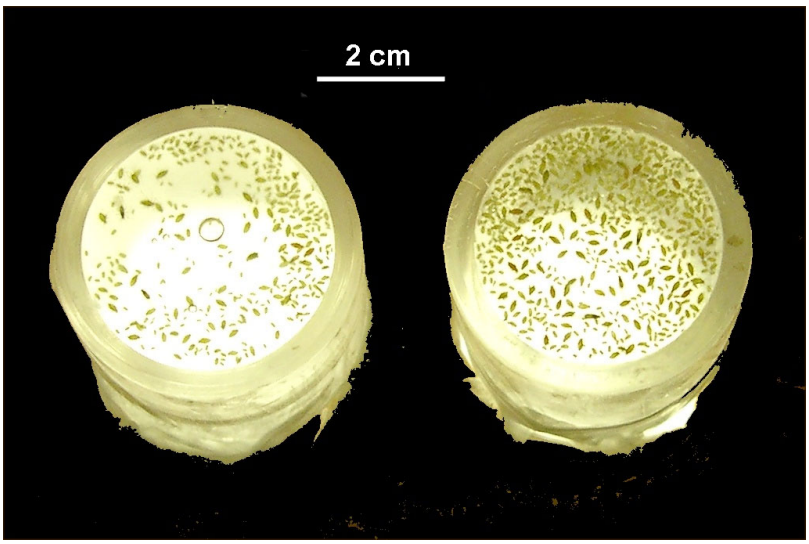

Figure 1 Cultures of *Myzus persicae* on the artificial diet.

to make a depression into the cylinder, and the loose Parafilm flaps are firmed around the sides of the cylinder by pressing gently with the golf ball in place. The golf ball is then removed and used on the next cylinder. We stretch the Parafilm although aphids will actually readily feed through unstretched Parafilm. However, this gets rather expensive.

The prepared cylinders are placed on a tray, and the remaining unstretched pieces of Parafilm to be used as the top membranes of the sachets are laid out on another tray. Both trays are put into the laminar flow cabinet, and the fan and UV light (to sterilise the exposed Parafilm) are switched on with the blackout curtain at the front of the laminar flow cabinet pulled down. The UV light should not be left on for longer than 35 min to prevent deterioration of the Parafilm. At the end of the sterilisation period, the UV light is switched off and the ordinary lights are switched on with the fan left running.

We have recently changed from re-usable metal filter units to disposable millipore (0.22 μm pore size) bacterial filter units (Figure 2B) for dispensing diet from a syringe (Figure 2C). Shake the tube of thawed diet well before filling the syringe. A sterile disposable 50-ml plastic test tube is then removed from its packet, and diet from the syringe is dispensed into it through the disposable filter. The dimensions are given for the syringes, tubes, etc. we have used, but they can be varied for convenience.

If using an automatic pipette (e.g., a Gilson) fitted with a disposable sterile tip, aliquots of 0.6 ml are dispensed onto the Parafilm lower membranes already on the Perspex cylinders (Figure 3A). Alternatively, guessed aliquots can be dispensed directly from the syringe through a disposable sterilised hypodermic needle (Figure 2E) fitted to the bacterial filter. Diet can be dispensed onto several cylinders at a time before adding the covering membranes if there is ample space to do so in the laminar flow unit without touching other cylinders.

The diet on each cylinder is covered with Parafilm by stretching a piece simultaneously in both directions and inverting it over the lower membrane. The edges of the Parafilm are gently firmed around the cylinder at the top, leaving the rest hanging free. When stretching the covers great care must be taken not to touch the centre of the cover (the technique is illustrated in Figure 3B); the cover is then turned over so that only the sterilised surface touches the diet. Finally, the made-up diet cylinders together with any unused tubes of diet are put into the freezer.

Before we switched to disposable bacterial filters, we used a re-useable metal filter unit (a Swinney holder for 13-mm filters) with a bacterial filter (13 mm diameter Metricel membrane filters of 45 μm pore size), and dispensed the diet through a metal hypodermic needle. The assembled filter unit with needle was wrapped in aluminium foil, and sterilised in a pressure cooker for 15 min at 103.4 kPa (15 psi) with a piece of autoclave tape attached. This changes colour (usually from beige to black) to verify the adequacy of the sterilisation. The foil-wrapped holder was then transferred to the laminar flow cabinet.

After use, the re-usable needles and the opened filter holder (with filter removed) were washed thoroughly and rinsed several times with distilled water. The metal grid from the filter unit was cleaned in an ultrasonic bath of distilled water for 7–8 min. Finally, the re-assembled filter holder with grid (without a filter) was syringed through with distilled water at least 3×.

Table 1 The diet recipe

Ingredient	mg per 100 ml water
Sucrose (analar)	15 000.0
di-Potassium hydrogen orthophosphate	750.0
Magnesium sulphate	123.0
Tyrosine[1]	40.0
L-Asparagine hydrate	550.0
L-Aspartic acid	140.0
L-Tryptophan	80.0
L-Alanine dextro-rotary	100.0
L-Arginine monohydrochloride	270.0
L-Cysteine hydrochloride, hydrate	40.0
L-Glutamic acid	140.0
L-Glutamine	150.0
Glycine	80.0
L-Histidine (free base)	80.0
L-Isoleucine (allo free)	80.0
L-Leucine	80.0
L-Lysine (monohydrochloride)	120.0
L-Methionine	40.0
L-Phenylalanine	40.0
L-Proline	80.0
L-Serine	80.0
L-Threonine	140.0
L-Valine	80.0
L-Ascorbic acid (vit. C)	100.0
Aneurine hydrochloride (vit. B)	2.5
Riboflavin	0.5
Nicotinic acid	10.0
Folic acid	0.5
(+)-Pantothenic acid (calcium salt)	5.0
Inositol (meso) inactive	50.0
Choline chloride	50.0
EDTA Fe (III)-Na chelate pure[2]	1.5
EDTA Zn-Na_2 chelate pure[2]	0.8
EDTA Mn-Na_2 chelate pure[2]	0.8
EDTA Cu-Na_2 chelate pure[2]	0.4
Pyridoxine hydrochloride (vit. B_6)	2.5
D-Biotin (crystalline)	0.1
Sodium acetate	320.0

[1]Tyrosine: at this point one may have a precipitate. This does not seem to cause a problem, and it usually disappears as the other ingredients are added.

[2]Some of these pure chelated compounds are becoming hard or even impossible to source. The only alternative is to use the less pure EDTA microelements sold for horticultural use; this may not actually be a bad thing.

Changing the diet cylinders

Aphid cultures on diet are best kept at 18–20 °C at L16:D8 and need to be changed every 2–3 days. Two days is preferable, but the working week will usually mean the sachets are changed on Monday, Wednesday, and Friday. The required number of new cylinders with diet from the freezer are thawed at room temperature for about 30 min. The loose flaps of membrane on a new cylinder are pulled out gently from the side. Cylinders with aphids will have an old cylinder attached, which needs to be removed by pulling the two connected cylinders apart. This old bottom tube is discarded. The Parafilm on it should be removed at this time; the longer it is left the more difficult it becomes to remove prior to washing the cylinders.

The new cylinder is abutted to the tube with the aphids and joined to it with the looser Parafilm at the sides of both cylinders. The old sachet is pierced with a hypodermic needle and the joined tubes inverted so that the old diet drains out onto some paper towel. The aphids quickly will climb to the new diet sachet leaving cast skins and dead aphids on the Parafilm of the now empty old sachet (Figure 4).

Results and discussion

The diet and techniques described above maintained a genotype of *M. persicae* without any return to plants for 32 years. Throughout this time, the aphid increased far more slowly on the diet than on plants (Douglas & van Emden, 2017). Population doubling time on diet of around 7 days contrasted with only 3 days on Brussels sprout plants. The main contributors to this difference were a long pre-reproductive period of 3–4 days after the moult to adult only on diet, and a reduction of mean total fecundity from 47 on plants to only 16.5 on diet. After the original genotype died out, an important modification to the diet has been the addition of sodium acetate. This resulted in the weight of adult aphids and the development time to reproduction matching those of plant-reared aphids. The beneficial effect of adding sodium acetate was an accidental discovery when working on the production of alarm pheromone by diet-reared aphids (van Emden et al., 2014b). The mechanism of this effect is unknown, although it is known that acetate is involved in lipid synthesis in insects (Robbins et al., 1960).

Our diet-reared culture of *M. persicae* died out very suddenly in 2008, and remarkably within a few days so did a daughter colony of the same genotype transferred to Imperial College a few years earlier (J Hardie, pers. comm.). Additionally, a genotype of *M. persicae* reared on detached leaves for insecticide resistance studies at Rothamsted Research similarly died out unexpectedly after about 30 years (SP Foster, pers. comm.). The reason for the ultimate failure of these diet-reared colonies is unknown, but raises the interesting speculation that totally parthenogenetic genotypes may have a limited lifespan due to accumulation of deleterious mutations.

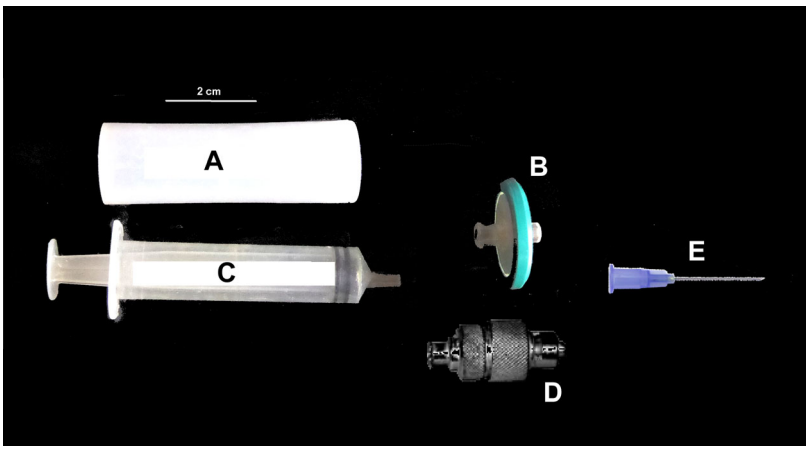

Figure 2 The syringe system: (A) tube of diet taken from the freezer; (B) disposable bacterial filter; (C) syringe body; (D) re-usable bacterial filter assembly; (E) disposable hypodermic needle.

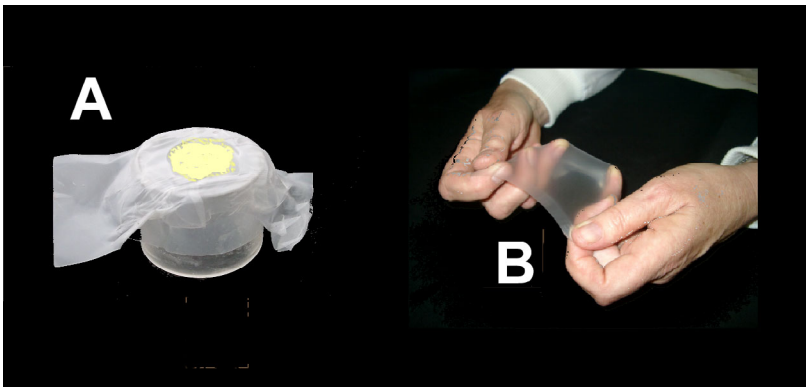

Figure 3 Assembling the diet cylinder: (A) the lower Parafilm membrane in place with an aliquot of diet pipetted onto it; (B) stretching the upper membrane before inverting it onto the cylinder.

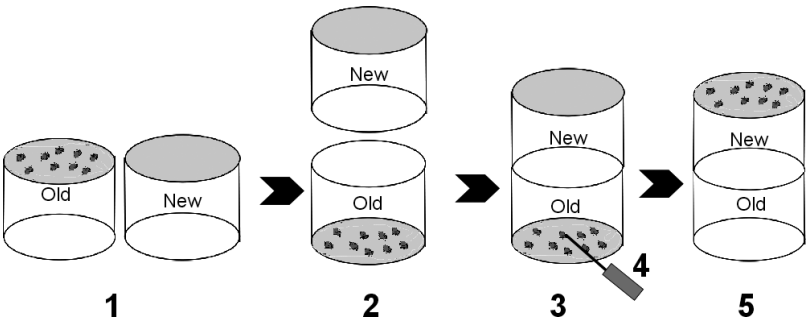

Figure 4 The sequence for changing the diet: (1) old and new cylinders; (2, 3) the new and old cylinders are joined by their open ends and held together with the tails of Parafilm from the sachets; (4) the old sachet is punctured with a needle and the diet drains out; (5) the aphids rapidly migrate from the drained empty sachet to the new diet.

The genotype which we could maintain for so many years was the only one of about 30 different genotypes started on artificial diet in 1976 that survived for any length of time, though other genotypes transferred to the diet in later years have also reared successfully for long periods. Although our success has no doubt followed from improvements we have made to the technique over the years, there is clearly considerable variation between *M. persicae* genotypes in the length of time they will survive. However, most should survive reasonably well unless they carry genes for organophosphate/pyrethroid insecticide resistance, when survival may be only a month or even less (van Emden et al., 2014a).

The quality of the water used is probably very important in relation to success. Ultrapure water may be 'too pure' – we have not been able to keep aphids for periods over a year since we started using ultrapure water instead of the water from a particular still with glass-covered heating elements. Other stills were not as successful, but it is probably worth trying 'purified' water from other pieces of equipment.

The diet is likely to be much less successful with other aphid species, increasingly so if they are host-specific and therefore require phagostimulation by secondary compounds. We have never achieved success by adding such compounds, and we have found that a better approach has been to increase the concentration in the diet of the general phagostimulant sucrose. In this way we have cultured *Aphis fabae* Scopoli, *Brevicoryne brassicae* (L.), *Macrosiphum albifrons* Essig, and *Sitobion avenae* (Fabricius) on diet for short periods (unpublished). We suggest raising the sucrose concentration from 15 to 30 g per 100 ml diet. There is also anecdotal evidence that it may help to have the correct host plant species in the culture room to provide the characteristic volatiles.

Over the 30 years we have used the diet, the uses and limitations of the technique have become apparent. Although we first started using artificial diet to study aphid nutrition, it has not proved useful for this purpose. The recipe was developed largely by trial and error to give the best population growth. The increases in individual amino acids we have tried other than methionine have lowered the performance of the aphid (van Emden, 1996). Basically, any change in the concentration of one compound changes the balance with others, and beneficial interactions between compounds may occur in the diet that do not occur in plants. In addition, although attractive for mass-rearing of aphids and also their parasitoids for biological control, the slow population increase, the expense, and the labour intensiveness probably rule the method out for this purpose. Parasitoids do rear on diet-reared aphids (van Emden & Kifle, 2002), but survival after the first generation is poor (Vamvatsikos, 2006).

The technique has proved useful for maintaining the integrity of various *M. persicae* genotypes for insecticide resistance studies, as aphids on diet cylinders can be kept for a few months in a refrigerator, which facilitates testing of the same strain over time (SW Bunting, pers. comm.). An obvious use of artificial diet is bioassay of toxins, phagostimulants, and deterrents (e.g., Kim et al., 2008; Paula & Andow, 2016). However, it is difficult to equate the dose taken up from the diet with uptake from phloem. This is not so serious with toxins. With compounds influencing behaviour, however, dose in the diet will determine whether the concentration of a compound is on the no-effect, positive, or negative part of the dose-response curve. Other applications include the use of antibiotics in diet to produce asymbiotic aphids, which has made a major contribution to the understanding of the role of the obligatory bacterial symbionts in aphids (Wilkinson, 1988). Finally, aphid saliva has been collected in diet for subsequent chemical analysis (e.g., Zhang et al., 2017).

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