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Artificial Diets and Rearing Techniques of Aphids

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SUMMARY

Aphids are small soft bodied prominent sucking crop pest causing economic losses either directly by sucking the phloem sap of from most of cultivated crops or indirectly by acting as vectors for most of viral diseases. As it is a Hemimetatabolous insect, life cycle includes egg, nymph and adult stages. There isneed to rear aphids under artificial lab conditions for better understanding of its complex life cycle, biotype development, insecticide resistance, mode of transmission of viral diseases, testing of efficiency of predators and parasitoids under lab conditions there by utilizing them under IPM practices.In this contest, it is important to us to rear the aphids in artificial controlled conditions either on their natural host plants or on the artificial diets to study their biology, understanding the resistance levels to various insecticides under lab conditions and then in field conditions. There are some of techniques available for rearing of aphids; Rearing on their natural host seedlings, Leaf disc method of rearing and rearing on the artificial diet.

INTRODUCTION

Aphids are small soft bodied sap-sucking pests belonging to super family Aphidoidea and family Aphididae. There are over 5000 different aphid species has been documented.Nymph and adult feed on plant juices.They attack everywhere on plants, starting from leaves, stem, buds, flowers and eventually the fruit of the crop involved. Some Specific Aphid species feed on the roots of the plants Ex: lettuce root aphid attacks the roots of plants.

Rearing techniques of aphids:

Rearing on plants in laboratory:

Field collection of Aphids: The adult stage of cotton aphid *Aphis gossypii* (Glover), bean aphid *Aphis craccivora* (Kock) and corn aphid *Rhopalosiphum maidis* (Fitch) (Homoptera: Aphididae), can be collected from okra, faba beans, and corn plants respectively.(Zein *et al.,* 1982).

Plants used for culturing in Lab: The aphid cultures should bemaintained on Kenaf plants *Hibiscus cannabinus,* broad bean *Vicia faba* and corn *Zea mays* (L.) *Wliczek* seedlings for rearing of aphids' *Aphis gossypii, Aphis craccivora* and *Rhopalosiphum maidis*, respectively (Zein *et al.,* 1982).

Procedure for culturing: Transfer aphids from infested to healthy seedlings after 7-15 by cutting the heavily infested leaves and placing them on the healthy seedlings. Prevent contamination between cultures by placing the seedlings in special chambers 50×50×60 cm which are covered with muslin on their sides. These cultures should be kept in a breeding room under the temperature of 25±2°C, 65±5 relative humidity (R.H) and 12 hours daily illumination by using two fluorescent bulbs of 40 watts each.

Leaf disc rearing technique (Hughes and Woolcock, 1965)

Maintaining of stock culture: Adult aphids from the field were used to produce nymphs at the start of experiments to avoid difficulties due to parasitism, disease, etc., (Laboratory colonies of aphids could be kept for indefinite periods on leaf-discs if fresh host-plant material was available.)

Newly-born aphid nymphs could be obtained for experimental purposes from stock cultures in which the aphids had reached the adult stage. Either apterous or alate females were used to produce young for critical experiments, as they showed variability depending on the form of the parent.

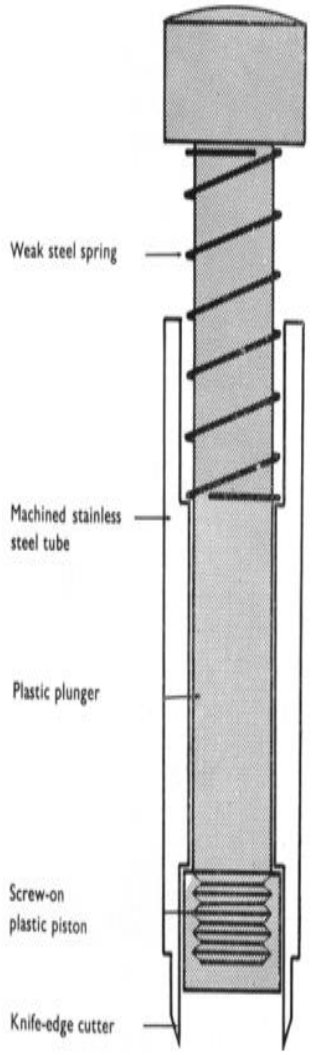

Fig. 1.—Design of leaf-disc cutter and handler, used in aphid studies.

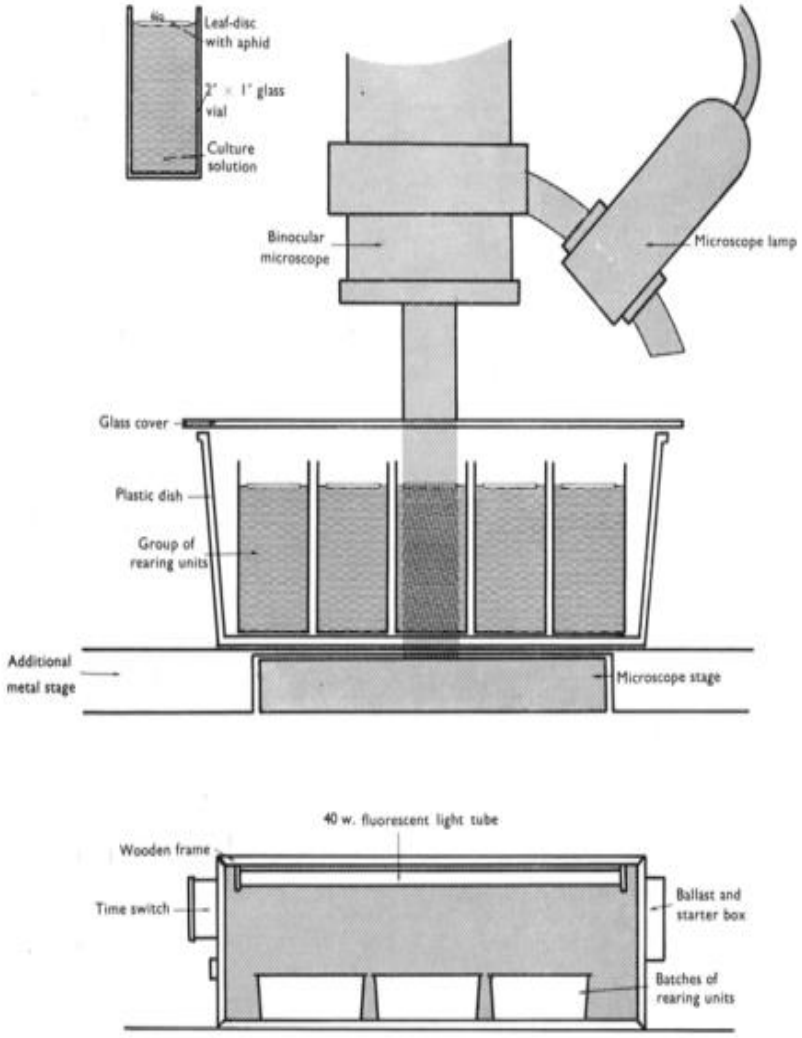

Fig. 2.—Sketches showing the arrangements of leaf-disc/culture solution units: top—as individually set up; centre—as handled in batches for observations; and bottom—as handled in batches during treatments.

Culturing on leaf discs:

- Fresh, turgid leaves of the aphids' host-plant were selected and discs cut from the laminae, care being taken to avoid main veins. A ~ in. diameter leaf-disc cutter and handler, C.S.I.R.O. workshops (Fig. 1).
- The discs were transferred directly to float on the surface of the liquid. Leaves with a hydrophobic surface layer are particularly suitable. as they float readily for long periods. Leaf-discs could be floated with either surface uppermost depending on the habit of the aphids.
- It was found that if the leaf-discs were floated on a culture solution instead of on plain water, at 200 e they grew actively, increasing in area by about 10 % week, and correspondingly increased their dry weight by about 20 %. This meant that the leaf-discs lasted longer on the culture solution.
- However, at low temperatures leaf-discs on standard strength culture solutions pigments more rapidly than when placed on dilute solutions.

- Procedures were standardised to use an optimal, one in four dilution of culture solution in all experiments. (A modified Hougland-Snyder formulation, including an antibiotic, streptomycin, to reduce bacterial decomposition, was used-see Appendix for details.)
- The solution should be held in 2 X I in. flat-bottomed glass vials, filled to within t in. from the top. The apparently large quantity of solution used for one leaf -disc was necessary to act as a thermal buffer when the discs were removed from experimental temperature treatments, for occasional observations to be made on the aphids.
- Transfer individual aphids or nymphs to rearing units by means of the fine point of a slightly damped paint brush. They generally settle to feed without difficulty.
- Remove periodically as per the need, the honeydew, wax, cast-skins, etc., produced by the insects, which may not only incapacitate them, but also encourage the growth of moulds on the leaf-discs, by the use of the paint brush.
- The leaf-disc/culture solution units, Fig. 2a, should banked together in groups in appropriately-sized containers made of plastic or metal. In this way they could be handled in groups of 20, 30, or more at a time.
- A clear glass plate should be held above the tubes to reduce evaporation and prevent deposit of dust during the periods of experimental treatment.
- Maintain uniformity in the height of fluid levels in the tubes to facilitate the observation of aphids on the leaf discs with a binocular microscope.
- A simply constructed, elongate metal staging below the microscope allow all the discs to be observed just by sliding the batch of tubes backwards and forwards, Fig. 2b.
- Maintain controlled environment conditions as per location of the experiment. The whole apparatus is small and portable (Fig. 2c).
- Record observations on specially printed cards. The frequency of records depends on the temperature used in the experiment and on the desired accuracy of the results.

Rearing on Artificial diet:

Artificial diets for Aphid rearing:

Artificial diet can be defined as "Any diet that is not the natural food of the insect". This comprises of synthetic, chemically defined, purified, holidic, meridic and oligidic diets.

Diet Preparation and composition:

- Dissolve 15 g of Analar sucrose in 100 ml ultrapure water.
- Add the diet ingredients and dissolve them completely one at a time in the order given in Table 1. Stir the contents on magnetic stirrer.
- Collect small, plastic tubes (25–30 ml, Figure 2A) fill them with the diet and deep freeze 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.

Table.1: **Diet composition for M. *persicae***

Ingredient	**M. *persicae* (Basic diet developed in 1960s) (Mittler and Dadd,1962)**	**M. *persicae*(Modified diet) (Emden and Wild,2020)**
	mg per 100 ml water	
Sucrose (analar)	18000.0	15 000.0
di-Potassium hydrogen orthophosphate	-	750.0
Magnesium sulphate	-	123.0
Tyrosine[1]	40.0	40.0
L-Asparagine hydrate	550.0	550.0

L-Aspartic acid	140.0	140.0
L-Tryptophan	40.0	80.0
L-Alanine dextro-rotary	100.0	100.0
L-Arginine monohydrochloride	270.0	270.0
L-Cysteine hydrochloride, hydrate	10.0	40.0
L-Glutamic acid	140.0	140.0
L-Glutamine	150.0	150.0
Glycine	40.0	80.0
L-Histidine (free base)	40.0	80.0
L-Isoleucine (allo free)	30.0	80.0
Leusine	40.0	-
Lysine	120.0	-
Methionine	10.0	-
Phenylalanine	10.0	-
Proline	40.0	-
Serine	80.0	-
Threonine	140.0	-
Valine	40.0	-
Thiamine	2.50	-
Riboflavin	2.50	-
Nicotinic acid	10.0	-
Pyridoxine	2.50	-
Folic acid	0.50	-
Calcium pantothenate	5.0	-
Meso – inositol	50.0	-
Choline chloride	50.0	-
Biotin	0.1	-
Ascorbic acid	100.0	-
K_3PO_4	500.0	-
$MgCl_3.6H_2O$	200.0	-
Cholesterol	20.0	-

Table 2: Diet Composition for *Aphis glycines*:

L – Amino acid	**Diet A0[a]**	**Diet A1 [a]**	**Diet B [b]**	**Diet C [c]**	**Diet D [c]**
Ala	20.1	20.1	5.4	4.9	4.2
Arg	14.1	14.1	3.5	5.7	6.6
Asn, H_20	19.9	19.9	179.0	7.4	4.9
Asp	6.6	6.6	11.9	15.6	14.7
Cys	2.4	2.4	1.6	2.2	1.9
Glu	10.2	10.2	4.9	20.3	11.5
Gln	30.5	30.5	5.9	36.6	24.9
Gly	22.2	22.2	1.7	2.1	1.5

His. HCl, H_2O	6.5	6.5	1.7	1.4	2.7
Ile	12.6	12.6	4.0	3.4	8.0
Leu	17.7	17.7	3.8	3.4	9.3
Lys, mono HCl	19.2	19.2	2.2	5.2	7.8
Met	4.9	4.9	0.8	2.2	2.9
Phe	10.3	3.8	3.0	4.6	8.7
Pro	11.2	11.2	4.3	4.9	4.2
Ser	11.8	11.8	10.4	8.1	5.7
Thr	10.7	10.7	6.8	9.6	9.1
Trp	2.1	2.1	1.4	5.3	8.9
Tyr	2.1	2.1	1.1	0.6	1.4
Val	16.3	16.3	5.9	6.2	2.9

a Febvay *et al*. (1972) *b* Febvay *et al*. (1999) *c* Karley *et al*. (2002)

Rearing procedure on artificial diet:

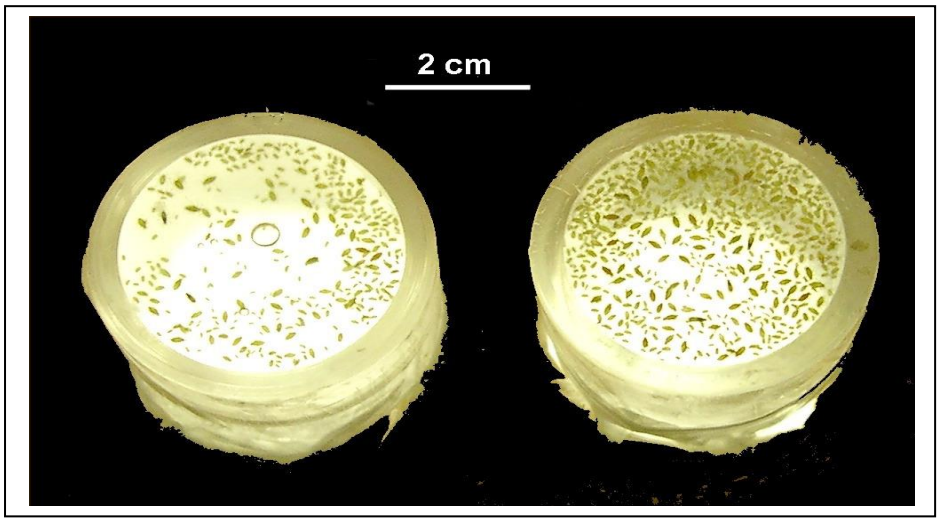

Figure 1: Cultures of *Myzus persicae* on the artificial diet.

Diet sachets:

The diet is enclosed in sachets made from two layers of stretched Parafilm 'M'. To ensure sterile conditions while making the sachets, the work should be conducted in a laminar flow cabinet with built-in UV lighting and a front blackout curtain. 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 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 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. 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.

Re-usable metal filter units can be replaced with 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.

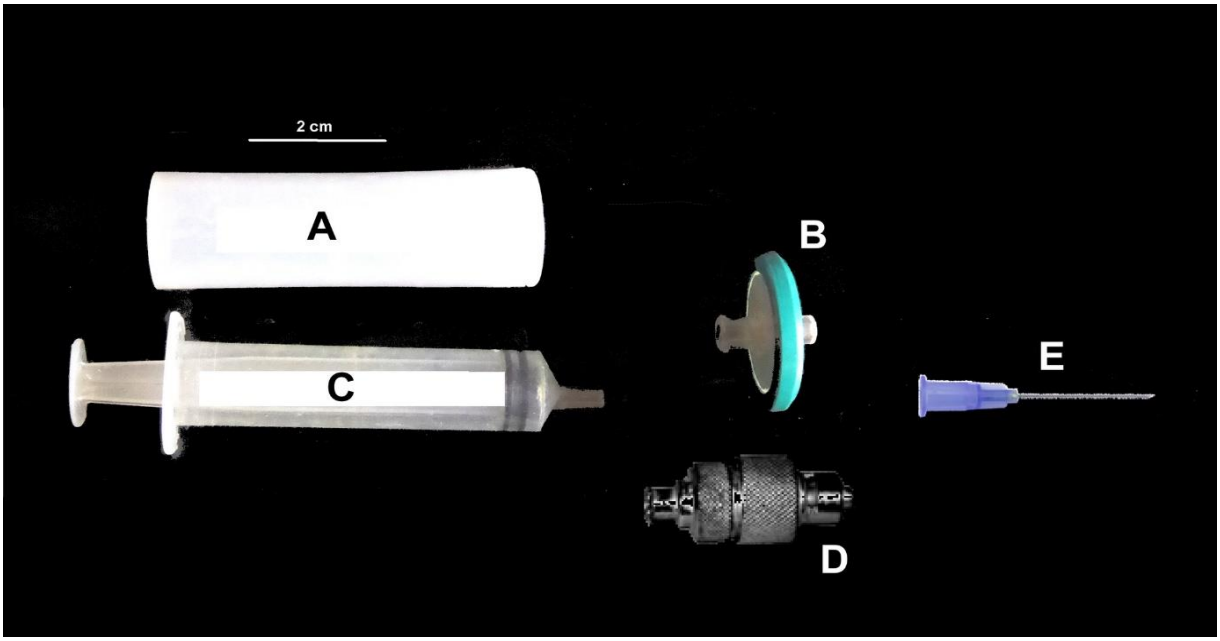

Figure 2: The syringe system: (A) tube of diet taken from the freezer; (B) disposable bacterial filter; (C) syringe body; (D) re-usable bacterial filter assembly; (E) disposable hypodermic needle

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.

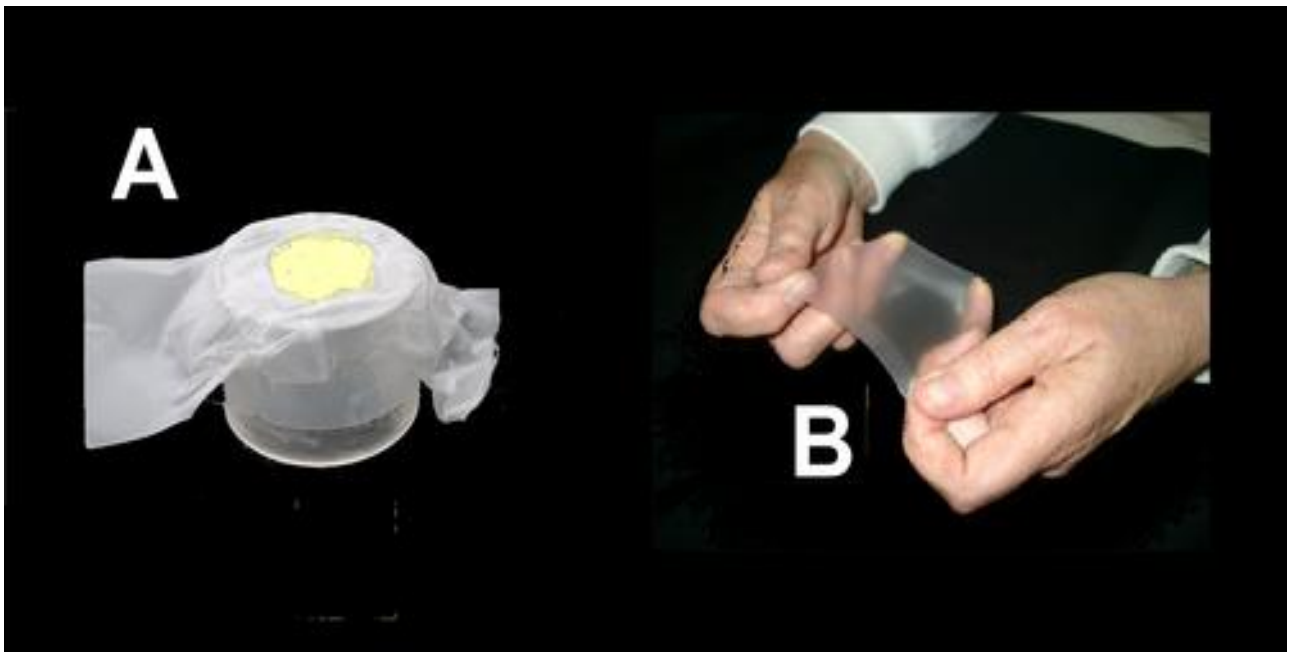

Figure 3: Assembling the diet cylinder: (A) the lower Parafilm membrane in place with an aliquot of diet pipetted onto it; (B) stretching the upper membrane before inverting it onto the cylinder.

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 should use 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), to dispense the diet through a metal hypodermic needle. The assembled filter unit with needle was wrapped in aluminium foil, and should 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) should be washed thoroughly and rinsed several times with distilled water. Clean the metal grid from the filter unit with an ultrasonic bath of distilled water for 7–8 min. Finally, re-assemble filter holder with grid (without a filter) and syringe it with distilled water at least 3 times.

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.

The required number of new cylinders with diet from the freezer are thawed at room temperature for about 30 min.

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 to be 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).

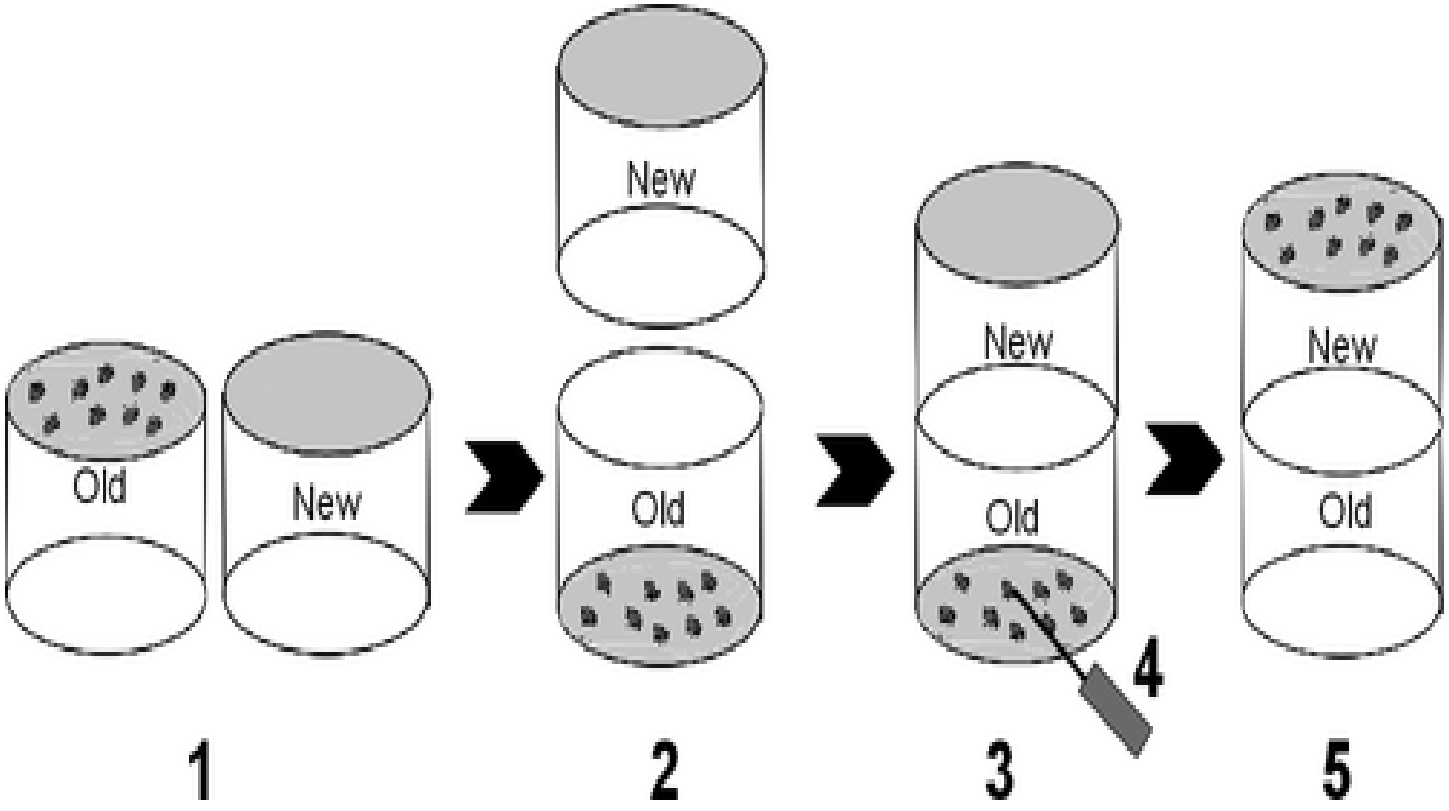

Figure 4: The sequence for changing the diet: (1) old and new cylinders; (2, 3) the new and old cylinders are joined by their open ends and held together with the tails of Parafilm from the sachets; (4) the old sachet is punctured with a needle and the diet drains out; (5) the aphids rapidly migrate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

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