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Mini review

Artificial diets used in laboratory rearing of the European grapevine moth, *Lobesia botrana* (Lepidoptera: Tortricidae).

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Abstract: The larval nutrition of the grapevine moth, *Lobesia botrana*, is determinant for its fitness; the amount and quality of the food ingested by larvae strongly influence the insect growth and reproduction. Utilizing appropriate artificial diets is a critical step in establishing a laboratory rearing colony. Generally, two types of diets are used in grapevine moth lab rearing, diets that stay moist and soft (agar-based diets) and those that dry out and harden with time (non-agar-based diets). Agar-based diets are satisfactory for producing small quantities of insects in small food containers, but with large containers, difficulties may occur. The relatively high cost of agar is another reason that stimulated the search for less expensive binders. To the contrary, non-agar based diets are generally used when large numbers of insects are required and where cost becomes a critical factor. In addition, many general-purpose diets (with or without agar) are used for rearing this insect. The selection of a particular diet, however, is a personal decision that should be based on the quality of the produced insects and the diet suitability for the rearing purpose. This paper discusses the artificial diets used by researchers for *Lobesia botrana* laboratory rearing.

Keywords: artificial diet, grapevine moth, insect rearing, *Lobesia botrana*

Background

The European grapevine moth, *Lobesia botrana* (Denis & Schiffermueller) (Lepidoptera: Tortricidae), is a key pest of vineyards in many countries, particularly in the Mediterranean basin and Southern Europe (Ifoulis and Savopoulou-Soultani, 2004; Moschos, 2006; Shahini *et al*., 2010). The insect has a wide geographical distribution including Europe, North Africa, Middle East, and West Asia (Roehrich and Boller, 1991; Moschos, 2006; Thiéry, 2008; Thiéry *et al*., 2014). *L. botrana* is a phytophagous non-feeding adult stage insect and its adoption to grapevine is relatively new (Thiéry and Moreau, 2005; Varela *et al*., 2010). Therefore, the larval nutrition is very important factor in its fitness; the amount and quality of the food ingested by larvae strongly influence the storage and accumulation of the resources that are allocated to reproduction (Awmack and Leather, 2002; Moreau, 2006). Consequently, a significant implication on its population dynamics is related to its larval feeding.

The interest in implementing artificial diets for *L. botrana* lab rearing developed with the growing interest in studying the biology, physiology and toxicology of this insect. These types of studies require continuous supplies of insect, sometimes in large numbers, throughout the year. Therefore, laboratory rearing became a necessity and enhanced the need to develop economical formulations of artificial diets, especially with the potential of applying the

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sterile insect technique (SIT) to control this pest (Saour, 2014; Steinitz *et al*., 2015).

Artificial diets have some advantages over natural foods. Grape berries, which are the most important natural food for *L. botrana*, may not be always available, particularly out of the season. To the contrary, artificial diets are available throughout the year, yield more moths than grape berries and produce uniform quality of insects. In addition, they provide steady production of insects year round and they are easier to apply sanitation procedures (Dyck, 2010).

Selecting an appropriate diet is the key factor to success in rearing insects in the laboratory, whether in a small scale, or in a large scale for mass rearing to be utilized in a sterile insect release program (SIT) for controlling this pest. This study provides information on the most commonly used diets by researchers for *L. botrana* lab rearing and discusses the advantages and drawbacks of each diet.

Nutrition requirements in insect artificial diets

Nutrients are raw materials and energy sources for metabolism, building blocks for biosynthesis, and co-factors for biochemical and enzymatic reactions. The optimal nutrition for the insect should provide the main classes of nutrients (proteins, carbohydrates, and lipids) in appropriate proportions to achieve a certain balance (House, 1961; Savopoulou-Soultani, 1985; Savopoulou-Soultani *et al*., 1994). The quantitative requirements of diet for each insect depend on the percentage of nutrients in that diet (Savopoulou-Soultani *et al*., 1994). Vitamins and minerals are also important for nutrition. Providing nutrients below the threshold level, in general, results in small, slowly developing, and less fertile insects (Dyck, 2010).

Insect artificial diets

Vanderzant (1969) defined the insect artificial diet as "any diet that is not the natural food of an insect". It may consist of nutritional ingredients from plant materials and chemical substances. The fundamental research in developing artificial diets for Lepidopteran larvae was done in the in the sixties and seventies of the last century (House, 1961; Bathon *et al*., 1991; Dyck, 2010). The principle goal of the artificial diet is to produce large numbers of suitable insects at the lowest possible cost, where female productivity exceeds that of the native population (Chambers, 1977). Selecting an adequate diet is the key factor to success in rearing insects in the laboratory. Diet adequacy is usually determined by survival rate, fecundity, body weight and size, adult longevity and reproduction rate (Navon, 1968; Dyck, 2010); developmental speed is not necessarily a good adequacy indicator (Rock *et al*., 1964).

Nutritional standards for artificial diets

Any artificial diet should meet several nutritional standards (Dyck, 2010). All necessary nutrients should be available, metabolically suitable, chemically and physically acceptable, and supplementary sources of nutrients can be provided as needed (Howell, 1970; Singh, 1984; Dyck, 2010). Ideal diets should provide all nutrients necessary to produce acceptable insects, meet the insect's behavioral needs, and economical with the optimum cost/benefit ratio. In addition, they should be easy to prepare from locally available ingredients and ingredients should be available throughout the year with a long storage life. Furthermore, diets should produce an average yield of adults of at least 75% from initial viable eggs (Singh, 1977, 1983; Dyck, 2010). The insect size and developmental rate should be similar to that in nature, the adults should mate and reproduce, lay viable eggs without loss of fecundity or fertility and the behavior of the insects should be normal (Singh, 1977, 1984; Dyck, 2010).

Artificial diets for *L. botrana* lab rearing

Moreau (1965) was, apparently, the first to attempt to rear *L. botrana* larvae on an artificial diet (Tzanakakis and Savopoulou, 1973). Two years later, Roehrich (1967) compared apples with several plants leaves, including grapevine leaves, as larval food. He found that apples covered with grapevine leaves or lettuce was better than apples or leaves alone. His diet was further developed using either ripe apples (Maison and Pargade, 1967) or green thinning

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apples (Touzeau and Vonderheyden, 1968). Guennelon and his co-workers (1970) reared over 30 generations of *L. botrana* on an artificial diet consisting of 15 ingredients including ripe grapes, dried grapevine leaves, grape seed oil, casein hydrolyzate, and brewer's yeast. As for many Lepidopteran species, the numerous diets used today for rearing *L. botrana* are quite similar. Differences are due to local attempts to simplify or reduce the cost of the diet, or to utilize locally available ingredients and not due to real differences in the nutritional requirements of the strain being reared (Dyck, 2010). In general, *L. botrana* artificial diets can be categorized in two groups: (a) diets that stay moist and soft (agar- based diets) and (b) those that dry out and harden (non-agar- based diets).

a. Diets that stay moist and soft (agar-based diets)

Many diets are intended to stay moist and soft throughout the period of larval development by incorporating a gelling agent such as agar. Agar is a gelatinous substance extracted from a group of red-purple marine algae (Class Rhodophyceae). The substance (agar) is widely used in preparing culture media for microorganisms and as a gelling and stabilizing agent in foods. It is added at early stages of diet preparation by dissolving its powder in hot water and boiling; the mixture forms a gel when it cools off.

In nature, wild mature *L. botrana* larvae exit the grapevine berries and seek out a dry place to spin their cocoons and pupate. In the laboratory, mature larvae mimicking the natural situation, tend to leave the soft diet medium and search for a dry place to pupate. Cardboard strips are usually used to provide suitable cocooning sites for mature larvae, and pupae can be obtained easily by opening the cardboard strips.

Tzanakakis and Savopoulou (1973) tested two agar-based diets for rearing *L. botrana.* The first diet was composed by weight (g) of tap water (50), agar powder (2), methyl ρ-hydroxy-benzoate (0.15), potassium sorbate (0.1), carrot baby food (5), tomato juice (25), alfalfa meal (10), brewer's yeast (10), vitamin mixture (2) and salt mixture (0.5). The second diet contained, in addition, ascorbic acid (1), wheat germ (3), and powdered cellulose (5) and this formulation was used by other researchers (Roditakis and Karandions, 2001).

At present, several general-purpose diets based on agar are used for rearing many lepidopteran species, including species of Tortricidae (Bathon *et al*., 1991) and *L. botrana* is one of them. Ivaldi-Sender (1974) diet is a widely used one that was originally developed for rearing the oriental fruit moth *Grapholita molesta* (Busck), and has also been used to rear the codling moth. The methodology of this soft diet is described in details by Bathon *et al*. (1991), the quantities of ingredients were: water (780 ml), agar powder (20 g), wheat germ (50 g), corn semolina (50 g), yeast powder (50 g) (brewer's or torula yeast), benzoic acid (1.8 g), ascorbic acid (4.5 g), and Methyl *p*-hydroxybenzoate (1.8 g).

Maher (2002) made some modifications to Ivaldi-Sender's (1974) diet, the ingredients of the diet were: distilled water (220 ml), agar (4 g), wheat germ (15.6 g), corn flour (13 g), corn oil (0.4 ml), yeast (13 g), ascorbic acid (1.3 g), benzoic acid, nipagin (0.4 g), and iprodione (hydantoion fungicide) (0.3 g). Ibrahim (2004) made another modification by adding a complementary components, in addition to the essential components, such as alfalfa seeds, sugar, Wesson salt, casein, cholesterol, nipagin, vitamin C, sorbic acid, propionic acid, Formaldehyde, aureomycin, and multivitamin solution. They used these diets successfully for rearing *L. botrana.*

In general, agar-based diets are satisfactory if small food containers are used, but with large containers, which are desirable for mass-rearing, difficulties may occur (Brinton *et al*., 1969). The mould growth is often so serious despite reasonable sanitation procedures; moulds retard larval development. Another problem is that unacceptably large numbers of neonatal larvae leave the medium and die, unless food containers are covered with tightly fitting lids. The use of lids, unfortunately, increases humidity in the containers which consequently encourages mould growth again.

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b. Diets that dry out and harden (non-agar based diets)

Researchers who work with mass rearing have a tendency to reduce the cost and the handling efforts of the rearing process. Omitting the agar and finding a suitable, cheaper, and locally available substitute is a good approach. However, omitting agar reduces the ability of the diet to retain the internal humidity content, which would cause rapid drying out and if the diet dries too quickly, young larvae will die. Therefore, it was necessary to introduce additional components to bind the other components, regulate drying to keep up with larval development, and prevent the rapid drying out.

Brinton *et al.* (1969) initiated a major departure from agar-based diets into non-agar-based diets. Their diet is a modification of a casein-wheat germ diet that was initially developed by Ignoffo (1963) for rearing the cabbage looper *Trichoplusia ni* H. (Lepidoptera: Noctuidae). The agar in the looper diet was replaced by wheat flour, wood sawdust, and wood pulp. Brinton *et al.* (1969) diet consists of quantities expressed by percentages (%): water (71.7), wood pulp (1.24), wood sawdust (6.89), citric acid (0.9), sucrose (2.69), wheat bran (1.8), whole wheat flour (9.86), wheat germ (0.9), Wesson's salt mixture (0.62), casein (2.69), choline chloride (0.1), and triturated ingredients (0.61). this diet is widely used for rearing the codling moth, *Cydia pomonella* (L.) and later for rearing other Lepidopteran species. Growth of microorganisms was controlled by addition of ascorbic acid and aureomycine, and by lowering the pH of the diet with citric acid to 3.5. Production costs were much less than agar-based diets. The diet slowly dries out as larvae grow and mature. By the time the larvae spin their cocoons in the upper portion of the drying diet, the diet becomes rather hard but adults can still emerge and fly out of the diet (Brinton *et al.*, 1969; Howell, 1971; Bloem *et al.*, 1997; Dyck, 2010).

Mohammad and his colleagues in 1997 made several modifications to Brinton *et al.* (1969) diet in order to make it more economical and convenient to the Syrian local circumstances (reviewed in Dyck 2010). They replaced sawdust and paper pulp by legume straw, wheat germ by barley germ, gluten by a 1:1:1 mixture of maize, barley and wheat flour, and the amount of water was readjusted. They used this diet to rear *C. pomonella* also Mansour and Al-Attar (2012, 2014) used this diet in rearing *L. botrana* with good results.

This type of diet is practical when insects are to be sterilized in the adult stage for release in SIT programs. However, when pupae are required, Carpenter *et al.* (2004) tested procedures for their extraction from the media using a de-silking chemical and pressure washing. It's important to monitor the medium humidity content, and maintain a control of relative humidity and air movement when using this type of diet (Brinton *et al.*, 1969).

Comparisons of the general propose diet ingredients for Lepidopteran insects have been made by Hathaway *et al.* (1971), Shumakov *et al.* (1974), Butt (1975), Singh (1977), and Dyck (2010). These comparisons allow laboratories to choose an existing diet or to modify its formulation to make it suitable for their local conditions. However, the selection of a particular diet or modifying a diet formulation is a self-governing decision based on the availability of the ingredients, cost, equipment, and the purpose of rearing. General purpose non-agar-based diets such as Brinton *et al.* (1969) and its modifications (Wildbolz and Mani, 1971; BCFGA, 1972) are widely used for rearing many insect species including *L. botrana*. These diets slowly dry out as larvae grow up, mature and spin their cocoons in the upper portion of the drying diet.

Conclusion

In laboratory rearing, utilizing a suitable artificial diet is a critical step for establishing a laboratory colony of any insect. The main objectives are to produce the largest possible number of insects with acceptable quality at the lowest possible cost. Many formulations of artificial diets are used in rearing *L. botrana*. Agar-based diets are satisfactory for producing small number of insects, especially when larval or pupal extraction is required. For mass rearing, however, it would be expensive and less practical. To the contrary, non-agar based diets are more practical for producing large numbers of insects at an acceptable cost, particularly for SIT programs. In general, each laboratory has developed its own unique different ingredients and procedures including the exact sequence of adding and mixing the ingredients. It is worth mentioning, however, that some used diets are probably more complex than necessary; there is no specific knowledge about the essential ingredients and those that were added somewhat accidentally during the historical development of the diet (Dyck, 2010).

Additional researches are required to improve the productivity and efficiency of *L. botrana* laboratory rearing (Savopoulou-Soultani, 1994; Herrera, 2016). Indeed, introducing diets based on local substitute components would be promising. Furthermore, accumulating the practical experience is fundamental to arrive at the optimal diet formulation. Brinton *et al*. (1969) stated that “It is most important to get the correct consistency, and this can be achieved only through experience”. Finally, the most important criterion in choosing a diet is the response and quality of the produced insects and its suitability for the rearing purpose.

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رژیم‌های غذایی مصنوعی مورد استفاده در پرورش آزمایشگاهی کرم خوشه‌خوار انگور *Lobesia botrana* (Lepidoptera: Tortricidae)

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چکیده: تغذیه کرم خوشه‌خوار انگور، *Lobesia botrana*، در دوره لاروی تعیین‌کننده است. مقدار و کیفیت غذای خورده شده توسط لارو به‌شدت بر رشد و تولید مثل حشرات کامل تأثیر می‌گذارد. استفاده از رژیم‌های غذایی مصنوعی مناسب گامی اساسی در ایجاد کلنی پرورش آزمایشگاهی است. به‌طور کلی، از دو نوع رژیم غذایی در پرورش آزمایشگاهی کرم خوشه‌خوار انگور استفاده می‌شود. رژیم‌های غذایی مبتنی بر آگار که با گذشت زمان مرطوب و نرم باقی می‌مانند و رژیم‌های غذایی مبتنی بر غیرآگار که با گذشت زمان خشک و سخت می‌شوند. رژیم‌های غذایی مبتنی بر آگار برای تولید مقادیر کمی از حشرات در ظروف کوچک غذایی مورد استفاده قرار می‌گیرند، اما در مقیاس بزرگ، ممکن است با مشکلاتی مواجه شود. هزینه نسبتاً بالای آگار دلیل دیگری است که جستجو برای رژیم‌های غذایی ارزان قیمت را توجیه می‌کند. اما رژیم‌های غذایی غیرآگار معمولاً در مواقعی که تعداد زیادی حشره مورد نیاز باشد و هزینه‌های اقتصادی آن مهم باشد، استفاده می‌شوند. علاوه بر این، بسیاری از رژیم‌های غذایی عمومی (با یا بدون آگار) برای پرورش این حشره استفاده می‌شود. با این‌حال، انتخاب یک رژیم غذایی خاص یک تصمیمی است که باید براساس کیفیت حشرات تولید شده و مناسب بودن رژیم غذایی برای اهداف پرورش باشد. در این مقاله رژیم‌های غذایی مصنوعی مورد استفاده محققان برای پرورش آزمایشگاهی کرم خوشه‌خوار انگور مورد بحث قرار گرفته است.

واژگان کلیدی: رژیم غذایی مصنوعی، کرم خوشه‌خوار انگور، پرورش حشرات، *Lobesia botrana*