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Synthetic Diets and Piercing-Sucking Insect Research[1]

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ABSTRACT

Artificial diets have played an important role in the study of many aspects of insect biology. The use of diets and feeding systems has gained wide use among those studying pathogen/insect interactions. We used an artificial feeding system for the production of thrips eggs, which aided efforts to develop thrips cell cultures. These cell cultures were then used to examine the interactions between the tospovirus, impatiens necrotic spot virus, INSV and thrips cells.

Thrips cells were infected with INSV as detected by immunofluorescence. This is one case study which demonstrates the usefulness of artificial diets to aid in the production of insect cell cultures and how this can lead to studies on virus/vector cell interactions.

Key Words: Aphids, Bunyaviridae, Cell culture, Diets, Feeding, Insects, Leafhopper, Thrips, Virus, Whitefly.

INTRODUCTION

Many of the problems associated with the study of insects are those directly related to the amount of plant material that is needed to rear many species. Even if the plant material can be grown, space is often a limiting factor. Where plant viruses are involved it is beneficial to have a system whereby insects may feed devoid of any plant material to assure that insects are free of viruses. To overcome many of these problems, artificial diets for many insect species have been developed by Bio-Serv®[2], and Southland Products

[1]Mention of a trademark, warranty, proprietary product, or vendor does not constitute a gu[illegible] by the U.S. Department of Agriculture and does not imply its approval to the exclusion c products or vendors that may also be suitable.

[2]Bio-Serv, one 8th St., Suite one, Frenchtown, NJ, USA, 08825, E-mail: sales@bio-serv.com; 996-2155, www.bio-serv.com.

Inc.[3] The use of artificial diets as a research tool has shown them to be of immense benefit (Table 1). While many chewing insects, such as the Lepidoptera and Coleoptera, can readily be reared on artificial diets, it has been particularly difficult to maintain piercing-sucking plant feeding insects (like aphids, leafhoppers, whiteflies, thrips, mealybugs) for more than a few generations on an artificial diet. The development of artificial diets have aided advances on many aspects of entomology (Table 1). Studies on insect control, physiology, toxicology, behaviour and biological control have also been advanced with the development of artificial diets (Table 1).

Table 1. Research conducted using artificial diets or feeding systems for the examination of various biological aspects of piercing-sucking insects.

Research	Insects	Reference
Artificial diets.	Aphids	Auclair 1969, Auclair and Boisvert 1980, Hamilton 1935, Maltais 1959, Mittler 1976, Mittler and Dadd 1962, Srivastava 1987
	Whitefly	Salvucci et al. 1997.
Nutrition and mortality.	Aphids	Boisvert and Auclair 1981, Srivastava 1987, Van Den Heuvel et al. 1998.
	Leafhopper	Kono et al. 1982.
	Whitefly	Jancovich et al. 1997, Salvucci et al. 1997.
Insect physiology.	Aphids	Ehrhardt 1968, Ho 1983.
	Whitefly	Salvucci et al. 1997.
Toxicology, behaviour.	Aphids	Ho 1983.
Endosymbionts.	Aphids	Hogenhout et al. 1998, Ishikawa 1984, Van Den Heuvel et al. 1997, 1998.
Behaviour and virus-transmission.	Aphids	Powell et al. 1995, Spak 1992.
	Leafhoppers	Harris et al. 1981, Kono et al. 1982.
Virus pathogenicity.	Aphids	Gildow and D'Arcy 1990.
	Thrips	DeAngelis et al. 1993.
Presence of helper component its function, and transmission factors.	Aphids	Blanc et al. 1993, Peng Yan Hua et al. 1998, Wu and Wei 1995, Wang et al. 1996.
	Leafhopper	Hunt, Nault and Gingery 1988.
Purified virus preparations to examine attributes of virus acquisition and transmission.	Aphids	Gildow and D'Arcy 1990, Pereira et al. 1989, Powell et al. 1995, Terhune et al. 1991, Wang and Pirone 1996, Wang et al. 1996, Van Den Heuvel et al. 1991, Zhou and Rochow 1984.
	Thrips	Hunter, Hsu and Lawson 1995.
	Mealybugs	La Notte et al. 1997
	Leafhoppers	Magyarosy 1980
	Whiteflies	Muniyappa et al. 1987

[3]Southland Products Inc., 201 Stuart Island Road, Lake Village, AR, USA, 71653, E-mail: bugfood@ipa.net; 1-870-265-3747).

Another advantage is that the desired insect species to be studied can be colonized and produced in the quantities needed, with minimal space, and at a reduced cost for both basic and applied investigations. Basic information gathered can be on the life history and behaviour of the insect, which may provide valuable insights into alternative control strategies. For example, the ability to rear all life stages of an insect under laboratory conditions allows the researcher to examine in detail the development, biology, and interactions of parasitoids with the target species. Studies such as these are crucial to the screening and development of biological control agents for use in pest management programmes. For some insects, rearing under artificial conditions may be the only way for a researcher to obtain specific information. Insect physiology, and effects of nutrition are best measured with holidic diets and with uniform populations, which can be established more readily under artificial conditions.

Early reviews on insect nutrition and diets were made by Lipke and Fraenkel (1956) and Friend (1958). More recently work on aphid nutrition requirements were reviewed by Srivastava (1987). Piercing-sucking insects are prime candidates for studies using artificial diets, because of their intimate relationship with their host plants, their importance as feeding pests, and their significance as vectors of plant diseases. Biological, insecticidal and pathological research on piercing-sucking insects has incorporated an artificial diet of some kind at one time or another to examine different questions (Table 1). The majority of the research shown in Table 1, has used artificial diets to focus on questions about the acquisition, retention and transmission of pathogens. The ability to conduct experiments without the influence of plant material has allowed researchers to examine highly specific questions about pathogen/insect interactions.

The feeding adaptation of many piercing-sucking insects to their host plants makes it difficult to produce a good artificial diet. Considerable progress has been made for some sucking insects, like aphids, with the earliest attempts to develop an acceptable artificial diet reported in the mid 1930's (Hamilton 1935). Efforts have continued until today, although there are no competent diets available for most piercing-sucking plant feeding insects (Table 1). An excellent review of aphid diets, nutrition, physiology and feeding can be found by Srivastava (1987). At one time commercial diets were available for some aphids including the potato aphid, *Macrosiphum euphorbiae* (Thomas), the pea aphid, *Acyrthosiphon pisum* (Harris) and the green peach aphid, *Myzus persicae* (Sulzer) (Bio-Serv®), but aphids would often only survive for a few generations and then they would produce progressively smaller individuals, a sign of a poor quality diet (Srivastava 1987). For these reasons most research conducted today with piercing-sucking insects utilizes feeding durations of only a few hours to weeks on artificial diets.

Tospovirus and Thrips

To study tospovirus acquisition and transmission by thrips, an *in-vitro* system was developed to allow the feeding of larval thrips on concentrated tospovirus that was re-suspended in a sucrose-histidine buffer solution (Hunter et al. 1995). This technique permitted the separation of compounds which were toxic to thrips from the tobacco extract. So the tobacco could be used for virus production as it is an excellent viral-host plant that supports tospovirus replication, and the virus-suspension could be fed to thrips for the examination of virus acquisition and transmission. The best plant for the production of high virus titres may not always be the best plant to elicit long-duration

feeding from the insect vector (i.e. Begomoviruses in tobacco for studies with whiteflies, Hunter, pers. obs.). Here, an artificial system allowed long-duration feeding from an artificial sucrose-virus preparation extracted from a non-host plant of the thrips, tobacco.

To rear thrips under laboratory conditions and without the use of plants, Murai (1982) developed a method whereby thrips were reared on a commercially-available apple juice. Thrips could be reared for several generations in this manner, which also provided an easy means whereby to collect eggs. This method allowed us to collect hundreds to thousands of eggs for the production of thrips cell cultures (as in Hunter and Hsu 1995).

Artificial Diets and Cell Culture

Often there is a need to remove plant influences to study virus interactions with a vector, or to conduct nutritional studies where a single component may be varied. Therein lies some of the advantages of an artificial diet system. Similarly, it is often advantageous if the researcher can isolate insect cells from the complex effects of plants and/or the whole insect. Such an *in-vitro* technique used to examine the interactions between piercing-sucking insects and plant viruses is the use of cell cultures. Sometimes it is a formidable task just to obtain eggs of some insects due to problems in finding and growing suitable host plants, or in collecting eggs oviposited randomly and sparsely over a plant, or deposited inside plant tissues. For these reasons, we used an artificial rearing system to aid in the collection of thrips eggs for the development of thrips cell cultures.

Tospovirus and Cell Cultures

Thrips are the only reported natural vectors of tospoviruses. These viruses occur worldwide and cause widespread economic losses (Ullman 1996). The tospoviruses are the only plant-infecting member among a group of animal infecting viruses in the family Bunyaviridae.

The successful development and use of many other primary cultures and insect cell lines (Mitsuhashi, 1989) have proved to be invaluable in the study of the molecular biology of other plant viruses (Adam and Sander 1976, Hsu et al. 1983). Fundamental studies into virus acquisition and transmission, virus component functions and virus replication within insect hosts provides clues to the management of virus diseases. Therefore, we used an artificial diet system to obtain thrips eggs for the development of primary cell cultures from the embryonic cells of *Frankliniella occidentalis* (Pergande) to examine the interactions of a tospovirus (INSV) on thrips cell cultures. Thrips were reared in round plastic cylinders, 10 cm diameter and 5 cm long. One end of the tube was covered with Parafilm® the thrips adults added along with a small amount of pine pollen, and the top covered with another piece of Parafilm®. This layer was depressed slightly to hold the diet solution of sterile water and honey, and covered again with Parafilm® (Hunter et al. 1995). Thrips eggs were then collected by removing the top membrane after 2 d and the eggs were washed off into a dish using a water squirt-bottle. Fresh water and honey was replaced over the colony so that a continuous supply of eggs were produced. Adults could be maintained under these conditions for several weeks. Embryonic eggs of *Frankliniella occidentalis*, were used at the red eye stage. Eggs were surface-sterilized with 70% ethanol, homogenized with a glass rod in Tyrode's solution

and then processed following Hunter and Hsu (1995,1996). For inoculation, thrips cells were grown in 8-well chamber slides (Lab-Tek®)[4] for 10 days. Impatiens necrotic spot virus inoculum was prepared from infected jimson weed, *Datura stramonium* L., inoculated by thrips. Leaves were surfaced sterilized and homogenized in buffer under sterile conditions in a Laminar flow hood, the virus inoculum was placed on the cells for 1 h. Cells were microscopically observed at 24, 48 and 72 h post-inoculation. For immunofluorescence, cells were fixed with 3.7% formaldehyde in PBS for 10 min, washed five times with PBS, incubated in primary antibody, washed, incubated with a secondary antibody goat anti-mouse IgG conjugated to fluorescein isothiocyanate (FITC)[5], 50 Fg mL^{-1} in PBS, 0.1% saponin, 4 mg mL^{-1} normal goat serum, for 15 min at 23°C. Fixed cells were, mounted in Aqua-Mount®, then examined under fluoresence microscopy (complete protocol in Willingham and Pastan 1985) (Figure 1).

RESULTS AND DISCUSSION

Artificial diets have provided many benefits to research on piercing-sucking insects (Table 1). Herein we describe the benefits of being able to rear thrips on an artificial medium, under controlled conditions, to collect thrips eggs easily and in large numbers which aided in the culturing of thrips cells. By culturing thrips cells we were able to demonstrate successfully that they could be inoculated with INSV (Figure 1). Successful inoculation of thrips cell cultures demonstrates their potential use for the examination of tospovirus isolates and thrips vectors. Furthermore, this study shows the importance of having an artificial diet for thrips available which enabled enough eggs to be collected to establish thrips cell cultures.

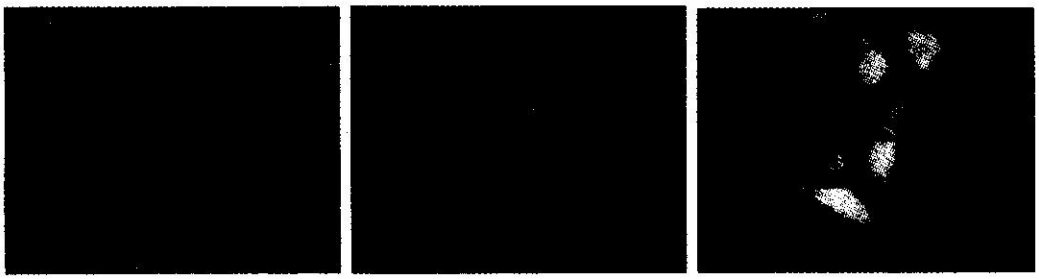

Figure 1. Light micrograph of thrips cells, *Frankliniella occidentalis*, in culture (Left image); Immunofluorescent image of thrips cells control treatment no virus (Middle); Immunofluorescent labeling of INSV in thrips cells (Right image).

[4]Lab-Tek®, Nalgene Nunc International, Naperville, IL, USA.
[5]Sigma Immuno Chemicals, P.O. Box 14508, St. Louis, MO 63178.

Artificial diets have been used and will continue to be an important tool in the study of piercing-sucking insects (Auclair 1969, Auclair and Boisvert 1980, Hamilton 1935, Maltais 1959, Mittler 1976, Mittler and Dadd 1962, Salvucci et al. 1997, Srivastava 1987). With improvements in insect diets more will be learned about insect nutrition, mortality (Boisvert and Auclair 1981, Jancovich et al. 1997, Kono et al. 1982, Salvucci et al. 1997, Srivastava 1987, Van Den Heuvel et al. 1998), insect physiology (Ho 1983, Salvucci et al. 1997), insect toxicology (Ho 1983) and insect behaviour (Ehrhardt 1968, Harris et al. 1981, Ho 1983, Kono et al. 1982, Powell et al. 1995, Salvucci et al. 1997). Much of what is learned may be applied in behavioural studies aimed at developing ways to reduce virus transmission (Kono et al. 1982, Spak 1992), and to elucidate the nature of virus transmission (Semipersistent or persistent), (La Notte et al. 1997). Artificial diets and solutions can be used with purified virus preparations to examine attributes of virus acquisition and transmission access periods, and virus retention within the insect vector (Gildow and D'Arcy 1990, Hunter et al. 1995, La Notte et al. 1997, Magyarosy 1980, Muniyappa et al. 1987, Pereira et al. 1989, Powell et al. 1995, Terhune et al. 1991, Van Den Heuvel et al. 1991, Wang et al. 1996, Wang and Pirone 1996, Zhou and Rochow 1984). Diet solutions can also be used to evaluate the activity of purified virus preparations and their transmissibility (Gildow and D'Arcy 1990, Hunter et al. 1995, Magyarosy 1980, Muniyappa et al. 1987, Terhune et al. 1991, Van Den Heuvel et al. 1991), to examine virus pathogenicity (Gildow and D'Arcy 1990), to screen for the presence of transmission factors (Blanc et al. 1993), or for the evaluation of helper components and their functions (Hunt et al. 1988, Peng et al. 1998, Wang et al. 1996, Wu and Wei 1995). Current technologies have begun to allow researchers to conduct studies on the interactions of the endosymbiotes found within most piercing-sucking insects. These bacteria are present in specialized cellular structures called mycetomes and are thought to contribute to the digestion and nutrition of the insect (Ishikawa 1984) and may contribute to an insects ability as a virus vector (Hogenhout et al. 1998, Van Den Heuvel et al. 1997, 1998).

Many aspects of insect feeding, host selection and biology will continue to be examined through the use of artificial diets. As more defined diets are created and used to analyse the various aspects of host plant/insect interactions we will begin to have a greater understanding of these insects. A greater understanding which will allow for the development of new management strategies to manage insect pests and the diseases they transmit.

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