



Comparison of seven methods for rearing western flower thrips *Frankliniella occidentalis* (Thysanoptera: Thripidae)

N. Mortazavi, M. Aleosfoor*, K. Minaei

Department of Plant Protection, College Of Agriculture, Shiraz University, Shiraz, I. R. Iran

*Corresponding Author: aosfoor@shirazu.ac.ir

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ABSTRACT- The species *Frankliniella occidentalis* (Pergande), western flower thrips, is a highly polyphagous pest and an important vector of tospoviruses on several crops and ornamental plants. Western flower thrips has been introduced throughout the world and has been subject to many biological studies including mass rearing. In this survey, seven common mass rearing methods of *F. occidentalis* were described in detail and compared with each other. As a result, using Persian cucumber in plastic boxes by producing 6.15 ± 0.4122 generations was selected as the best method. This method enables rearing multiple thrips generations on Persian cucumbers by using minimal supplies.

INTRODUCTION

Approximately 5800 species are recognized in the insect order Thysanoptera (Mound, 2013) about one hundred of which are considered as crop pests that cause damage by feeding or transmission of plant viruses (Lewis, 1997; Teulon and Niesen, 2005; Inouet et al., 2010; Chen et al., 2011). Most pests in this order are members of the family Thripidae (Mound, 1997). The genus *Frankliniella* with about 230 species is the third most species-rich genus in the order Thysanoptera (McDonald et al., 1998). One species, *F. occidentalis* (Pergande) known as western flower thrips (wft) has been introduced throughout the world and is a major pest of many crops (Kirk and Terry, 2003). The species was recorded for the first time in Iran from Tehran and Mahalat regions on Ornamental plants (Jalili Moghadam and Azmayesh Fard, 2004). Economic importance effects of these thrips lead to increased interest in culturing thrips for various purposes (Loomans and Murai, 1997). Experiments on thrips require a constant supply of healthy and live thrips and it is often necessary to rear thrips in controlled environments (Kirk and Terry, 2003). Various techniques for rearing phytophagous thrips have been described by some authors like Lewis, Murai and Loomans (Lewis, 1973; Murai and Loomans, 2001). However, there is no study dealing with the comparison of various methods in order to choose the best rearing method among them.

In this survey, seven simple and efficient methods are described and the numbers of produced thrips generations are compared with each other during five months. By understanding the best rearing method, the

maximum number of thrips generation can be achieved and waste of time can be avoided.

MATERIALS AND METHODS

Thrips specimens were collected from cockscombs (*Celosia argentea* var. *cristata*) (Amaranthaceae) garden in the College of Agriculture, Shiraz University, Shiraz, south of Iran. Thrips were inactivated by placing in refrigerator for a few minutes and then were identified. Two of seven rearing methods (2-1 and 3-1, see below) were done in incubator conditions ($27 \pm 1^\circ\text{C}$, $65 \pm 5\%$ R.H and 16:8 L: D) and others were conducted in greenhouse conditions, with the same microclimate. Various methods used in this study are shown in Table 1. The number of generations was compared in five months. All plants were raised from seeds, but sown at different dates to obtain leaves with the same size to cut equal-sized discs. Each experiment was replicated 20 times and the average numbers of generations produced in different methods were compared. Data were analyzed using one-way ANOVA followed by LSD test, while significant differences were considered at $\alpha=0.01$, with SAS, 13 software (SAS Institute, 1990).

Using the whole plants

Heavily garden thrips-infested cockscomb bushes with soil around them were planted individually in pots and covered by acrylic cylinders cap which had 100-mesh nylon screen for ventilation. (Fig.1a).

Table 1. Methods for rearing *Frankliniella occidentalis* in incubator and greenhouse according to literatures

Method(s)	Device	Place	Reference (s)
1	Cages, cylinder cap, cockscomb celosia bushes, red bean plants	Greenhouse	Brodsgaard (1993); Steiner and Goodwin (1998)
2-1	Microtube, bean leaf	Incubator	Arthurs and Heinz (2002); Brodeure and Cloutier (1992); Steiner and Goodwin (1998)
2-2	Cockscomb celosia flowers, plastic food storage box	Greenhouse	Van Dijken et al. (1994)
2-3	Bean pods, glass jar	Greenhouse	Lewis (1997)
2-4	Cucumber fruits, plastic food storage box	Greenhouse	Degraaf and Wood (2009)
3-2	Red bean leaf, plastic box, fabric wick, water bottle	Greenhouse	Brodeure and Cloutier (1992)

Plants were transferred to greenhouse and maintained in screen cages (70×50 cm) without cylinder caps near the 3-week old uninfested bushes (Fig. 1b).



Fig. 1a. Lateral view schematic Pots with acrylic-cylinder cap that had 100-mesh nylon screen: C.c= Cylinder cap, Ns= Nylon screen

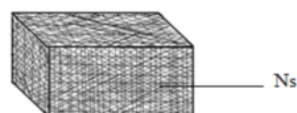


Fig. 1b. Lateral view schematic screen cages: Ns= Nylon screen

Thrips were removed from the infested bushes to healthy ones. The colony was maintained by removing infested old plants and replacing new and fresh ones. For enhancing the possibility of mating, after five days, 2 males and 1 female from isolated colony were transferred to 10-16 old red kidney bean pots and covered by acrylic cylinder cap which had 100-mesh nylon screen and have been left for 4-5 days oviposition period in $27\pm 1^\circ\text{C}$, $65\pm 5\%$ R.H and 16:8 L: D conditions. After this time, the adults in each pot were removed and the cohorts of even-aged old larvae, emerging on plants, were maintained in red kidney bean pots in laboratory conditions until maturation. The new adults were regarded as a new generation.

Using the plant parts

2-1- Microtube (2 ml) with 100-mesh nylon screen on its lid for ventilation was autoclaved (160°C) for one hour. A small piece of moist cotton to maintain high humidity and washed fresh kidney bean leaf disc (1.5 cm in diameter) as a temporary food source with 2 males and 1 female were placed within each of the tubes and were maintained in incubator for 4-5 days oviposition period. After this time, the adults were removed and the new cohorts of even-aged larvae, emerging on a disc, were transferred to new fresh kidney bean leaf disc and new tubes with the mentioned specifications and were maintained in incubator. After 2-3

weeks, emerged new adults were accounted as a new generation. New culture tubes were set up every 2-3 weeks by taking adults, from the parent colony. The number of generations has been calculated during five months.

2-2- The infested cockscomb flower with *F. occidentalis* and little water bottles blocked by cotton rolls were placed in plastic food storage boxes. Top of the boxes were covered by 100 mesh nylon screen and the leaves to prevent thrips from escaping and to maintain the humidity (Fig. 2).

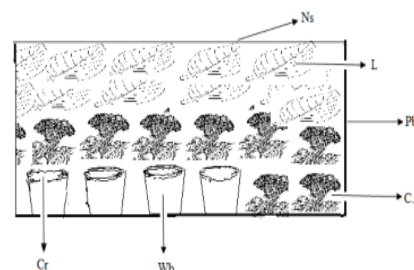


Fig. 2. Lateral view schematic flowers in plastic box: Cf= cockscomb flower, Cr= Cotton rolls, L=Leaves, Ns=Nylon screen, Pb= Plastic box, Wb= water bottles

After five days, 2 males and 1 female from isolated colony were transferred to new boxes with the mentioned specifications and in 20×7.5 cm size. Every three days, the flowers were checked and old flowers having no thrips were replaced with the new ones and every five days, the bottles were filled with fresh water. After 4-5 days, the adults were removed and after 2-3 weeks, we had new adults that would be regarded as a new generation. By continuing these processes and transferring new adults to new boxes and removing old generation larvae, the numbers of generations were calculated.

2-3- Two males and one female were placed in covered glass jar by 100-mesh nylon screen. Blocked little water bottle by cotton roll and three bean pods were placed in glass jar to maintain humidity and food source, respectively. Furthermore, 10 layers of tissue papers were placed on the bottom of glass jar to provide pupation site (Fig. 3).

The pods were purchased from market and washed with water and dipped in fungicide to avoid any contaminations. Cultures were maintained in greenhouse condition for 4-5 days as oviposition period. After this time, adults were removed and egg-infested pods were transferred to rearing glass jar to provide larvae. Larvae were maintained until they were matured. Every three days, the old pods were replaced with new

ones. The new adults were regarded as a new generation. By transferring adults to new glass jars and continuing these processes (removing adults after oviposition period and transferring egg-infested pods to rearing jar), the number of generations was calculated.

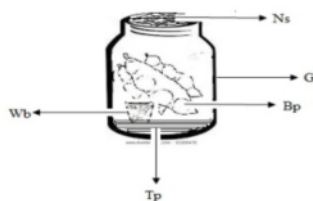


Fig. 3. Lateral view schematic Bean pods in glass jar: Bp= Bean pods, G= glass jar, Ns= Nylon screen, Tp= tissue paper, Wb= water bottle with cotton roll

2-4- The foam sheet with the same size of food storage box was perforated and placed in the middle of the box. Some tissue papers were placed on the foam and 4 or 5 fresh and thin Persian cucumbers were placed on the tissue papers. Blocked little water bottles by cotton rolls were set under the foam in the box for moisture supply. The plastic box was covered by 100-mesh nylon screen and maintained in the greenhouse conditions for preparing stock culture (Fig. 4).

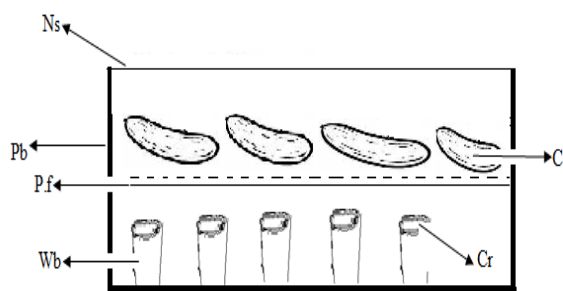


Fig. 4. Lateral view Schematic Persian cucumbers in plastic box: C= Persian cucumber, Cr= Cotton roll, Ns= Nylon screen, Pb= Plastic box, Pf= Perforated foam, Wb= water bottle

Purchased thin Persian cucumbers from local grocery store were washed and cleaned with alcoholic cotton then incubated in 37 °C for one day and replaced every five days with old cucumbers. After five days, 2 males and 1 female from isolated colony were transferred to new boxes with mentioned specifications and in 20×7.5 cm size. After 4-5 days, the adults were removed and larvae were maintained until they were matured. The new adults were regarded as a new generation. The newly matured larvae in each experimental unit were considered as a generation.

Leaf disc

3-1- Each Petri dish (9-cm diameter) with 2-cm diameter mesh-covered holes in its lid for ventilation, was filled with 1-2 mm deep layer of 0.5% agar. Two leaf discs (2-cm diameter) with 2 males and 1 female were placed in each Petri dish. Petri dishes were placed in incubator conditions (27±1°C, 65±5 % R.H and 16:8 L: D) for 4-5 days. Adults were removed and the egg-infested leaf discs were transferred to new Petri dishes

by thin brush to avoid contamination. The Petri dishes were kept in incubator until hatching eggs. Cohorts of even-aged larvae were kept in another Petri dish containing fresh leaf disc until maturation. Every three days, the old leaves were replaced with new ones. The new adults were regarded as a new generation.

3-2- The water bottles were placed under the small plastic box which had a hole on the top. Fabric wick was passed through the box hole, placed inside the water bottle and in contact with filter paper which was on the top of the box. Five cm diameter bean leaf disc surrounded by cotton rolls was placed on filter paper and then the Petri dishes lid (9cm diameter) with mesh-covered hole was used to cover the leaf disc. (Fig. 5).

The adults in each Petri dish were kept for 4-5 days oviposition period. Newly hatched larvae were transferred to fresh leaf discs in Petri dishes and maintained in greenhouse conditions. The new adults were regarded as a new generation. The number of generations was calculated by counting the number of new adults.

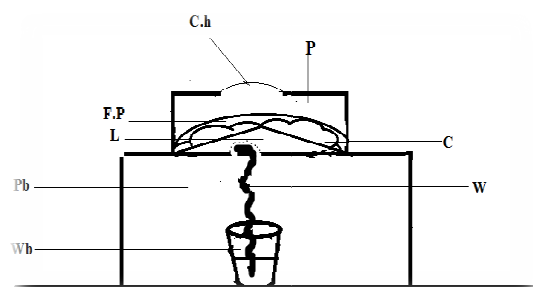


Fig. 5. Lateral view Schematic bean leaf disc, C= Cotton roll, C.h= Covered hole, L= Leaf, F.p= Filter paper, P= Petri dishes lid, Pb= Plastic box, W= Wick, Wb= water bottle

The adults in each Petri dish were kept for 4-5 day oviposition period. Newly hatched larvae were transferred to fresh leaf discs in Petri dishes and maintained in greenhouse conditions. The new adults were regarded as a new generation. The number of generations was calculated by counting the number of new adults.

RESULTS AND DISCUSSION

Results demonstrated that there was a significant difference among various methods of thrips rearing in laboratory conditions ($df=6$, $df=19$, $F= 57.76$, $P= 0.0001$). Based on the results, method 1 and 2-4 showed maximum number of generations (6.05 ± 0.3283 and 6.15 ± 0.4122) and the minimum number of generations (0.35 ± 0.1313) has been observed in method 2-3. There was no significant difference between method 3-1 and 3-2 (2.3 ± 0.3253 and 2.5 ± 0.235) (Fig. 6).

Usually mass rearing of target species in biological experiments is a problem. The problem would be more when dealing with a small and cryptic species such as thrips. Their unique and special characters such as small and easily damaged body and wonderful ability in

hiding and escaping from the gaps make studying them more problematic. It is often necessary to rear an acceptable number of thrips in controlled environments during experiments to provide the healthy colony and cohorts of even-aged larvae in a certain age for serial test and bioassay. Because of the diet, temperature, space stress, lack of suitable humidity and other important factors, insects grown in artificial environments are usually more sensitive than those grown in natural environments; so, mass culture of them poses numerous challenges such as reduction in fecundity, longevity and infection with different types of pollution and disease (Grundy et al., 2000). Many of these risks can be avoided by choosing the best rearing method and managing it properly.

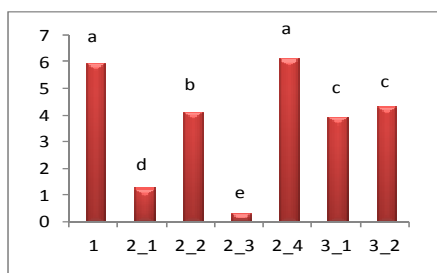


Fig. 6. Mean number of *F. occidentalis* generations in each treatment during 5 months (Different letters between the methods denote that they are significantly different ($P=0.0001$))

In our survey, seven rearing methods in three current groups were compared. Method 2-4 was the best (Table 1). This was inspired by the method described by Degraaf and Wood (2009). However, this method was more time-consuming and had more steps for rearing. Fungus and bacteria-free sterilized Persian cucumbers as a food source that has longer life than bean pods or bean leaves (Degraaf and Wood, 2009), alternative colony for possible dead thrips (Tanigoshi and Nishio-Wong, 1981), a big and suitable area as a life and oviposition place, sufficient and proper ventilation, moisture and temperature as a rearing microclimate (Katayama, 1997; Lublinkhof and Foster, 1997; McDonald et al., 1998.) were the factors that increased thrips generation numbers as well as the efficiency of this method. In addition to being inexpensive, its contamination and other risks are also very low.

In method 1, generation number was not significantly different from method 2-4 (Fig. 6). It had an alternative colony and a big area with sufficient food source for oviposition and life. Also, by transferring infested garden flowers to greenhouse and proper management, we will always have thrips colony in all years. Method 1, by using whole plants (generally used to maintain stock colony or for mass rearing of all life stages in certain time) compared to method 2-4, had some problems such as troublesome in synchronization of cohorts of even-aged larvae, and handling tissue containing eggs. In method 2-4, handling host plant material is easy and desired tissue can be kept until a suitable time (Loomans and Murai, 1997).

Also, method 2-2 is good form of parts of the plant method. Flowers are the best parts of the plants for *F. occidentalis* feeding and oviposition (Lewis, 1973) and compared to other parts of the plants seem to be less susceptible to various fungi and diseases. In this method, Amaranthus flowers due to their specific tissue dry later than other flowers and can be kept for a longer duration. Although this method has some disadvantages like more labor requirements and timely management for replacing flowers, it can be a good rearing method due to alternative colony, big area with sufficient and permanent food by replacing old flowers with fresh ones. There are some problems with leaf discs for thrips rearing; for instance, limited life time, higher vulnerability to contamination and hard and time-consuming transferring but it can be good for rearing individuals for life-history studies (Loomans and Murai, 1997). Method 3-1 is common form of leaf disc method. Agar media is a nutrient solution that can keep the leaves fresh for a few days and so, in this method, there is no need to change the leaves or wet the filter papers daily. Most problems of this method are factors like small area for oviposition and life and insufficient food source. Also, agar media is relatively expensive and susceptible to various diseases and fungi which can cause thrips to die or damage their oviposition. In fact, incubator is not available in all of the situations. The results of method 3-2 did not show a significant difference with method 3-1 (Table 1), but had less contamination and did not need an incubator. In this method, instead of agar which is subject to contamination, fabric wick and filter papers that were made wet by water bottles have been used to keep the bean leaves fresh. In method 2-3, thrips did not survive for a long time which was probably due to bad conditions like a warm place or unpalatable bean pods as a food source. Iranian bean pods seem to be hard for thrips stylet and they couldn't eat from them. Also, the glass jars in the laboratory conditions were highly absorbent of the sun heat which made their inside warm.

In this research, some simple, less-laborious and inexpensive rearing methods were proposed and compared to select the best ones. Any of these methods had their own advantages and disadvantages, but among them, methods 1 and 2-4 were the best for rearing thrips in all year round.

CONCLUSIONS

Mass rearing of a desired insect is usually a boring job in biological experiments. Western flower thrips is one of the most important key pests throughout the world. During five months, seven common rearing methods were compared with each other and results showed that method 2-4 which used Persian cucumbers in plastic boxes (inspired by Degraaf and Wood, 2009) was the best one. By using this method, we can build a live and healthy colony with the least possible equipment.

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مقایسه هفت روش پرورش تریپس غربی گل

Frankliniella occidentalis (Thysanoptera: Thripidae)

نسیمه مرتضوی، مریم آل عصفور*، کامبیز مینایی

بخش گیاهپزشکی، دانشکده کشاورزی، دانشگاه شیراز، شیراز، ج.ا. ایران

*نویسنده مسئول

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واژه های کلیدی:

Frankliniella occidentalis

توسپوویروس

پرورش انبوه

خیار ایرانی

چکیده - گونه‌ی *Frankliniella occidentalis* یا تریپس غربی گل، یک آفت بسیار پلی فاز و ناقل مهم توسپو ویروس ها به بسیاری از محصولات زراعی و گیاهان زینتی می باشد. این تریپس انتشار جهانی داشته و به همین دلیل موضوع بسیاری از مطالعات بیولوژیکی از جمله پرورش انبوه بوده است. در این مطالعه ۷ روش رایج پرورش تریپس غربی گل، به تفصیل شرح داده و با یکدیگر مقایسه شده است. بر اساس نتایج به دست آمده، روش استفاده از خیار ایرانی در ظرفهای پلاستیکی با تولید $6/15 \pm 0/4122$ نسل به عنوان بهترین روش انتخاب شد. این روش قادر به تولید نسل های زیاد تریپس بر روی خیار ایرانی با استفاده از کمترین لوازم است.