An immuno-marking technique for thrips

Poonam Jasrotia & David Ben-Yakir*

Department of Entomology, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel

Accepted: 29 March 2006

Key words: *Thrips tabaci*, *Frankliniella occidentalis*, Thysanoptera, Thripidae, mark–release–recapture, ELISA, protein marking

Abstract

Rabbit immunoglobulin G (R-IgG) was used successfully as an external mark for thrips. Females of both *Thrips tabaci* Lindeman and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) were marked with 1 mg ml^{-1} R-IgG solution with 1% 'Tween 20' by the contact exposure method. Determining the retention of the mark was by running the rinsing solution of individual thrips in an enzyme-linked immunosorbent assay (ELISA). The sandwich ELISA method was used with an additional biotin–avidin step. The threshold for a positive marking score was defined as three times the mean optical density readings of the negative control thrips. Under laboratory conditions, on bean pods, all marked thrips scored positive up to 6 days after marking (DAM). When marked thrips were kept in the laboratory on marigold flowers for 2 days, they all scored positive. When marked and unmarked thrips were placed together on these flowers, the mark was transferred to 10–20% of the unmarked thrips and they became positive. Under field conditions, on sticky traps covered with water-base glue, 100, 80, and 20% of the marked *T. tabaci* scored positive by the 3rd, 6th, and 9th DAM, respectively. Under the same conditions 100, 90, and 10% of the marked *F. occidentalis* scored positive by the 3rd, 6th, and 9th DAM, respectively. The retention of the R-IgG decreased significantly under conditions of wetness and high humidity. After 6 days on chive plants kept at 80–100% r.h., all marked thrips scored negative while on plants kept at 40–60% r.h., 85% of the marked thrips scored positive. Rabbit IgG can be used as an external marker for thrips. The suitability of this marking method for dispersal studies of these important pests needs to be evaluated.

Introduction

Thrips (Thysanoptera: Thripidae) are opportunistic insects exploiting intermittently occurring host plants. They are adapted to succeed in habitats in which optimal conditions are brief (Funderburk, 2002). Thus, thrips migration between habitats is an essential component of their life strategy. Following thrips migration can enhance our understanding of their biology, zoography, and ethology. Insect movement is often studied by following a group of marked individuals. For mark–release–recapture (MRR) studies of insects the marker should be persistent, cost effective, easy to apply, and with no effect on the insects' normal behavior. A wide variety of markers have been used for labeling insects, of which paints, dyes, and tags are the most common (Southwood, 1978; Akey et al., 1991). However, these markers are usually unsuitable for very small insects because they are difficult to apply and often alter the insects' normal behavior (Costa & Byrne, 1988; Naranjo, 1990). Protein markers have been used successfully in MRR studies of several insect pests and natural enemies (Hagler & Jackson, 2001). These markers are especially suitable for small and fragile insects. The protein markers can be applied to a large insect population through contact with moist surface or in a mist form with minimum handling time and efforts (Hagler & Jackson, 1998). These markers can be detected at very low concentrations using an enzyme-linked immunosorbent assay (ELISA).

Thrips are very small sized, highly mobile, and weak fliers. It is expected that marking thrips with paints, dyes, dusts, or tags will inhibit their normal dispersal behavior (Steiner, 1965). Indeed, when thrips were marked with fluorescent dyes, their flight was impeded (Lewis, 1973). New Zealand flower thrips (*Thrips obscuratus*) were successfully marked by incorporating rubidium to their diet (Ellis et al., 1990). However, we have not found any report on the use of this method for MRR studies.

The method used for recapturing externally marked insects is expected to affect the retention of the marker.

*Correspondence: E-mail: benyak@volcani.agri.gov.il

Thrips are often monitored by sticky traps and they may be stuck on traps for several days before they are tested for the presence of the marker. While it seems that marking thrips with fluorescent powder was not affected by sticky traps (Rhainds & Shipp, 2004), the protein marker is expected to be more labile on sticky traps under field conditions.

In this study we evaluated the protein marking method, originally developed by Hagler & Jackson (1998), for thrips. We modified the marking and detection methods to enable the following of single-marked thrips. The retention of the marker on the thrips was evaluated under both laboratory and field conditions.

Materials and methods

The insects

Adults of *Thrips tabaci* Lindeman and *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) were collected from various locations in Israel and kept in laboratory culture for over 2 years using the rearing method described by Loomans (2003). Thrips were maintained on fresh pods of kidney bean (*Phaseolus vulgaris* L.) in round-bottom glass rearing jars (12 cm in diameter × 20 cm high) with a circular piece of foam-rubber sponge at the bottom for pupation. Jars were kept at 25 ± 2 °C, r.h. of 70 ± 5%, and photoperiod of L16:D8.

The marking procedure

The marking was done by the contact exposure method described by Hagler & Jackson (1998) for labeling minute parasitoids. Initially, we compared marking with a distilled water solution of 1 mg of rabbit immunoglobulin G (R-IgG, No. I5006; Sigma Chemical Co., St. Louis, MO, USA) with and without 1% 'Tween 20' (Sigma). Because the addition of 'Tween 20' improved the marking (see Results section), we used it in all further studies. A marking unit consisted of a small Petri dish (3 cm in diameter) containing two layers of filter papers drenched with 500 µl of the marking solution. Each unit was used for marking 50 females. Thrips were removed from the marking dish after 30 min and dried for 1 h at room temperature. Right after drying, these thrips were used for the various studies. Ten marked thrips were placed in a freezer (–20 °C) at the end of each marking session to be used later as positive controls.

Experimental arrangement

In all studies we kept both marked and unmarked (negative control) populations under the same conditions. In each study, at every time interval, we randomly selected thrips from both populations (20 marked/10 unmarked). Unless stated differently, each experiment was run three times (replication in time).

Retention of the marker under laboratory conditions

Laboratory conditions were as described above for the thrips' culture. The retention of the marker was determined for thrips kept on bean pods in rearing jars. There were 16 treatments [thrips species × type of marking solution (with or without 'Tween 20') × marking state (marked vs. unmarked) × retention time] and we used one jar per treatment on each run. There were 40 thrips in each jar. The levels of the markers on the thrips were determined on the marking day, as well as 3 and 6 days after the marking (DAM). Later, the retention of the marker on thrips infesting marigold flowers was studied. We placed a detached flower head in each small Petri dish. There were four treatments (thrips species × marking state) and we used 10 dishes per treatment on each run. We placed one thrips in each dish. The levels of the markers on the thrips were determined only 2 DAM because the detached flowers dried out. We also used these flowers to determine the risk of marker transfer from marked to unmarked thrips. In this study, we placed two thrips in each dish one marked and the other unmarked. When we determined the level of the marker on these thrips, the one with the higher marking level was considered as the marked one.

Retention of the marker under field conditions

The retention of the marker was determined for thrips affixed on sticky traps. Traps consisted of plastic transparency films (35 × 5 cm) covered with either water-base glue ('Thripstick II', Aquaspersions Ltd, Yorkshire, UK) or non-water-base glue ('Rimifoot', Jewnin-Joffe Industry, Petah-Tiqwa, Israel). There were 24 treatments (thrips species × type of glue × marking state × retention time) and we used one film per treatment on each run. We affixed 40 thrips on each film. Affixed thrips were encircled with a marker (on the non-sticky side of the film) to distinguish them from thrips that would be trapped naturally in the field. The films were wrapped around a pole (4″ in diameter) at heights between 1.5 and 2.5 m. The pole was placed in an open field at the Volcani center. There were seven runs (replications in time), of 10 days each, between 3 April and 29 June 2005. The levels of the markers on the thrips were determined 3, 6, and 9 DAM. Climatic conditions during this study were obtained from a local meteorological station.

The retention of the marker was determined for thrips infesting potted chive plants kept in a 50-mesh screen house (Antivirus, Klayman Meteor Ltd, Petah-Tiqwa, Israel). In this study only *T. tabaci* was used because *F. occidentalis* did not develop well on chive. There were eight treatments (humidity level × marking state × retention time) and we used 10 pots of chive infested with 20 thrips per treatment on each run. Half the plants were covered

with plastic jars (15 cm in diameter × 20 cm high) with small screened ventilation openings, in order to achieve high r.h. (85–100%). Uncovered plants had a mean daily r.h. of 60–75%. There were three runs (replications in time), of 10 days each, between 15 May and 15 June 2005. The levels of the markers on the thrips were determined only 3 and 6 DAM because after that time most females had died.

Extracting the marker from the thrips

The markers were extracted from individual thrips by placing each one in an Eppendorf tube (SARSTEDT AG and Co., Nümbrecht, Germany) containing 150 µl of phosphate-buffered saline with 0.05% Tween 20 (pH 7.4; PBS-T). This tube was agitated by hand and then centrifuged at 2822 ***g*** for 5 min. The supernatant collected from each tube was immediately frozen (–20 °C) until used for ELISA. The marker of the thrips that had been on the sticky traps was extracted by cutting a small piece of the film surrounding the randomly selected affixed thrips. Then, we proceeded as above except centrifugation time was extended to 15 min to dislodge the thrips from the film.

ELISA procedure

A sandwich ELISA in a 96-well microplate was used for detecting the marker as described by Hagler (1997a), with a few modifications. Unless stated differently, all reagents were obtained from Sigma Chem. Co. and they were used at 50 µl per well, there were two wells per sample, and plates were incubated for 2 h at 37 °C after each step. Wells were first coated with 1 µg ml^{-1} goat antirabbit IgG (R2004) in carbonate–bicarbonate buffer (pH 9.6). In the following blocking step each well was filled with 100 µl of 1% bovine serum albumin (B4287) in PBS-T and the plates were kept overnight at 4 °C. In the following step, thrips extracts were added. At this step we also added to each plate(s) 3–5 extracts of the positive control thrips (day 0), 5–7 extracts of the unmarked control thrips, and reference solutions of R-IgG in the concentration range of 2.5–10.0 ng ml^{-1} PBS-T. Next, goat antirabbit IgG conjugated to biotin (B8895) diluted 1:10 000 was added. In the following step, avidin conjugated to alkaline phosphatase (B7294) diluted at 1:500 was added. In the last step, alkaline phosphatase substrate (p-nitrophenyl phosphate; N9389) at 1 mg ml^{-1} was added. The optical density was read after 30 min of incubation at 405 nm. The threshold for a positive score was defined as three times the mean optical density (OD) readings of the unmarked (negative) control thrips on each plate, i.e., only thrips that had OD readings equal or above this threshold were considered positive.

Results

In all experiments the unmarked controls had low OD values (overall mean ± SEM ranged between 0.18 ± 0.02 and 0.24 ± 0.07). In most studies there were no significant differences between the results obtained for *T. tabaci* and *F. occidentalis.*

Retention of the marker under laboratory conditions

On bean pods, all thrips that had been marked with R-IgG solution containing 'Tween 20' scored positive up to 6 DAM (Figure 1). However, only 10–20% of the thrips that had been marked without 'Tween 20' scored positive at 6 DAM. The mean OD values at 3 and 6 DAM remained high (above 2.0), which equal an R-IgG concentration of about 10 ng ml^{-1}. On detached marigold flowers, all marked thrips scored positive 2 DAM (Figure 2). When marked and unmarked thrips were placed together, 10–20% of the unmarked thrips scored positive. This indicates some transfer of the marker.

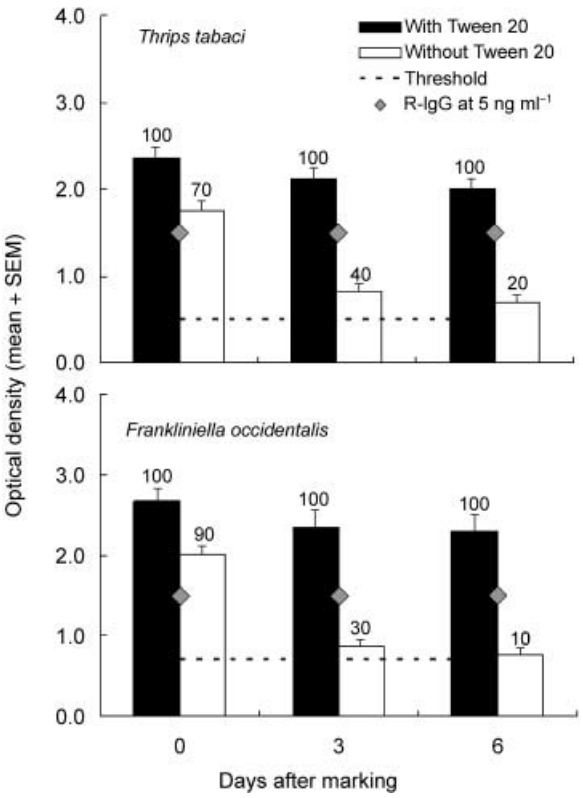

Figure 1 ELISA readings for the retention of rabbit IgG on females of *Thrips tabaci* and *Frankliniella occidentalis* (n = 20) kept on bean pods under laboratory conditions. The numbers above the error bars are the percentage of individuals scoring positive. The unmarked controls (n = 10) were all negative.

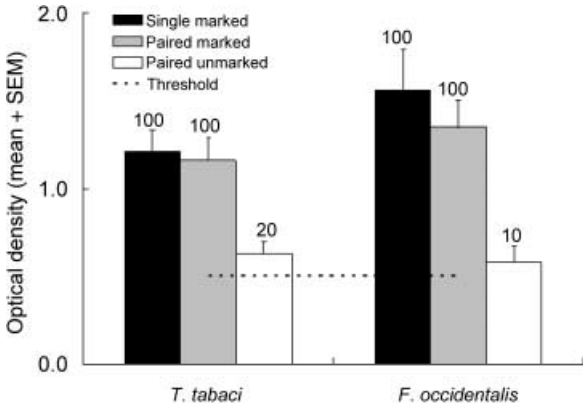

Figure 2 ELISA readings for the retention and transfer of rabbit IgG on marked (n = 20) and unmarked (n = 10) females of *Thrips tabaci* and *Frankliniella occidentalis* kept on flowers under laboratory conditions, 2 days after marking. The numbers above the error bars are the percentage of individuals scoring positive.

Retention of the marker under field conditions

During this study, the mean daily temperature ranged from 18 to 25 °C and the mean daily r.h. ranged from 65 to 75%. On the sticky traps, a gradual decrease in the mean OD values of marked thrips was observed over time (Figure 3). At 6 DAM, 80–90% of the thrips affixed by the water-base glue scored positive while among the thrips affixed by the non-water-base glue less than 10% scored positive. A steep decline in the retention of the marker occurred after 6 days and by 9 DAM as only 10–20% of the marked thrips scored positive.

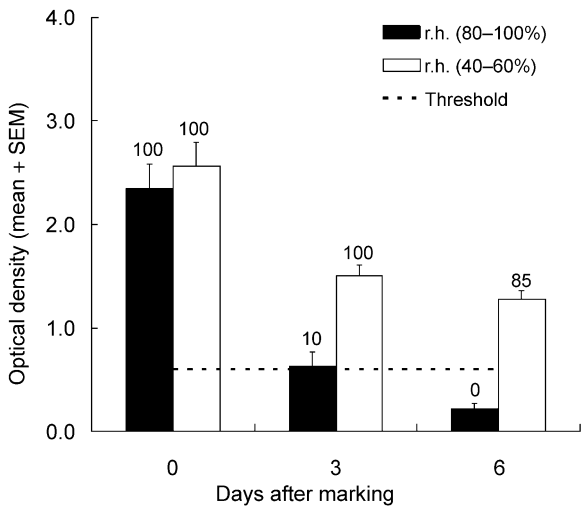

Figure 4 ELISA readings for the retention of rabbit IgG on females of *Thrips tabaci* (n = 20) kept on chive plants in a screen house. The numbers above the error bars are the percentage of individuals scoring positive. The unmarked controls (n = 10) were all negative.

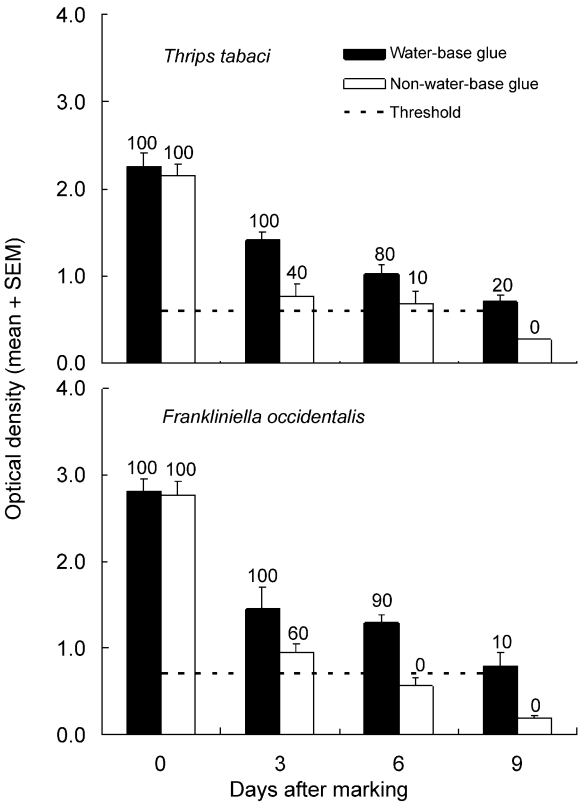

Figure 3 ELISA readings for the retention of rabbit IgG on females of *Thrips tabaci* and *Frankliniella occidentalis* (n = 20) on sticky pole traps under field conditions. The numbers above the error bars are the percentage of individuals scoring positive. The unmarked controls (n = 10) were all negative.

At 6 DAM, 85% of the marked thrips on chive plants kept at low humidity scored positive. In contrast, all the marked thrips on plants kept at high humidity scored negative (Figure 4).

Discussion

Proteins have been used to mark and follow the movement of phytophagous, predatory, and parasitic insects (Hagler et al., 1992; Hagler, 1997b; Hagler & Jackson, 1998). We report here the successful adaptation of a protein marking method for thrips. The marking method we used is quick and gives a uniform marking of a large number of thrips with a small quantity of a relatively inexpensive protein. The addition of the surfactant ('Tween 20') to the marking solution increased the uniformity and the retention time of the marker.

The ELISA procedure is relatively simple and economical. The addition of the biotin–avidin step to the ELISA procedure used by Hagler (Hagler & Jackson, 1998) increased the assay's sensitivity by 3–5 fold (Kendall et al.,

1983). Using this ELISA procedure, we detected R-IgG at concentrations of 3–10 ng ml^{-1} with very low OD readings for negative samples.

The retention time of the R-IgG marker on thrips was about 6 days under field conditions. A similar retention time was reported for R-IgG marked moths of the pink bollworm, *Pectinophora gossypiella* (Saunders) caught with sticky traps (Hagler & Miller, 2002). A much longer retention time, 19 and 21 days, was reported for the sharpshooter, *Homalodisca coagulata* (Blackmer et al., 2004) and the lady beetle, *Hippodamia convergens* (Hagler, 1997a), respectively. Thus, it seems that the retention time of this marker does not correlate with the size of the insect.

The retention time of the water soluble protein we used was significantly shortened under wet or very humid conditions as shown on the chive plants. This may pose a problem because under natural field conditions, thrips tend to reside in hidden parts of plants where humidity is relatively high.

Retention rates were significantly higher when marked thrips were extracted from traps coated with water-base glue compared to these rates from non-water-base glue. However, the use of water-base glue is restricted to dry periods and to fields with no above-ground irrigation.

The retention of the R-IgG marker on thrips may be improved by using a marking solution with higher IgG concentration and adding other surfactant(s). Internal marking may be an alternative method for extending the retention time of the marker, especially under high humidity conditions. Indeed, feeding the minute parasitoid *Anaphes iole* on honey solution containing IgG gave a better retention of this marker in comparison to marking them externally (Hagler & Jackson, 1998). The retention time of IgG ingested by thrips needs to be determined in order to assess this marking method.

The results of this study show that R-IgG can be used as an external marker for females of both *T. tabaci* and *F. occidentalis* for about 1 week. Females of both thrips species are expected to live 2–3 weeks under field conditions (Bailey, 1938). Thus, this marking technique permits tracking female thrips for only part of their lifespan. Usually, adult thrips flight and dispersal occur only at a certain part of their life (Lewis, 1997). The frequency and duration of flight vary with species and gender, and is influenced by the weather, suitability of food, and, possibly, by crowding (Funderburk et al., 2002). Thus, the suitability of this marking method for the study of thrips dispersal needs to be evaluated under field conditions.

Acknowledgements

The authors are grateful to Michael Chen, The Volcani Center, ARO, for his generous assistance. This study was supported partly by the Fresh Herb Growers Association as Project no. 131-1242. This paper is a contribution from the Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel, no. 514, 2005 series.

References

Akey DH, Hayes JL & Fleischer SJ (1991) Use of elemental markers in the study of arthropod movement and trophic interactions. Southwestern Entomological Society, Dallas, TX, USA.

Bailey SF (1938) Thrips of Economic Importance in California. University of California Agricultural Experimental Station Circular no. 346, Berkeley, CA, USA.

Blackmer JL, Hagler JR, Simmons GS & Canas LA (2004) Comparative dispersal of *Homalodisca coagulata* and *Homalodisca liturata* (Homoptera: Cicadellidae). Environmental Entomology 33: 88–99.

Costa HS & Byrne DN (1988) Neutron activation of a rare element to mark sweet potato whitefly, *Bemisia tabaci*. Ecological Entomology 13: 465–467.

Ellis EC, Chapman RB & Penman DR (1990) Rubidium marking of New Zealand flower thrips for dispersal studies. Proceedings of 43rd New Zealand Weed and Pest Control Conference, New Zealand (ed. by AJ Popay), pp. 117–118. New Zealand Plant Protection Society, Dunedin, New Zealand.

Funderburk JE (2002) Ecology of thrips. Thrips and Tospoviruses: Proceedings of the 7th International Congress on Thysanoptera (ed. by R Marullo & LA Mound), pp. 121–128. CSIRO Entomology, Canberra, Australia.

Funderburk J, Stavisky J, Olson S & Momol T (2002) Biology and Economic Importance of Flower Thrips. University of Florida Cooperative Extension Service, Gainesville, FL, USA.

Hagler JR (1997a) Field retention of a novel mark–release–recapture method. Environmental Entomology 26: 1079–1086.

Hagler JR (1997b) Protein marking insects for mark–release–recapture studies. Trends in Entomology 1: 105–115.

Hagler JR & Jackson CG (1998) An immunomarking technique for labeling minute parasitoids. Environmental Entomology 27: 1010–1016.

Hagler JR & Jackson CG (2001) Methods for marking insects: current techniques and future prospects. Annual Review of Entomology 46: 511–543.

Hagler JR & Miller E (2002) An alternative to conventional insect marking procedures: Detection of a protein mark on pink bollworm by ELISA. Entomologia Experimentalis et Applicata 103: 1–9.

Hagler JR, Cohen AC, Bradley D & Enriquezl FJ (1992) New approach to mark insects for feeding and dispersal studies. Environmental Entomology 21: 20–25.

Kendall C, Ionescu-Matiu I & Dreesman GR (1983) Utilization of the biotin/avidin system to amplify the sensitivity of the enzyme-linked immunosorbent assay (ELISA). Journal of Immunological Methods 56: 329–339.

Lewis T (1973) Thrips, their Biology, Ecology and Economic Importance. Academic Press, London, UK.

Lewis T (1997) Flight and dispersal. Thrips as Crop Pests (ed. by T Lewis), pp. 175–196. CAB International, Wallingford, UK.

Loomans AJM (2003) Parasitoids as Biological Control Agents of Thrips Pests. PhD Thesis, Wageningen Agricultural University, Wageningen, the Netherlands.

Naranjo SE (1990) Influence of two mass-marking techniques on survival and flight behavior of *Diabrotica virgifera virgifera* (Coleoptera-Chrysomelidae). Journal of Economic Entomology 83: 1360–1364.

Rhainds M & Shipp L (2004) Dispersal of adult western flower thrips (Thysanoptera: Thripidae) in greenhouse crops. The Canadian Entomologist 136: 241–254.

Southwood TRE (1978) Ecological Methods, 2nd edn. Chapman & Hall, London, UK.

Steiner LF (1965) A rapid method for identifying dye-marked fruit flies. Journal of Economic Entomology 58: 374–375.