The efficiency of several sampling methods for *Frankliniella occidentalis* (Thysan., Thripidae) in strawberry flowers

J. E. González-Zamora¹ and F. García-Marí²

¹Departamento de Ciencias Agroforestales, Universidad de Sevilla, Sevilla, Spain; ²Institut Agroforestal Mediterrani, Universitat Politécnica de València, València, Spain

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Abstract: Methods for sampling western flower thrips *Frankliniella occidentalis* (Pergande) (Thysan., Thripidae) in strawberry flowers were evaluated in three commercial plots by monitoring the population densities of adult and immature thrips throughout the growing season. Three types of flowers were sampled: open, senescent and petal fall, and three procedures were compared: a visual examination of flowers, a turpentine-funnel method and a shake method. Open flowers showed higher population densities of adults, whereas senescent or petal fall flowers had a higher number of eggs and more damage symptoms. Seasonal changes in population density were similar for the three sampling procedures, although the turpentine method recovered more insects, especially larvae, than the other two methods. The turpentine procedure was the most efficient as it extracted almost 100% of adults and, in plots with no chemical sprays, nearly 100% of second instars and 50% of first instars. Regular control of thrips with pesticides reduced the percentage of larvae recovered with this procedure. The visual method spotted 80% of adults and 33% of larvae. The shake method extracted nearly 80% of adults but only 18% of larvae. The visual method is recommended for routine field samplings, especially for sampling adults and in plots regularly sprayed with pesticides, as it constitutes a cost-effective method when time and efficiency are considered.

Key words: *Frankliniella occidentalis*, flowers, sampling methods, strawberries, thrips

1 Introduction

Strawberries are an important crop in Spain, with a production of around 2 000 000 tonnes/year, much of which is exported. Damage by thrips can become a major problem in this crop, the most common species of thrips being the western flower thrips, *Frankliniella occidentalis* (Pergande) (Ribes-Koninckx et al., 1990; González-Zamora et al., 1992). Feeding by thrips causes strawberry flowers to turn brown and wither prematurely. As the fruits develop, thrips feeding may also cause russetting of the fruit receptacle around the achenes and this can become pronounced especially in certain varieties. Different methods have been developed to study the population dynamics of thrips and its natural enemies in strawberry in Spain (González-Zamora et al., 1992; Ribes-Koninckx and Coscolla-Ramon, 1992; González-Zamora et al., 1994). *Frankliniella occidentalis* is usually controlled with insecticides or predators such as anthocorids, which are considered promising biological control agents (González-Zamora et al., 1992).

Efficient and accurate methods for estimating the population density of thrips are of great relevance in research and pest management (Southwood, 1978) considers three categories of sampling methods for thrips on plants: (i) washing methods that extract thrips using a liquid; (ii) mechanical or tapping methods for dislodging thrips and (iii) irritation methods, based on the repellent effect of certain chemicals or heat on insects, causing them to fall inside a collecting device. Washing methods can extract all the insects in the plant, even dead ones, which may result in overestimating the population. Tapping or shaking methods are easily applicable, but part of the population can remain in the plant. In methods that use chemical repellents some individuals or stages that move slowly or are inactive are not well extracted from the sample, which results in underestimating the total number. The use of heat may cause a sample to desiccate quickly and can also kill individuals if the temperature is high (over 52°C). Coloured sticky traps have commonly been employed, but they only capture adults and the correlation between thrips catches and the population in the plant must be interpreted with caution (Lewis, 1997).

Several workers have compared different sampling methods for western flower thrips. On sweet pepper, Shipp and Zariffa (1991) made comparisons between four methods. Counting thrips in all the plant was used as an absolute sampling method of reference. Sampling
blossoms was reported as the most cost-effective method. Garcia et al. (1988) studied the sampling of different arthropods, including *Frankliniella occidentalis*, in cotton. They found apparent deficiencies in each of the three sampling methods tested and suggested a combination of techniques for establishing population trends of *F. occidentalis*. Also in cotton, Rummel and Arnold (1989) found a washing method in the laboratory to be the best for estimating the number of thrips compared with visual, in-field sampling. In apple blossom clusters, Terry and Degrande-Hoffman (1988) studied the accuracy of the 'shake' sampling method for thrips. They compared three types of blossom in their work: pink, open and petal fall. Carnero and Torres del Castillo (1989) found the Berlese-Tullgren funnel to be an accurate and easy method to determine the population densities of *F. occidentalis* in different types of plants.

The aim of this study was to find the best method for sampling the population of western flower thrips on strawberry flowers with procedures that could be applied in field conditions and be used to determine economic threshold values. Three sampling methods were compared: shaking the flowers against a mesh, visual (inspecting each flower with a 10× hand lens) and turpentine as a repellent combined with a funnel collector.

### 2 Materials and methods

#### 2.1 Plots

The study was carried out during 1991 in three plots of commercial strawberries (*Fragaria × ananassa*) of the Chandler variety, located in the province of Valencia (Spain). The plots were named Alginet-1, Alginet-2, and Bolbaite and the area of each plot was between 175 and 395 m². The Alginet-1 plot was a second year plantation and the two others had been planted during the year of the study. The three plots were managed under the normal cultural practices of the area. In Alginet-1 and Bolbaite no pesticides were applied throughout the season, while the Alginet-2 plot received six pesticide applications against thrips during the sampling period.

#### 2.2 Sampling

Three types of flowers were sampled according to their stage of development: (i) 'open', recently opened, with all the petals fresh, anthers starting to open and fresh pistils; (ii) ‘senescent’, with senescent petals and with drying anthers and styles; and (iii) 'petal fall', with no petals, anthers completely dried, and ovaries starting to swell.

Three sampling procedures were used: (i) 'shake', in which flowers held upside down were shaken (tapped) 15 times against a mesh that rested on a Petri dish base in which a layer of dishwasher soap was spread on the interior surface. The mesh was 1 cm above the Petri dish base, which avoided contact between the flowers and the soap. After the thrips from the flower got stuck on the soap, the Petri dish was covered and carried to the laboratory where all the thrips were counted under a microscope; (ii) 'visual', in which the flower was carefully examined *in situ* to count all the thrips that could be seen with the help of a magnifying lens (10×) and (iii) 'turpentine' in which 10 flowers were placed on a mesh located in the upper third of a plastic funnel, and a lid containing a cotton bud soaked in turpentine was placed on top of the funnel. After 1 h the insects were collected in a vial with 70% ethanol placed in the narrow end of the funnel. The contents of the vial were examined in the laboratory, with the aid of a microscope.

The three procedures were evaluated with the three types of flowers, using 10 flowers for each sampling procedure, thus giving 90 flowers collected on each sampling date. The plots were sampled once a week. Flowers were always selected at random, walking in zigzag around the plot. A total of 2880 flowers were sampled during this study and 13 397 thrips were counted.

Each of the three methods was judged on the basis of its efficiency compared with absolute counts. The total number of thrips in the flower was obtained by completing the extraction of flowers previously extracted by another method. As we did not know previously which was the best, two procedures were considered and compared: (i) 'ethanol', in which each flower previously sampled by the 'shake' method was placed in a separate plastic vial with 70% ethanol and carefully examined in the laboratory under the microscope and (ii) 'Berlese', in which the 10 flowers used in the 'turpentine' procedure were taken to the laboratory inside a paper bag lined externally with plastic and placed in a Berlese funnel with a 25 W incandescent bulb for 2 days. The bags were carefully checked for thrips stuck to the inside. Thrips extracted by the funnel were collected in a vial with 70% ethanol.

Counts of the first and second instar larval and the adult thrips were then made. In addition, for the 'ethanol' procedure, the eggs of thrips inserted in the flower calyx were also counted by examining the calyx under light and the symptoms of injury by thrips were estimated. Each flower was given a relative index of damage according to the following scale: 0, no damage; 1, small rust spots in the base of the stamens, pistils and achens; 2, larger rust spots, extending to the sepals and areas between stamens; and 3, rust areas all around the flower receptacle and necrosis of sepals. The adults were always identified to the species level and 95% of them belonged to the species *F. occidentalis*. Most of the remaining thrips were *Thrips tabaci* Lindeman. Only *F. occidentalis* adults were considered in this study, but larvae were not identified, so they probably included a small proportion of other species.

#### 2.3 Statistical analysis

All statistical tests were performed using SAS/STAT statistical software (SAS Institute, 1990). Data on the number of thrips in different types of flowers and on the percentage of thrips captured by each sampling procedure and type of flower were analysed by one-way *ANOVA*. The LSD-test was used to find differences between mean values. Percentages were transformed (arcsine) to stabilize the variance. The comparison of the two methods considered to collect all the insects was carried out using Student’s *t*-test for paired samples.

### 3 Results and discussion

#### 3.1 Abundance of thrips in relation to flower age

The abundance and development stage of the thrips varied depending on the age of the flower (table 1).
Adults were significantly more abundant in ‘open’ flowers \( (F = 31.12; \text{d.f.} = 2, 90; P < 0.0001) \), whereas larvae were found equally among the three types of flowers studied \( (F = 1.39; \text{d.f.} = 2, 90; P = 0.256) \). Adults prefer pollen as a food source \((Trichilo\text{ and }Leigh, 1988)\) and consequently they are more abundant in flowers that have recently opened. As larvae feed preferentially on plant tissues, they stay on the flower for longer periods; larvae move easily between flowers, from older flowers to younger flowers, and densities of larvae can be much higher than those expected based on the density of eggs found in the buds \((Terry, 1991)\). Terry and Degrandi-Hoffman \((1988)\) also found more adult \(F. \text{occidentalis}\) in open apple blossoms than in the petalless buds.

There were significantly fewer eggs in flowers recently opened than in older flowers \((table \ 1; F = 5.74; \text{d.f.} = 2, 75; P = 0.006)\). This is presumably a consequence of the cumulative effect of egg laying, with eggs increasing in numbers as the flowers develop, a result also found by Terry \((1991)\).

‘Senescent’ or ‘petal fall’ flowers have allowed more time for female thrips to lay their eggs than ‘open’ flowers. Many eggs still remain unhatched when flowers develop into fruitlets. Damage by adult and larval thrips, also increases as the flower ages and is maximum in ‘petal fall’ flowers \((F = 24.97; \text{d.f.} = 2, 72; P < 0.0001)\).

### Table 1. Influence of flower development on the mean (±SE) number of thrips per flower and damage index \((n = 32 \text{ samples})\). Data obtained from the shake plus ethanol method of extraction

<table>
<thead>
<tr>
<th>Type of flower</th>
<th>Eggs</th>
<th>Larvae</th>
<th>Adults</th>
<th>Damage index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open</td>
<td>6.98 ± 1.58 a</td>
<td>3.53 ± 0.94 a</td>
<td>4.23 ± 0.64 a</td>
<td>0.54 ± 0.09 a</td>
</tr>
<tr>
<td>Senescent</td>
<td>12.35 ± 2.81 b</td>
<td>3.93 ± 0.89 a</td>
<td>2.20 ± 0.36 b</td>
<td>1.15 ± 0.15 b</td>
</tr>
<tr>
<td>Petal fall</td>
<td>10.77 ± 2.39 b</td>
<td>2.60 ± 0.42 a</td>
<td>0.56 ± 0.09 c</td>
<td>1.23 ± 0.15 b</td>
</tr>
</tbody>
</table>

Values in column with the same letter are not statistically different \((P > 0.05)\).

### 3.2 Population trend of \(F. \text{occidentalis}\) throughout the season

Adults of \(F. \text{occidentalis}\) showed similar population density and seasonal changes in abundance with the three procedures tested in this study \((figs \ 1 \text{ and } 2a)\). Seasonal changes in the larval population throughout the season were also similar for the three procedures, but population densities were different. Immature populations were always higher when evaluated with the ‘turpentine’ procedure, followed by the ‘visual’ procedure, and the lowest larval population was found with the ‘shake’ procedure \((figs \ 1 \text{ and } 2b)\). Differences between procedures were especially important when populations were high, as in Alginet-1 in April and May.

In Alginet-1 \((fig. \ 1)\) the population of larvae and adults was low until the end of winter, but there was a sharp increase in the population of larvae at the beginning of April, quickly followed by a decrease in May. The adults had a first peak in April, at the same time as the larvae, and their numbers increased again at the end of the season, just when the crop finished.

![Fig. 1. Seasonal population density of Frankliniella occidentalis adults (a) and larvae (b) on strawberry flowers in two plots, Alginet-1 (continuous line) and Alginet-2 (broken line), with three sampling methods: turpentine funnel (●), shake (□) and visual (×)](image)

The Alginet-2 plot \((fig. \ 1)\) was treated repeatedly \((six \text{ times})\) against thrips during the monitoring period. Unlike the observations in the other two plots, there was a higher number of adults than larvae right through the sampling period. This could reflect the higher sensitivity of larvae to pesticides and the higher mobility of adults, which can reinfest the strawberry flowers from nearby plants and crops.

In Bolbaite \((fig. \ 2)\) the population of adults and larvae was low throughout the sampling period, and there was no larval peak. There were more adults than larvae at the end of the period, in June-July.
Overall, the population trend of *F. occidentalis* observed was similar to previous reports in strawberries, with an increase in spring, beginning in March–April, and with high populations until June–July, when numbers decreased (González-Zamora et al., 1992; Ribes-Koninckx and Coscolla-Ramon, 1992).

### 3.3 Efficiency of the three sampling procedures

There were no differences between the types of flowers in the efficiency of extraction. The relative number of thrips extracted by each procedure as a percentage of the total number of thrips extracted by the sum of the three procedures was similar in the three types of flowers for the shake method ($F = 1.14$; d.f. = 2, 75; $P = 0.326$), for the visual method ($F = 0.15$; d.f. = 2, 75; $P = 0.865$) and for the turpentine method ($F = 0.36$; d.f. = 2, 75; $P = 0.696$) (table 2). Accordingly, the comparison in efficiency between procedures was calculated by putting together the 10 flowers of each type, 'open', 'senescent' and 'petal fall', and considering the 30 flowers collected in each date as a unique sample.

To calculate the absolute efficiency of the three procedures it was previously necessary to obtain the total number of thrips in the flower. The comparison of the mean number of thrips recovered by the two methods intended to collect all the insects, from 32 samples of 30 flowers, 10 of each type (table 3), showed no significant differences between those with second instars (paired *t*-test; $t = -0.15; P = 0.88$) and adults (paired *t*-test; $t = 0.19; P = 0.85$). However, the 'shake plus ethanol' method recovered more first instars than the 'turpentine plus Berlese' (paired *t*-test; $t = 3.67; P = 0.001$). Accordingly, the average of the two methods was used as the reference value for the total number of second instars and adults in the flower, and the 'shake plus ethanol' method was used as the reference value for the total number of first instars and total larvae (first and second instars) in the flower.

Compared with these reference values, recovery percentages were calculated separately for each plot, sampling procedure and thrips development stage (table 4). Combining the three sampling procedures, there were significant differences in the average efficiency depending on the plot ($F = 3.51$; d.f. = 2, 298; $P = 0.03$): the Alginet-2 plot had a lower efficiency rate in recovering thrips (44.9%) compared with the other two plots, Alginet-1 and Bolbaite (59.8 and 58.4% respectively), because of the low extraction of larvae with the turpentine procedure (39.7%), whereas it was
80.7 and 87.8% in Alginet-1 and Bolbaite, respectively; table 4). Chemical sprays were regularly applied to Alginet-2 and dead larvae or those affected by pesticides were detected and counted with the shake and visual procedures, but in the extraction with turpentine those dead or dying larvae were not recovered.

Considering only the two plots with no chemical sprays (Alginet-1 and Bolbaite), the efficiency in the extraction of thrips (all developmental stages) differed significantly between the three procedures (extraction of thrips (all developmental stages) differed sprays (Alginet-1 and Bolbaite), the efficiency in the procedures, but in the extraction with turpentine clusters. Rummel and Arnold (1989), in a trial in which instars were not separated in the countings, stated that the visual method was less appropriate for counting thrips in cotton than a washing method. Shipp and Zarifia (1991) found that sampling flowers in greenhouse sweet pepper and observing them visually was the most cost-effective sampling method for determining the adult population of F. occidentalis. Peasall and Myers (2000) found that the beating of branches, the flicking of buds and visual estimation methods were not accurate for estimating F. occidentalis in nectarine buds, and that the best method was to collect nectarine buds and count the thrips in the laboratory.

The relationship between the abundance of F. occidentalis in flowers (measured as the average of the two absolute methods for all adults and larvae in 30 flowers) and the relative efficiency of the three procedures was also analysed. A linear regression between abundance and efficiency was calculated with 51 samples from the plots Alginet-1 and Bolbaite. The results showed that the two parameters were not related in any of the three procedures. It was found that shake ($F = 3.10$; d.f. = 1, 49; $P = 0.08$), visual ($F = 0.25$; d.f. = 1, 49; $P = 0.61$) and turpentine methods ($F = 0.26$; d.f. = 1, 49; $P = 0.61$) extracted the thrips with similar efficiency across the range of thrips population densities, in this study.

In conclusion, the changes in abundance in different thrips stages and damage as flowers become older highlights the importance of carefully defining the type of flower to be sampled when developing a sample procedure in order to avoid variability in the results. The type of flower to be sampled will depend on the objectives of the sample. If it is directed at estimating larvae and adults, then recently opened flowers should be sampled. If we are trying to estimate eggs or damage, senescent or petal fall flowers are the best choice.

The best method for sampling thrips populations in strawberry flowers in the field was the turpentine procedure, but taking into account the material needed to make the extraction and the time needed for processing the samples in the laboratory, the visual method is cost-effective considering time and efficiency, especially for sampling adults and in plots usually sprayed with pesticides where larvae are not well extracted with dynamic procedures.

References


Authors’ addresses: J. E. Gonzalez-Zamora (corresponding author), Departamento de Ciencias Agroforestales, Universidad de Sevilla, Carretera de Utrera, km 1, E-41013, Sevilla, Spain; F. Garcia-Mari, Institut Agroforestal Mediterrani, Universitat Politècnica de València, Cami de Vera, 14, E-46022, Valencia, Spain. E-mail: fgarciam@caf.upv.es