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Accuracy, Precision, and Economic Efficiency for Three Methods of Thrips (Thysanoptera: Thripidae) Population Density Assessment

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ABSTRACT Western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), is a major horticultural pest and an important vector of plant viruses in many parts of the world. Methods for assessing thrips population density for pest management decision support are often inaccurate or imprecise due to thrips' positive thigmotaxis, small size, and naturally aggregated populations. Two established methods, flower tapping and an alcohol wash, were compared with a novel method, plant desiccation coupled with passive trapping, using accuracy, precision and economic efficiency as comparative variables. Observed accuracy was statistically similar and low (37.8–53.6%) for all three methods. Flower tapping was the least expensive method, in terms of person-hours, whereas the alcohol wash method was the most expensive. Precision, expressed by relative variation, depended on location within the greenhouse, location on greenhouse benches, and the sampling week, but it was generally highest for the flower tapping and desiccation methods. Economic efficiency, expressed by relative net precision, was highest for the flower tapping method and lowest for the alcohol wash method. Advantages and disadvantages are discussed for all three methods used. If relative density assessment methods such as these can all be assumed to accurately estimate a constant proportion of absolute density, then high precision becomes the methodological goal in terms of measuring insect population density, decision making for pest management, and pesticide efficacy assessments.

KEY WORDS *Frankliniella occidentalis*, sampling, accuracy, precision, efficiency

The western flower thrips, *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), causes feeding damage on a wide range of agricultural crops and serves as a vector for tospoviruses such as Tomato spotted wilt and Impatiens necrotic spot (Robb 1989). Integrated pest management (IPM) can be used to monitor and control pests such as western flower thrips, maintaining populations below an economic threshold (Stern et al. 1959). Knowledge of pest densities resulting from an accurate and precise sampling plan is necessary for successful IPM. Evaluations of pesticide efficacy, also crucial to IPM in terms of pesticide selection, resistance management, and plant selection (breeding), are likewise dependent on meaningful sampling protocols.

Accurate and precise sampling for western flower thrips and related species is often complicated by inherent aspects of thrips biology and population dynamics. At the individual level, thrips seek out tight crevices (exhibiting positive thigmotaxis), such as developing composite flowers and leaf axils, for feeding and oviposition (Mallmann 1959, Lewis 1997). This biological attribute may contribute to low sampling accuracy (depending on the method used) because a proportion of insects will be always be visibly unapparent, necessitating extraction by some means. In addition, populations of thrips, and especially western flower thrips, are usually highly aggregated rather than randomly distributed, both among and within individual host plants (Shipp and Zariffa 1991, Salguero Navas et al. 1994, Cho et al. 1995, Joost and Riley 2004). The power law (Taylor 1961 states that variance within population samples accelerates as a fractional power of the mean population density, has been used many times to characterize and corroborate this aggregative nature of western flower thrips populations (Taylor et al. 1998). Categorical (treatment) comparisons using density assessments of such clumped, or aggregated, populations are often hampered by high variation (Joost and Riley 2004), resulting in apparent statistical similarity. Clearly, a meaningful western flower thrips sampling method is necessary for various aspects of IPM, but biological and logistic challenges to its development exist. Many different sampling methods have been used for western flower thrips in the past. Southwood (1978) attempted to divide all sampling methods for thrips on plants into three categories: 1) liquid washing methods, 2) mechanical dislodgement methods such as tapping, and 3) desiccation and/or irritation methods coupled with some collection device. Washing methods have the poten-

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Table 1. Thrips sampling methods, within an open population greenhouse study, compared by overall mean thrips recorded, ability to detect apparent effects of block, bench quadrant, and week on thrips density (*P* values from Kruskal–Wallis test shown), RV, and RNP

Sampling method	Overall mean ± SEM	Block effect	Bench quadrant effect	Week effect	RV[a] ± SEM	RNP[b]
Tap	2.61 ± 0.18a	0.001*	0.002*	0.004*	18.8 ± 0.81a	177
Alcohol wash	1.91 ± 0.14b	0.005*	0.966	0.004*	25.6 ± 1.79b	15
Brown bag	2.74 ± 0.26a	<0.0001*	0.005*	0.195	25.3 ± 2.38b	56

Values within columns followed by the same letter are not significantly different ($P > 0.05$; Tukey's HSD test). Asterisk (*) indicates significant effect, as detected via nonparametric Kruskal–Wallis test ($\alpha = 0.05$).

[a] RV, (SEM/mean) × 100.

[b] RNP calculated as 100/[(mean RV) × (cost)] (see text).

tial to capture all thrips on a sample and have been used as an absolute measurement of total thrips on plant samples (Warnock and Loughner 2002, Palumbo 2003, Joost and Riley 2004, Liu and Chu 2004, Aliakbarpour and Md Rawi 2010). It has been suggested that washing methods can even overestimate thrips density because dead insects can be extracted and counted (Gonzalez-Zamora and Garcia-Mari 2003). Tapping or shaking methods have been criticized as underestimates because only easily dislodgeable thrips are harvested and counted (Gonzalez-Zamora and Garcia-Mari 2003). Previous work with *Thrips tabaci* Lindeman (Shirck 1948, Edelson 1985) used desiccation coupled with funnel collection as an absolute measurement, and Carnero and Torres del Castillo (1989) found a similar method to be the most accurate for western flower thrips on several species of plants. In this study we compare and discuss several western flower thrips population density assessment methods, one from each of the categories of Southwood (1978), in terms of accuracy, precision, and economic efficiency.

Materials and Methods

Experimental Units. *Accuracy Comparison.* Mature (containing pollen) flowers were removed from containerized (15-cm-diameter) floriculture chrysanthemum, *Chrysanthemum* × *morifolium* Ramat ('Limit', Grolink Plant Company, Oxnard, CA), plants. Three flowers were then situated within each of 45 arenas, composed of a screened (No-Thrips, Bioquip Products, Rancho Dominguez, CA) plastic food container (11 cm in diameter by 10.5 cm; Bare Deli Containers, Solo, Urbana, IL) containing three glass vials (1.5 cm in diameter by 4.5 cm; Fisherbrand 1 dram vial 03-338A, Thermo Fisher Scientific, Waltham, MA) filled with water and sealed with a paraffin square (Parafilm, Pechiney Plastic Packaging, Menasha, WI); one flower was inserted into each vial via a small perforation in the paraffin. Exactly 15 adult western flower thrips were filter-aspirated by mouth from an open population on greenhouse-grown roses, *Rosa* 'Radcon': Rainbow Knockout, into 45 plastic-screened (No-Thrips) tubes (2-ml Boil-Proof Tube, Genemate/Bioexpress, Kaysville, UT). Each tube was then snapped into place over a hole in each arena, allowing the 15 thrips within to venture out and colonize the flowers over a period of 48 h while arenas were housed within greenhouse-associated laboratory or office space (ambient fluorescent lighting, ≈22°C). Each arena represented one experimental unit.

Open-Population Greenhouse Study. A floricultural crop, *Zinnia elegans* L. ('Short Stuff', Goldsmith Seeds, Inc., Gilroy, CA), was grown hydroponically, by using drip emitter irrigation, on four expanded-metal benches in a small (≈380-m^2) greenhouse on the University of California–Davis campus during late summer. There were 72 plants (15-cm-diamter pots, 20-cm spacing between pots) per bench for a total of 288 plants in the greenhouse. Each plant was labeled as to its position relative to the cooling pad (Table 1) and its quadrant position on the bench relative to the cardinal directions (NW, SW, SE, and NE). Environmental conditions were moderate (23.9°C ± 4.4°C and 50–80% RH). A naturally occurring western flower thrips population was augmented in the greenhouses through weekly releases of 1,000 adult thrips (250 released per bench) collected from heavily infested field alfalfa, *Medicago sativa* L., on the University of California–Davis campus. Five of these releases were made before initiation of sampling. Thrips were allowed to disperse throughout the greenhouse from the point of release, resulting in an open and naturally aggregated population. Each plant represented one experimental unit.

Sampling Methods. Each experimental unit was assigned to one of three population density assessment methods: mechanical tapping of flowers over white paper, maceration of flowers in an alcohol wash followed by removal of flower parts and examination of the filtrate from the resulting suspension, and desiccation of flowers within paper bags coupled with sticky card trapping. Therefore, in the accuracy comparison there were 15 arenas per treatment, and in the open-population greenhouse study there were 24 plants per treatment per bench, or 96 plants per treatment total. For all methods used, the sampling unit consisted of three mature (containing pollen) flowers and the response variable was the total number of western flower thrips (adults plus pupae plus larvae). In the open-population greenhouse study, the sampling frequency was 1 wk, and the sampling day and time were the same throughout the study.

The tap method was adapted from a thrips sampling study in greenhouse cucumbers (Boll et al. 2007) and was quite similar to methods used in tomato, *Solanum lycopersicum* L. (Joost and Riley 2004), and straw-

berry, *Fragaria* × *ananassa* Duchesne (Gonzalez-Zamora and Garcia-Mari 2003) fields. Three mature flowers were removed from each unit and, while held with corolla facing down, sequentially struck three times each with a bamboo rod over a white paper boxtop. The number of total dislodged thrips was then recorded.

The alcohol wash method used in this study was from existing published protocol (Robb 1989) for western flower thrips on floricultural crops. Similar methods have often been used for thrips sampling on other greenhouse crops (Warnock and Loughner 2002, Ugine et al. 2006, Boll et al. 2007); on fieldgrown tomatoes (Salguero Navas et al. 1994, Cho et al. 1995, Joost and Riley 2004); on fieldgrown onions, *Allium cepa* L. (Liu and Chu 2004); on fieldgrown lettuce, *Lactuca sativa* L. (Palumbo 2003); and on mango (*Mangifera indica* L.) inflorescences (Aliakbarpour and Md Rawi 2010). In all cases, the sampling unit was removed in situ, placed in a collection vessel containing 70% ethyl alcohol or, rarely, water (Liu and Chu 2004); agitated or macerated to dislodge thrips; and the filtrate from the suspension later examined under a dissecting microscope in the laboratory. For this study, three mature flowers were removed from each unit and immediately enclosed within a 110.9-ml polystyrene vial (30-dram Polystyrene Clear Vials, United States Plastics, Inc., Lima, OH) containing ≈40 ml of 70% ethyl alcohol. In the laboratory, the contents of these vials were manually macerated with steel forceps to separate and remove all flower parts. The resulting alcohol suspension was then filtered through standard coffee filter paper (100 g/cm^2) by using a Buchner funnel and a laboratory vacuum line. Finally, the number of thrips on the filter paper was counted and recorded, facilitated by a dissecting microscope.

The desiccation or collection method reported here has been used previously as a population density assessment device for *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) (Kaspi and Parrella 2003), and in various insecticide performance trials (M.P.P. laboratory, unpublished). The general method, hereafter referred to as the brown bag method, involved the placement of the sampling unit (e.g., flowers, leaves, or entire plants) into an opaque brown paper bag containing a portion of a yellow sticky card (Horiver Pest Monitor, Koppert Biological Systems, Inc., Romulus, MI). The sealed bag and its contents were then stored in a low-humidity environment for 1–3 wk, allowing for plant desiccation, insect emergence, emigration, and arrestment on the sticky card. The card was then removed, and trapped insects were counted and recorded. For this study, three mature flowers were removed from each unit and immediately enclosed within bags containing one sticky card portion (5 by 10 cm) affixed to the upper inside of the bag via staples. Bags were then sealed, moved to the laboratory (≈23°C and 20–30% RH) and stored on shelves for 2 wk, at which time cards were removed and examined, and the number of trapped western flower thrips was recorded.

Methods Comparisons. *Accuracy Comparison.* During the sampling period, when flowers were removed from the arenas, the number of western flower thrips remaining inside the arena was carefully recorded. This number was subtracted from the 15 western flower thrips known to have been released to determine how many western flower thrips were present on samples. Accuracy (as percentage) was determined by dividing the number of western flower thrips recovered from the sample by the number known to have been present on the sample. Accuracy was compared across methods by means of one-way analysis of variance (ANOVA). All data handling and statistical analysis was performed using the software package JMP version 8 (SAS Institute 2008).

Open-Population Greenhouse Study. The three sampling methods were first compared by considering the distribution of data resulting from each for overall mean, SE of the mean (SEM), normality, and number of zeroes recorded. The overall mean number of western flower thrips recorded by each method was compared by the Kruskal–Wallis nonparametric test (Zar 1996). Next, to determine the utility of each method for facilitating the detection of apparent effects of week, block and bench quadrant on the number of western flower thrips recorded, nonparametric analyses for these effects were performed on the raw data by using the Kruskal–Wallis test. Mean separations, where applicable, were conducted using Tukey's honestly significant difference (HSD) test (Zar 1996). All data handling and statistical analysis were performed using the software package JMP version 8 (SAS Institute 2008).

Sampling precision was expressed as the relative variation (RV) of each method over time and space. The RV values were calculated as $RV = (SEM/mean) \times 100$. Smaller RV values indicated higher levels of precision. To show differences in levels of precision, mean RV for each method was calculated by averaging the different spatiotemporal RV (block × week), and then compared using ANOVA and Tukey's HSD test. Economic efficiency was expressed as relative net precision (RNP), calculated as $100/[(mean\ RV) \times (cost)]$, with mean RV as above, and cost as person-hours per sample (Buntin 1994, Joost and Riley 2004). Larger RNP values indicated greater economic efficiency.

Results

Accuracy. There was no difference in terms of sampling accuracy (no. thrips recovered/no. thrips on sample) between methods ($F_{2,42} = 1.89$; $P = 0.164$). The highest observed mean accuracy resulted from the alcohol wash method (0.536 ± 0.044) and the lowest from the brown bag method (0.378 ± 0.074). Observed accuracy was intermediate within tap method samples (0.483 ± 0.054). Recorded zeroes were low overall, with none from the alcohol wash, one from the tap method, and two from the brown bag method.

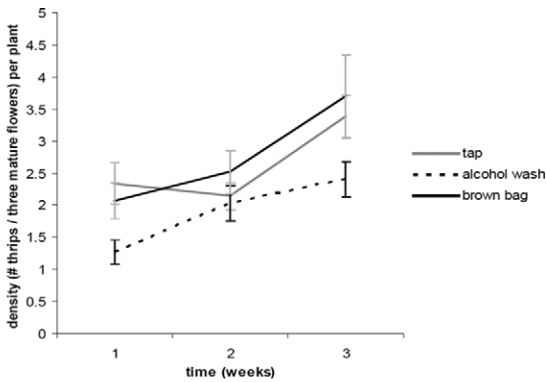

Fig. 1. Mean density of western flower thrips over time in an open and aggregated population on floricultural zinnia, *Z. elegans*, in a hydroponic greenhouse in Davis, CA, as reflected by three different sampling methods.

Open-Population: Distributions and Effects Tests. Mean western flower thrips recovered per unit was highest (2.74; $n = 249$) using the brown bag method, lowest (1.91; $n = 234$) using the alcohol wash method, and intermediate (2.61; $n = 266$) using the tap method; the SEM was likewise highest for the brown bag (0.26), lowest for the alcohol wash (0.144), and intermediate for the tap (0.175) methods (Table 1). All methods yielded distributions that were highly non-normal ($P < 0.0001$; Shapiro–Wilk W). All methods resulted in many recorded zeroes (no thrips encountered). Highest incidence of zero records (66.3%) was in the brown bag dataset, lowest (32.9%) in the alcohol wash, and intermediate (46.2%) in the tap data set. Overall mean recorded western flower thrips was significantly higher when using the tap and brown bag methods than when using the alcohol wash method ($\chi^2 = 9.74$, df = 2, $P = 0.008$) (Table 1). Graphical representation of western flower thrips density over time shows a similar, increasing trend portrayed by data resulting from each sampling method, but with significantly more thrips recovered using the brown bag and tap methods than the alcohol wash during weeks 1 and 3 (Fig. 1). All three sampling methods were effective in detecting a block effect on western flower thrips density (Table 1). The alcohol wash method was unable to detect an apparent effect of bench quadrant on western flower thrips density, and the brown bag method was unable to detect an apparent effect of week on western flower thrips density (Table 1).

Open-Population: Precision and Economic Efficiency. The tap method required 3 h with one worker (1.88 person-minutes, or 0.03 person-hours per sample) for the sampling of all 96 units (U). The alcohol wash method required 1 h with one worker for greenhouse sampling, 16 h with one worker for laboratory processing, and 8 h with one worker for microscope counting and recording (15.63 person-minutes, or 0.26 person-hours per sample) for all 96 U. The brown bag method required 2 h with two workers for greenhouse sampling and bag sealing, and 3 h with one worker for counting and recording (4.38 person-minutes, or 0.07 person-hours per sample) for all 96 U. The overall RV was lowest (18.8; $n = 12$) in data from the tap method and highest (25.6; $n = 12$) in data from the alcohol wash (Table 1). The tap method RV was significantly lower than the RV of the other methods ($F = 4.66$, df = 2, $P = 0.016$). The overall RNP of the tap method (177) was almost 12 times higher than that of the alcohol wash method (15), and >3 times higher than that of the brown bag method (56) (Table 1). This large difference was attributed to the low cost (0.03 person-hours) per sample and low RV using the tap method coupled with the high cost (0.26 person-hours) per sample using the alcohol wash method. There was no statistical difference between RV of the brown bag and alcohol wash methods, but cost per sample using the brown bag method (0.07 person-hours) was 3.7 times lower.

Discussion

The low mean recorded using the alcohol wash method for open-population samples was surprising because similar methods have been touted as measurements of absolute density for western flower thrips (Warnock and Loughner 2002, Palumbo 2003, Joost and Riley 2004, Liu and Chu 2004) and considering that the alcohol wash method resulted in the highest accuracy observed (53.6%) when thrips populations per sample were known beforehand. Generally, however, washing methods require several processing steps, and the possibility exists that through human error, protocol inefficiencies, or both western flower thrips may be lost when inadvertently discarded with plant debris or when stuck on filter materials. For example, Warnock and Loughner (2002) reported only a maximum 27.8% recovery rate of known numbers of released western flower thrips by using a similar wash technique. Interestingly, and as expected, the alcohol wash method resulted in the lowest incidence of recorded zeroes, indicating that it was the best method for extraction of difficult-to-dislodge western flower thrips individuals. Within the open-population study, the overall high mean recorded using the tap method was surprising considering that only easily dislodgeable thrips were sampled. One possible reason for this high mean could be an outlier in week 1, when one unit in the tap method treatment yielded 22 western flower thrips. The brown bag method resulted in the highest mean number of recovered western flower thrips during week 1, three and overall within the open-population study, suggesting that it represented the most accurate estimate of absolute density. When density was known beforehand, however, observed accuracy was lowest within brown bag samples (37.8%). One possibility for this discrepancy may lie within specific protocol differences. Brown bags were stapled closed during the accuracy comparison and sealed with tape during the open-population study. Gaps between staples could have allowed for escape of small, thigmotactic western flower thrips. It is possible, however, that the tape-

sealed bags may have allowed for some escape as well, because the highest incidence of recorded zeroes was seen in the brown bag samples during the open-population study as well as during the accuracy comparison. Whether an absolute measurement method exists in this particular system remains to be seen. Given the poor accuracy exhibited by these three methods, it may be prudent to consider all thrips population density assessments, especially on structurally complex, three-dimensional sample units, as relative measurements. Techniques yielding absolute density measurements are expensive to implement, and so are rarely used for management purposes; more often, relative estimate techniques are calibrated to absolute density through ratios or equations (Pedigo 1989). Those workers which do attempt an absolute insect density measurement sometimes concede it is "assumed" (Browde et al. 1992) or a "rough estimate" (Horton 1994).

The appropriate sampling method for western flower thrips should depend on goals, resources available, and the level of precision required. For example, at the low densities evident in this study, direct damage was not observed, and so a hypothetical treatment threshold, corresponding to prevention of the economic injury level, was not reached. However, western flower thrips-vectored tospovirus are known pathogens of *Z. elegans* (Morales-Diaz et al. 2008), and so in the presence of such viruses, treatment thresholds may be much lower and required levels of precision much higher. In monitoring for IPM, the conventional tap method may be desirable due to the low cost (person-hours) per unit. A distinction should be made, however, between specifically educated and nonspecifically educated person-hours. Insect identification requires specialized training or education and so may call for a higher wage than that of a general employee. The tap method requires such an educated employee during the entire sampling process, whereas the alcohol wash and brown bag methods only require specialized training during insect identification and recording (samples can be collected without any special training or education). Because the brunt of the cost in person-hours of the alcohol and brown bag methods involves sample collection and processing, a savings is possible if general labor is used. Furthermore, the brown bag method may offer a more complete population assessment than other methods because it may recover more total western flower thrips as it accounts for cloistered pupae in the sample, which emerge as adults during the desiccation period of 1–3 wk. This required desiccation period, however, may be detrimental in situations where treatment decisions should be made immediately. A general note should be made as to the cost of the equipment required for the three sampling techniques compared, because this factor is important in the choice of an appropriate method. The alcohol wash method is best suited for a laboratory, and, in addition to the supplies need for sample collection, requires a vacuum line, large glass flasks, ceramic funnel attachments, laboratory-grade ethyl alcohol, filter paper and a dissecting microscope for sample processing. The other two methods presented require no additional equipment other than supplies needed for sample collection.

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