Research Article

Efficiency of two conventional sampling methods and spatial distribution pattern of the soybean stem fly, *Melanagromyza sojae* (Diptera: Agromyzidae) in soybean field

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Abstract

Relative efficiency of direct observation, and sweep sampling methods for soybean stem fly, *Melanagromyza sojae* (Zehntner) adults were evaluated by relative variation (RV) criteria in soybean field. The mean RV obtained from the direct observation yielded lower RV than the sweep net method and adequately for extensive sampling program. Periodic sampling of the adult flies between October 2019 and March 2020 at 3rd and 4th week after planting revealed that *M. sojae* produced the greatest number of adults in November and declined in January but increased again during February through March. There was a positive correlation between adult populations of *M. sojae* and the levels of infestation of plants. The observed and expected frequency distributions of immature stages among 2,400 plants were tested by a Chi-square goodness-of-fit test. The results indicated that *M. sojae* populations are not randomly and independently dispersed among their host plants.

Keywords: Chi-square goodness-of-fit, *Melanagromyza sojae*, Relative variation, Soybean stem fly

Introduction

Soybean, *Glycine max* (L.) Merr., is one of the most important crops which is extensively grown for oil and protein in Thailand. However, 95% of soybean demand is imported from America continent while only 5% of soybean demand is produced in the country [1]. One of the major constraints to successful soybean production in Thailand is the damage caused by insect pests [2, 3]. Research experiences revealed that 15-20 percent of total soybean production is lost directly or indirectly by the attack of insect pest every year [3-6]. Titayavan [5] listed 29 species of insects that attacked soybean in northern Thailand. Among these insect pests, the soybean stem fly, *Melanagromyza sojae* (Zehntner) (Diptera: Agromyzidae) is presently the most injurious species attacking soybeans in the seedling stage throughout the year [4]. *Melanagromyza sojae* is a polyphagous species feeding on soybean plants as its main host along with other cultivated legume crops [7, 8]. The maggot of *M. sojae* enters the stem through the leaf stalks, then feeds on the stem pith by mining both upwards and downwards and makes a reddish feeding tunnel in the affected plant [9]. In Thailand soybean represents one of 55 economically important crops that are available to *M. sojae* [10]. Together with the substantial soybean areas available in neighboring countries such as Myanmar, Cambodia, Viet Nam, Malaysia, Brunei, and Philippines, the *M. sojae* has the potential to establish across these diverse eco-climate zones [1]. They can establish and sustain population growth via the high reproduction rate and short generation time (four to five generations per year with an average duration of developmental time from egg to adult is approximately 21 days) [11] which probably render containment or eradication expensive and unachievable. During the seedling stage in soybean, crop loss up to 100% have been reported in some parts of northern Thailand [10]. Curioletti, Arnemann [12] indicated that the first 3- 4 weeks after the planting is the most susceptible periods for *M. sojae* to damage in soybeans. In addition, early stages of infestations are difficult to detect because of their small size of flies and inconspicuous oviposition scars [7].

In order to evolve economically feasible, ecologically sound and social acceptable pest management strategies detailed information on the pest complex are of great importance [13]. For most pest management program, population estimates and their nature field distribution patterns are essential for the pest-damage assessment that can rapidly classified the situations in decision making [14]. The method most often used to sample above-ground insect populations have also been extensively operated in soybean insect research and surveys. There are four of the principal methods including direct observations, ground or beat cloth, sweep net, and vacuum or suction net (usually a D-Vac). However, all methods require proper calibration against reliable absolute sampling techniques that allow conversion of population samples to density estimates [15]. Southwood [16] indicated that the description of patterns of distribution of the animal in space is a key factor of considerable ecological significance. Shepard and Carner [17] also reported that the distribution of a population does affect the sampling program which is needed before developing a sequential sampling plan. The spatial distribution patterns of insect population are often predicted using frequency distribution models [18]. In these models, the data are adjusted to the frequency distribution (e.g., negative binomial, Poisson or binomial positive) which indicates distribution pattern of insect is aggregated or random or uniform [19].

For making pest management decision, knowledge of the mathematical distribution is essential not only for construction of the sequential sampling plans but also determination of the spatial patterns [14]. Ruesink and Kogan [20] pointed out that chosen sampling method for arthropods in soybeans is also necessary to compromise between manpower expended and the accuracy of results in a comparative study. Kogan and Pitre [15] developed the concept of relative variation to compare the efficiency of various sampling methods. However, currently knowledge on *M. sojae* is currently lacking with regard to the description of dispersion patterns in discrete units and sequence of appearance during the crop period [6]. In addition, no published information is available on sampling techniques or distribution patterns of the soybean stem fly in Thailand. The major objectives of this research were to evaluate the efficiency of the two sampling methods commonly used in agricultural arthropod survey (1) direct observation, (2) sweep netting and to measure the spatial distribution pattern of *M. sojae* during early stage in unsprayed soybean field.

Materials and Methods

Sampling and analysis of adult soybean stem fly, *Melanagromyza sojae* (Zehntner) populations

Experiments were carried-out at the Agricultural Research and Training center, Chiang Mai university between October 2019 and March 2020. A total area of 1 rai block (1,600 m^2) was divided into 6 alternating test blocks ca. 260 m^2 each. A variety of soybean SJ5 was planted in the test block with 25x15 cm row x plant spacing. Planting dates were sequenced, so each subsequent test block adjacent to the neighboring block was seeded once every 2-week throughout the study periods. All recommended soybean cultural practices viz land preparation and planting, plant population, plant row-spacing, planting date, irrigation, fertilizer application and weed control were used except the insecticide application. Each of non-protected plots was sampled at 3rd and 4th week after planting by relative procedures, including direct observation and sweep net technique. Direct observation, or visual search for adult flies was conducted by walking along a length of row and randomly selecting 100 non-adjacent plants. After the initial scan, each of plant canopies was carefully examined and searched for adult *M. sojae* flies. The total number of files per 100 plants was recorded and calculated the average number of flies. Three replicates were made for this experiment. A sweep net being used was made of muslin with 38 cm in diameter and 75 cm deep. The wood handle was 2.2 cm diameter and 90 cm long. When sampling adult *M. sojae* flies, a collector moved forward along the middle row, stretched out a handle, and kept the net about 30 cm above soybean canopies. As the hop reached the top of the plants, the sweep net was positioned ahead of the collector and turned 180° downward and then quickly twisted the handle. The design experiment based on ten sweeps were performed with 3 replicates.

The collected specimens were transferred into a labeled plastic bag and consequently identified. The average number of adult *M. sojae* flies was calculated. Knowing the row width and number of plants per meter of row, the plant population per rai was computed by using formula Number of plant per hectare = (Number of plants per meter of row X 1000)/ (Row spacing in meters) [15]. The number of infested plants was recorded and calculated the percentage of infested plants. According to Kogan and Pitre [15], a relative variation (RV) was used to compare the efficiency of conventional sampling methods between direct observation and sweep net technique as RV=100 $S_{\bar{x}}$ / $\bar{x}$, where $S_{\bar{x}}$ is the standard error of the mean, $\bar{x}$ is the mean individuals counted, $S_{\bar{x}}$ = s / $\sqrt{n}$, s is the standard deviation and n is the number of observations respectively. For each sampling methods, the relationship between number of adult *M. sojae* fly and percentage of infestation in soybean was examined by mean of linear equation; $y = b_0 + b_1x$, where y is the percentage of infested plant. x is number of adult *M. sojae* fly, b_0 is the intercept of the line indicating the change in y for each unit change in x, b_1 is the slope of line.

Spatial pattern of soybean stem fly, *Melanagromyza sojae* (Zehntner)

A sample unit of 200 plants in non-protected blocks was un-rooted randomly at 3rd and 4th week after planting. The plants were dissected under a dissecting microscope and number of immature insects was recorded. To identify the fly species, specimens were mounted in a Permount's solution on glass microscope slide and determined the species under a phase contrast microscope. During each plant dissection, the total number of larvae and pupae was recorded. The frequency distribution of *M. sojae* in soybean stem in the field was evaluated using a Poisson distribution given by the function; $P_x = a^x. e^{-a}/ x!$ where x is the number of individuals per unit, and a is the mean number of individuals per unit. The symbol x! stands for x factorial and e is the base of natural logarithm. Data from this experiment was evaluated by a chi-square goodness-of fit test as $x^2 = \sum_{x=0}^{N} \frac{(O-E)^2}{E}$ where *O* is the observed frequency of *x*, *E* is the expected frequency, and *N* is the number of frequency groups. The observed chi-square value is compared to a chi-square table with *N* – 2 degree of freedom [21, 22].

Results and Discussion

Sampling and analysis of adult soybean stem fly, *Melanagromyza sojae* (Zehntner) populations

Data given in Table 1 revealed that many overlapping generations were produced by the soybean stem fly under natural conditions. It would appear that the combinations of suitable weather conditions and food plants approached the optimal conditions for both breeding and multivoltinism of *M. sojae*. The average number of adult flies obtained during 3rd week after planting reached their highest number in November 2019 with 18.12±1.59 and declined to 7.00±2.00 in March 2020. Similar results were obtained from data collected on 4th week after planting when the greatest average number of 14.93±2.40 adult flies were detected in November 2019. Adult flies continued to be caught through March 2020 but declined to 7.75±0.25. Factors involving the population dynamics of *M. sojae* cannot be described in terms of geographic range alone, but natural enemy effects on this insect could not be ruled out [16]. Five species of hymenopteran, the very parasitoids comprising *Eurytoma melanagromyzae*, *Syntomopus carinatus*, *Aneuropria kairali*, *Sphegigaster* sp, and *Cynipoidea* sp. were frequently found parasitizing *M. sojae* larvae and pupae. They were the most prevalent parasitoids throughout the season and had a significant impact on *M. sojae* population with a peak parasitism of 20% during the mid-season. Titayavan [6] reported this phenomenon with a total of 8 species of hymenopteran parasitoids comprising *Eurytoma melanagromyzae*, *Syntomopus carinatus*, *Aneuropria kairali*, *Sphegigaster agromyzae, Opius phaseoli, Pediobius naunai, Cynipoidea* sp., and *Stenomalina* sp. with parasitism rates ranging from 3% to 20% in soybean field. These parasitoids which have much shorter life cycles than that of *M sojae* and subsequence the next season may favorable to the parasitoids whose numbers then increased further. Additionally, these natural enemies may also act along with other mortality agents to inflict levels of mortality on population of *M. sojae*. However, a complex of parasitoid-host interactions and host-plant attributes on disruption of population mortality and dispersion of stem fly under field conditions is poorly understood.

Table 1 Monthly mean sampling of soybean stem fly, *Melanagromyza sojae* (Zehntner) in soybean field and the percentage of infested plants at the Agricultural Research and Training Center, Chiang Mai University during October 2019 and March 2020

Sampling Date	**Sampling Methods**	**Mean number of adult files ±SE**						**Relative Variation**
		Oct.	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**Mar.**	
3rd weeks after planting	Direct observation	12.50±0.30	18.00±1.52	9.67±0.32	6.00±0.79	5.50±0.41	9.00±1.04	18.72
	Sweep net technique	2.75±0.16	18.25±1.59	6.75±1.46	3.13±0.47	4.70±0.50	5.00±0.84	35.05
	Average	7.62±4.86	18.12±1.59	8.21±1.46	4.56±1.43	5.10±0.40	7.00±2.00	
		Percentage of infested plants						
		31.00	52.50	19.67	8.50	12.00	26.00	
4th weeks after planting	Direct observation	6.00±0.56	12.50±0.58	8.50±0.45	6.50±0.43	8.00±0.56	7.50±0.34	11.58
	Sweep net technique	9.00±0.68	17.37±1.17	7.25±0.94	2.00±0.41	2.38±0.28	8.00±0.88	29.77
	Average	7.50±1.50	14.93±2.40	7.88±0.62	4.25±2.25	5.19±2.81	7.75±0.25	
		Percentage of infested plants						
		41.00	68.50	42.00	18.50	11.50	39.00	

The highest percentage of infested plants observed was 68.50% sampled in November 2019 at 4th week after planting and declined thereafter. The percentage of infested plants dropped to 11.5% during February 2020 but increased again in March 2020. The lowest percentage of infested plants was 8.50% observed during January 2020 at the 4th week after planting. Our data suggest that the increase in number of flies during the study periods coincide closely with the increase in the percentage of plant infestations. A regression function or relationship between two sets of correlated variables was presented in Figure 1. A significant positive correlation at 5% level of significance was found between the adult populations estimated and percentage of infested plants recorded in the field. Chiang and Talekar [23] reported a similar result that a strong positive correlation ($P<0.01$) exists between the number of *M. sojae* and the percentage infested plants in Taiwan. Note that, the two regression lines of the pest damage relationship as indicated in Figure 1 do not differ significantly from each other. The increased in the percent damage to plants due to increments in fly densities at 3rd and 4th after planting were not significantly different by testing homogeneity of regression coefficients at 5% level of significance.

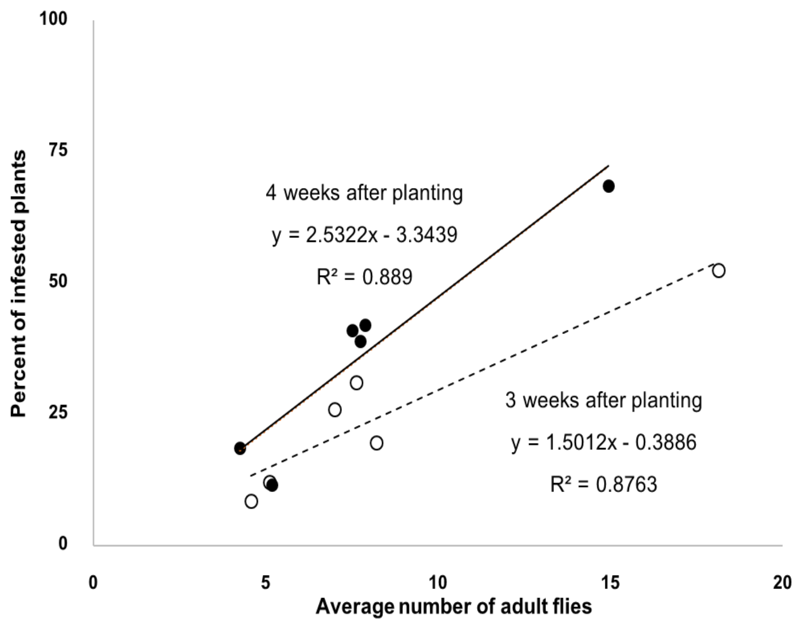

Figure 1 Relationship between average number of *Melanagromyza sojae* (Zehntner) adult files and percentage of total infested plants under field conditions at the Agricultural and Research Training Center, Chiang Mai university 2019-2020

To compare the efficiency of various sampling methods, direct observation showed lower RV value (3rd weeks: 18.72, 4th weeks: 11.58) than sweep net method (3rd weeks: 35.05, 4th weeks: 29.77) (Table 1). The lower RV, the more efficient sampling method [15] According to Southwood [16], an RV less than 25 is adequate for the most extensive sampling programs, thus the sweep net technique was less efficient than the direct observation. The sweep net technique was perhaps the most convenient sampling procedure but appeared to be used only early in the season where soybean plants were small and tender. Vegetation structure and taxon being sampled can also affect sweep net collection. The sweep net method more often yielded the greatest RV, although its value was sometimes lower than 25.00 because the variation in sampling methods can be influenced by sample size, the time of day and season when samples are taken [24, 25]. Direct observation might be considered the beast overall method for sampling adult *M. sojae* flies since number of flies were accurate counted of individuals on plant without disturbed insects and least time consuming [24]. This method caused no seriously damaging the plants and without removal of individuals in the population. However, the efficiency of direct observation was extremely dependent on the collector's responsibility and visual ability. These were the factors that certainly limit its usefulness in comparing data gathered by different collectors. An additional disadvantage of the method was the chance for misidentification of insects due to the collectors had only a glimpsed at the sampled specimens, positive identification was possible.

Spatial pattern of soybean stem fly, *Melanagromyza sojae* (Zehntner)

During the study periods, 200 plants were un-rooted at 3rd and 4th weeks after planting and dissected under a dissecting microscope. The total of 2,400 collected plant samples yield 877 live immature stages of *M. sojae* and population levels of more than 4 live immature stages per plant were observed. The calculation of the frequency distribution and/or the expected number of units with a certain number of individuals in the sample plants is illustrated in Table 2. The observed and expected frequencies of immature in stems are given. The expected frequencies are those given by the assumption that the insects are independently and randomly distributed among plants [21].

Table 2 The observed and expected frequencies of number of *Melanagromyza sojae* (Zehntner) larvae and pupae per stem in the soybean field at the Agricultural and Research Training Center Chiang Mai University during the sampling period of October 2019 – March 2020

Number of larvae and pupae	Frequency observed (*O*)	Frequency expected (*E*)	$(O\text{-}E)^2/E$
0	1.769	1,759.969	0.046
1	432	617.774	55.865
2	157	108.431	21.755
3	37	12.699	46.503
+4	5	1.124	13.366
Total	2,400 stems		χ^2 = 137.535

The fit of the observed and expected frequency distribution was tested by a Chi-square goodness-of-fit test. The fit results revealed that the pooled Chi-square value (137.535) was greater than the table χ^2 value (11.34) at 1% level of significance with 3 degree of freedom. Hence, the tests indicated that the observed frequency distributions deviated significantly from the random distribution. Based on the data of Table 2 showed that, there were too many stems with no insects and too many stems with many insects. Thus, the expected frequencies of the Poisson did not fit the observed frequencies. Consequently, the *M. sojae* were not randomly and independently dispersed among the plants. The results consistent with the report that in nature insect populations are rarely found to be randomly dispersed. In fact true randomness represents an infinitely small point between regular and aggregated dispersion patterns [16]. Poole [21] indicated that if the sample is large enough, it will be always possible to prove the population is not randomly dispersed except under the most improbable circumstances. Regarding to the ratio of s^2/x (1.173) which was larger than 1.00 and therefore a contagious distribution was suspected [21, 25]. Titayavan [6] reported similar results with *Ophiomyia centrosematis* (de Meijere) and *O. phaseoli* (Tryon) in mungbean.

Conclusions

Periodic sampling of the adult *M. sojae* by direct observation and sweeping at 3rd and 4th weeks after planting revealed that the fly populations reached their highest number in November with 18.12±1.59, 14.93±2.40 and declined to 4.56±1.43 and 4.25±2.25 in January 2020 but increased again during February through March 2020. The percentage of infested plants increased from 31.00 and 41.00 in October to 52.50

and 68.50 in November at 3rd and 4th week after planting, respectively. The highest percentage of infested plants was 68.5 occurred in November but declined thereafter. The data suggested that every generations of the *M. sojae* were well synchronized with the percent of damaged plants. The mean RV of 15.12 obtained from the direct observation on 100 plants was lower than 25, adequately suggested for extensive sampling program [16]. A t-test performed on these data indicated that there was no significant ($P<0.05$) between two sampling methods. Based on comparing the test regression slope, there was no significantly difference between direct observation and sweep net. The sweep netting was perhaps the most convenient sampling procedure for adult *M. sojae* but the effect of sample size on the likelihood of finding insects should be considered when conducting a survey. The 877 total live immature stages of *M. sojae* were counted from the 2,400 plant samples taken during the sampling period of October 2019 and March 2020. The observed and expected frequency distributions were tested by a Chi-square goodness-of-fit test. The pooled Chi-square value of 137.535 was greater than the table χ^2–value of 11.34 at 1% level of significance with n–2 degree of freedom, indicated that the expected frequencies of the Poisson distribution did not fit the observed frequencies. Data obtained in these experiments suggested that *M. sojae* populations were not randomly and independently dispersed among soybean plants. However, any sampling method will have inherent advantages and limitations and it is essential that researchers be aware of these so that results can be properly interpreted. Different sampling methods for the same taxonomic group will often yield different results. This means that consistency in methodology is essential for results to be comparable. Results of different sampling method may be complementary to an extent, and that use for exploratory research design.

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