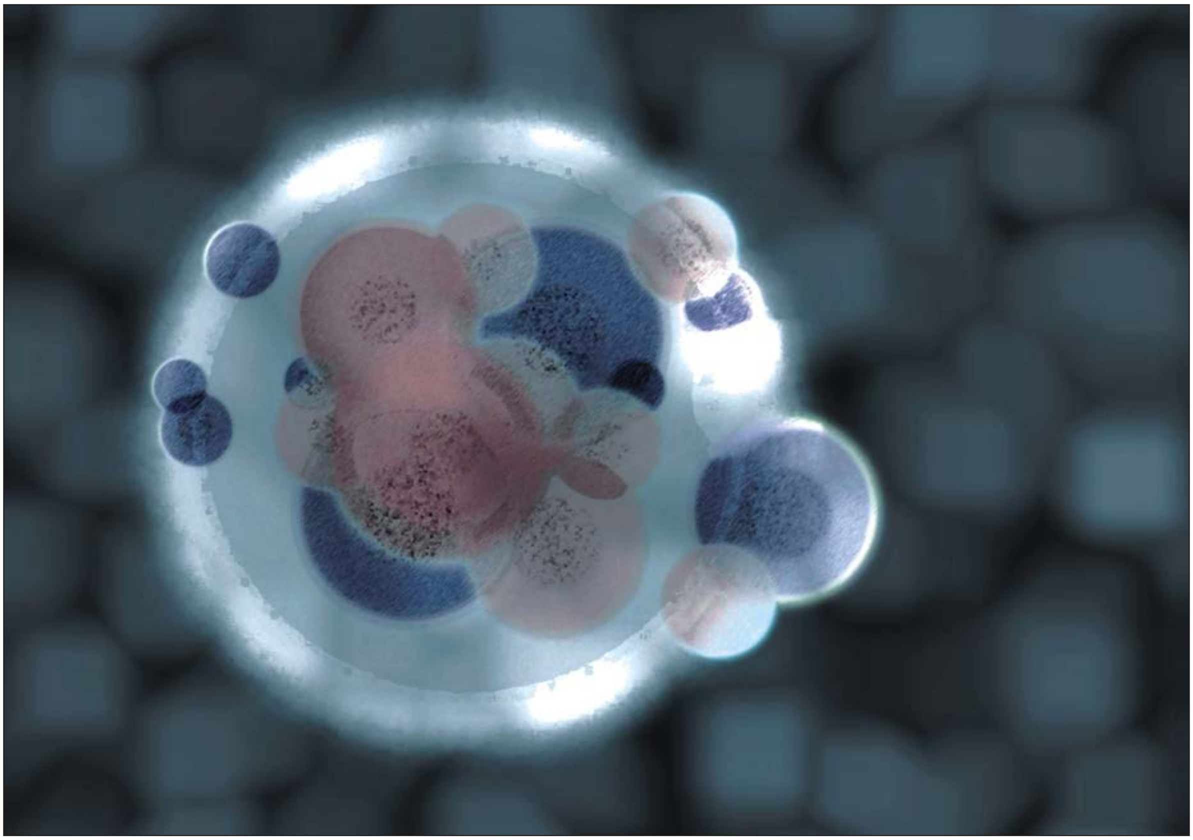

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Spatial Distribution and Sampling of *Icerya purchasi* Mask. (Hom.: Margarodidae) on Orange Trees in Southwest Iran

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Abstract: The Cottony-Cushion Scale, *Icerya purchasi* Maskell, is an important pest of citrus and ornamentals. The first objective of this study was to analyze the spatial distribution of this insect on the orange trees, in southwest Iran. The second objective was to develop sampling plans to determine sample sizes for fixed levels of precision and fixed-precision-level stop lines for sequential sampling. Population estimates were made by registering the number of various stages of the scale on 40 twigs at 10 days intervals from 2003 to 2004. Taylor's power law and Iwao's patchiness regression were used to analyze the spatial distribution of the pest. Taylor's power law fitted the data better ($R^2 = 0.80$) than Iwao's regression model ($R^2 = 0.72$), indicating that *I. purchasi* populations were aggregated. Optimal sample sizes for fixed precision levels of 0.10 and 0.25 were estimated with Taylor's regression coefficients. Required sample sizes increased dramatically with increased levels of precision. The sampling plans presented should be a tool for research on population dynamics and pest management decision.

Key words: Icerya purchasi, spatial distribution, sampling plan, southwest Iran

INTRODUCTION

The Cottony-Cushion Scale (CCS), *Icerya purchasi* Maskell (Margarodidae) is an important pest of citrus and ornamentals throughout the world. Much has been published concerning the observed successful biological control of CCS in many tropical and subtropical countries (Bodenheimer, 1951; Quezada and DeBach, 1973; Caltagirone and Doutt, 1989). In southwest Iran, the hermaphroditic CCS was first found in citrus orchards of north Khuzestan province in 2003, while it infested 41 species of 22 different families of plants apart from citrus and citrus trees supported heavy CCS populations, showing some branch dieback and other signs of stress (Esfandiari *et al*., 2007). In north Khuzestan citrus encompasses about 5000 planted hectares.

The spatial distribution of organisms is an intrinsic characteristic of the species and it is shaped by behavioral and environmental factors (Taylor, 1984). Thus, Knowledge on the spatial distribution patterns of insect populations may provide information on the behavioral traits of the insect species and on the effects of environmental factors on the populations (Nestel *et al*., 1995; Southwood and Henderson, 2000; Martinez-ferrer *et al*., 2006). Methods that are used to describe distribution of arthropods populations have been summarized by Southwood and Henderson (2000). Several estimates based on the relationship between sample mean (m) and variance (S^2) are used as indices of aggregation (Taylor, 1984; Southwood and Henderson, 2000). Sampling plans based on these indicators reduce sampling effort and minimize variation of sampling precision (Kuno, 1991). None of previous studies considers the spatial distribution and sampling of CCS. To fill this gap, we report results from 2003 to 2004 study to investigate basic information for interpreting spatial distribution, designing efficient sampling programs for population estimation and pest management of CCS on orange trees in citrus orchards of southwest Iran.

MATERIALS AND METHODS

Sampling populations of CCS was carried out from July 2003 to June 2004 at Sharafabad district of Dezful in north Khuzestan province, southwest Iran. The study was conducted in a pesticide-free orchard (3 ha), consisted of 25-year-old trees and was characterized by high population levels of the scale. The main orange variety was Siavaras, the native variety of the area and the spacing between trees was 4.0 by 5.0 m.

Five orange trees of approximately the same height and diameter were selected at random. At 10 days intervals, one twig, 15 cm in length, was cut from the upper and lower halves (above and below 1.5 m,

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respectively) of each quadrant (north, west, south and east). Thus a total of 8 twigs were selected from each of the 5 trees per sampling date. Numbers of 1st, 2nd and 3rd instars as well as adult females of CCS per twig were counted and recorded.

Variance-mean relationships: The mean density (m) per twig and variance (S^2) were calculated for twigs per sampling date and related to each other using Taylor's power law (Taylor, 1984) and Iwao's patchiness regression (Lloyd, 1967; Iwao, 1968) to describe the distribution of CCS among twigs.

Taylor's power law states that the variance (S^2) of a population is proportional to a fractional power of the arithmetic mean (m): $S^2 = am^b$. To estimate a and b, the values of log (S^2) were regressed against those of log (m) using the model

$$\log(S^2) = b\log(m) + \log(a)$$

where, a is the anti-logarithm of the intercept, a scaling factor related to sample size (Southwood and Henderson, 2000), the slope b gives a measure of aggregation with; b<1 uniform (regular); b>1 aggregated (contagious) and b = 1 random.

Iwao's patchiness regression method quantifies the relationship between Lloyd's (1967) mean crowding index (m*) and mean (m) by:

$$m* = \alpha + \beta m$$

where, (m*) was determined as $[m+(S^2/m-1)]$ (Lloyd, 1967). The intercept (α) is the index of basic contagion, indicates whether single individuals (α = 0), a colony ($\alpha>0$) or a negative association of individuals ($0>\alpha>-1$) is the basic component of the distribution. The slope (β) is the density contagiousness coefficient, suggests whether the basic components distribute themselves in their habitat at random (β = 1), aggregatively ($\beta>1$) or uniformly ($0<\beta<1$).

Sampling plans

Representative stage: Linear regression of all living individuals per twig on living individuals of 1st, 2nd, 3rd instars and adult females per twig were calculated, respectively. After linear regression analysis based on the simplicity of discrimination and counting and it's representative to scale population, an appropriate representative stage was determined.

Fixed-sample sampling: We determined the sample sizes for fixed levels of precision by substituting Taylor's variance-mean relationship into the usual expression for the standard error of the mean and rearranging:

$$n = am^{b-2}/D^2$$

where, n is the sample size and D is the required level of precision expressed as a proportion of the mean and a and b are the coefficients from Taylor's power law (Walker and Allsopp, 1993; Tsai *et al*., 2000). Southwood and Henderson (2000) suggested that a value for D of 0.25 is sufficient for pest management and of 0.10 for research purposes.

Sequential count plan: The number of samples after which sampling can be terminated (T_n) for a constant precision, D, of the mean $[D = (S^2/n)^{-2}/m]$, was determined using the equation derived by Green (1970):

$$\log T_n = [\log(D^2/a)/(b-2)] + [(b-1)/(b-2)]\log n$$

where, (T_n) is the stop line for sample size n, a and b are from Taylor's power law and D is defined as above.

RESULTS

Variance-mean relationships: The results of Iwao's regression of (m*) on (m) and Taylor's power law analysis for various stages are listed in Table 1.

Considered as a total, without any distinction between developmental stages, Taylor's power law provided a highly significant relationship between variance (S^2) and mean density (Table 1 and Fig. 1). Estimate of b was significantly >1 for various stages as well as total counts indicating an aggregated distribution among twigs (Table 1). Slop for adult females was significantly lower than that for 1st (t_{72} = 2.95, $p<0.01$), 2nd (t_{72} = 2.52, $p<0.01$) and 3rd (t_{72} = 2.20, $p<0.05$) stages.

Iwao's patchiness regression described well the relationship between mean crowding and density for CCS (Table 1 and Fig. 2). Estimates of β was not significantly different from unity for adult females and total counts, but it is significantly >1 for other stages (Table 1). Even in the case of adult females and total counts in which β does not differ significantly from unity, the value of t is significant at p = 0.10 for a one-sided test. Slop for total counts was significantly lower than that for 3rd

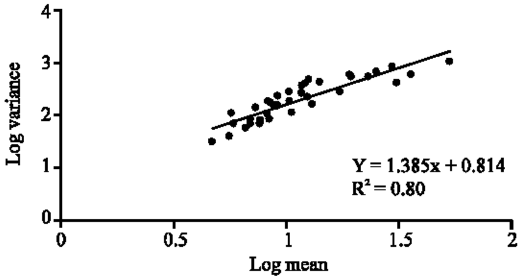

Fig. 1: Regression analysis of Taylor's power law for CCS total population on orange trees

Table 1: Parameters estimation for CCS spatial distributions on orange trees and tests for the difference of slopes from unity (1), for 1st (I), 2nd (II), 3rd (III) instars, adult females (A) and total population

Stages	Parameters estimations					Test for slop#1
Taylor's[a]	Log (a) ±SE	Slope±SE	R^2	F	p-value	t^b
I	0.697±0.053	1.547±0.067	0.94	531.573	0.000	8.155**
II	0.569±0.065	1.555±0.106	0.86	214.562	0.000	5.228**
III	0.531±0.025	1.452±0.066	0.93	479.649	0.000	6.820**
A	0.561±0.028	1.178±0.105	0.78	124.855	0.000	1.689*
Total	0.814±0.128	1.385±0.119	0.80	135.880	0.000	3.242**
Iwao's[a]	α±SE	β±SE				
I	9.638±1.864	1.411±0.166	0.67	72.250	0.000	2.476**
II	4.814±1.847	1.692±0.402	0.34	17.745	0.000	1.723*
III	1.688±0.549	1.908±0.333	0.48	32.803	0.000	2.726**
A	2.262±0.445	1.527±0.370	0.33	16.997	0.000	1.422
Total	14.365±2.160	1.213±0.129	0.72	87.886	0.000	1.644

[a]Number of observations = 37, [b] Significantly different from 1. **$p<0.01$; *$p<0.05$ ($t = [\text{slope}-1]/SE_{\text{slope}}$, $df = N-2$)

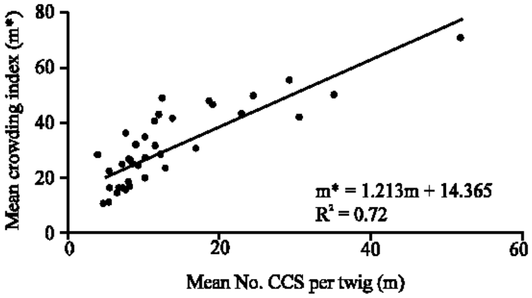

Fig. 2: Regression analysis of Iwao's mean crowding index (m*) on mean density (m) for CCS total population on orange trees

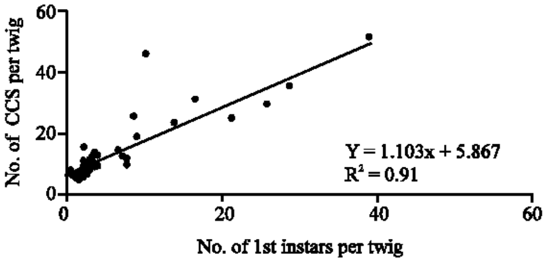

Fig. 3: Linear regression of population densities of 1st instars to total densities of CCS

stages (t_{72} = 1.95, $p<0.05$). The value of α was > 0 in various stages as well as total counts, indicating that basic component of the population is a colony (Table 1).

Sampling plans

Representative stage: There was a significant relationship between all living scales and 1st instars (t_{36} = 20.02, $p<0.01$; Fig. 3) as well as 2nd instars (t_{36} = 5.43, $p<0.01$) per twig. The relationship between 1st instars and total living scales (R^2 = 0.91) was higher than that for the 2nd instars (R^2 = 0.44). Directly counting 1st instars was easier because of characteristic lozenge shape of 1st instar which has 3 separated yellow waxy portions on the back. Therefore the scale numbers of 1st instars per twig were suggested as the sampling target.

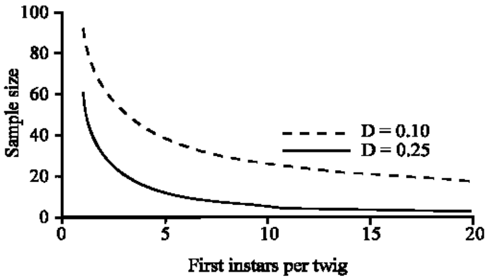

Fig. 4: Relationship between required sample size and mean density for achieving a fixed precision level of 0.10 and 0.25 for CCS populations on orange trees using enumerative sampling procedures

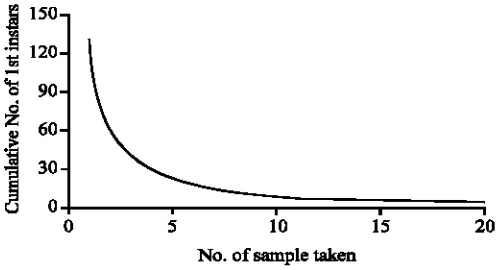

Fig. 5: Sequential count plan for CCS populations on orange trees, showing the stop line at a fixed precision level of 0.25

Fixed-sample sampling: The a and b indices of Taylor's power law for 1st instars were 4.98 and 1.55, respectively. According to equation $n = am^{b-2}/D^2$, mean number above 2 scales per twig with precision levels of 0.10 and 0.25, for the same condition would reach the optimum samples of 50 and 8 twigs per tree, respectively (Fig. 4).

Sequential count plan: The stop line of the fixed-precision-level of 0.10 of the mean was not presented because of the requirement for extremely large samples from the field. A person sampling CCS could use Fig. 5 by plotting T_n (accumulated 1st instars) and n (number of twigs sampled) after each sample was taken. When the plot falls above the line, sampling is stopped and the mean density (m) is within 0.25 of m = T_n/n.

DISCUSSION

Taylor's aggregation indices have a narrower range with from 1.18 to 1.55 than Iwao's do with from 1.21 to 1.91. Taylor's power law consistently provided a good fit to the data (R^2 from 0.78 to 0.94) and Iwao's patchiness regression provided erratic fit to the data (R^2 from 0.33 to 0.72). These results were predictable because the patchiness regression is not subject to the stabilizing effect of a log transformation (Tonhasca *et al*., 1996).

Taylor (1984) contends that the slope (b) is an index of the spatial distribution characteristic of the species, but some studies have shown that b is not species specific and varies among environments and developmental stages (Banerjee, 1976; Downing, 1986). Scale insects differ from other insect taxa because their distribution pattern in subsequent stages is shaped by mobile first instars (the crawler stage). The adult females are sessile or move very short distances. The typical thigmotactic behavior of the crawler (Bodenheimer, 1951), as the major factor, as well as other intrinsic behavior of the scales (e.g. tendency to settle closely to the parent female, phototaxis during crawlers dispersal) determine the distribution of the scale population (Nestel *et al*., 1995). The tendency to aggregate in the CCS could, therefore, be explained by short distance crawlers tend to move away from the mother scale or the ovisac and by their characteristic thigmotactic behavior for settle along the mid-ribs of the leaves (Bodenheimer, 1951). Whereas early stages tend to settle on the central vein of the leaves, adult females migrate to branches and twigs (Bodenheimer, 1951) which result in different degree of aggregation between nymphs and adult. These explains why the aggregated index, b, of nymphal stages is significantly greater than that for adult female (Table 1). The similar observations were also reported by Nestel *et al*. (1995) for the olive scale (*Parlatoria olea* Colvee), the Israeli pine bast scale (*Matsucoccus josephi* Bodenheimer et Harpaz) and the citrus mealybug (*Planococcus citri* Risso).

Since 1st stages had a high representation, selecting this stage as the sampling target can presumably reduce counting errors and sampling costs. Orchards with high scale densities require considerably fewer twig samples for a given level of precision than low density orchards. Based on computer simulation, the performance of the sampling procedures improved with increasing scale density. The sequential-count plan permits researchers and pest managers to describe the mean density more accurately than before. It may lead to a better determination of the economic threshold in the future.

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