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Spatial Distribution and Binomial Sampling of *Aphis gossypii* Glover (Homoptera: Aphididae) Infesting Protected Cucumber and Melon in Northern Italy. (*)

Introduction

Aphis gossypii Glover, the main pest of protected cucurbits in Italy (Celli et al., 1991) and northern Europe (van Lenteren & Woets, 1988), is also becoming an increasing threat to other greenhouse crops like sweet pepper. The development of strains resistant to pirimicarb is a serious drawback to the use of beneficial arthropods, and many countries are attempting to develop biological control strategies (van Steenis, 1992).

Studying the spatial distribution pattern of a pest is the first step to designing a sampling method. Common approaches in entomology are determinations of mean-variance relationships (Taylor, 1961; 1984), frequency distribution (Anscombe, 1949; Bliss & Owen, 1958), mean crowding (Lloyd, 1967; Iwao, 1968) or, more recently, a new survey based on an improvement of Iwao's model (Xu et al., 1993). Other techniques, like geostatistics, determine the degree of association (correlation) among samples based on the direction and distance between them (Schotzko & Knudsen, 1992). The latter is a kind of analysis that quantitatively evaluates variations or changes in spatial orientation within a defined area or volume. The present study reports two-year of data for *A. gossypii* in cucumber and melon crops of northern Italy's Po Valley in an attempt to determine viable sampling strategies for scouting and research of this aphid.

MATERIALS AND METHODS

1992. The samples of A. gossypii were taken in cucumber tunnels (250 m² each), two at Granarolo (Bologna Province) and four at Cesena (Forlì Province), from early May to July-August, with the end date depending on site and infestation severity. The number of aphids per leaf and their development stage were recorded weekly on 200 randomly selected leaves of 100 plants.

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1993. These samples involved two 300 m² tunnels of the cucumber cv. 'Darina' at Granarolo and four 250 m² tunnels of melon at San Pietro in Casale (Bologna Province). The cucumber data were recorded as in 1992, although leaf number that year ranged from 150 to 400; the melon count included 10-12 leaves per plant, for a total of 400 leaves.

Statistical analysis. The mean number of A. gossypii per leaf (m) and the variance (s²) were calculated for total leaves sampled per tunnel and per sampling date. Taylor's power law ($s^2 = am^b$) (Taylor, 1961; 1984), which describes the correlation of means to sampling variances, was employed to study the spatial distribution of A. gossypii, the estimates of parameters (a and b) being calculated by regression of $\log (s^2)$ over $\log (m)$ to yield:

 $\log (s^2) = \log a + \log (m)$,

where the intercept (a) is a parameter essentially dependent on sampling method and the angular coefficient (b) is defined as the index of aggregation; as such the latter is a constant per species and varies continuously from a regular distribution for $b \to 0$, to random for b = 1, to clumped for b > 1 (Taylor, 1961). To calculate the optimum sample size (OSS) of A. gossypii for direct count, the sampling variance from Taylor's power law was introduced in Karandinos's formula (1976), n = $[Z_{\alpha/2}/D]^2 s^2/m^2$, to generate n = $[Z_{\alpha/2}/D]^2$ am^{b-2},

where D is the required level of accuracy expressed as a decimal (0.2-0.3-0.5) or permitted percentage of error, and $Z_{\alpha/2}$ is the standard normal deviate; for n >30 and with α = 0.05, $Z_{\alpha/2}$ = 1.96 (Karandinos, 1976).

The model of Gerrard & Chiang (1970) was used to predict mean aphid density (m) by the proportion of empty sample units (p_0) :

 $(^{3})$ $\ln(m) = \alpha + \beta \ln \left[- \ln(p_0) \right],$

where α and β are constants. This equation yields

 $m = e^{\alpha} \left[-\ln (p_0) \right]^{\beta}$

(Binns & Bostonian, 1990). The variance of a predicted mean is the sum of a prediction variance [varp(m)] and a sampling variance [vars(m)] (Nyrop & Binns, 1991). The prediction variance is

 $varp(m) = m^2 \{ mse/N + [\ln [-\ln (p_0)] - avglnln(p_0)]^2 s\beta^2 + mse \},$

where mse is the mean square error from the regression, N is the number of data points in the regression, $avglnln(p_0)$ is the average of the independent variable in the regression and $s\beta^2$ is the variance of the regression slope. The sampling variance is

 $vars(m) = p_0 (1 - p_0) \beta^2/n,$

where b is the slope of the relationship between m and p_0 as per Eq.(3), and n is the sample size to estimate m from p_0 .

Analysis of covariance (ANCOVA) was used to compare the parameters of Taylor's power law, i.e. to determine if the regression for year, crop and location were coincidental or not. The comparison between year and location for cucumber was made first and then that between cucumber and melon.

RESULTS AND DISCUSSION

The fit of Taylor's model to the data per crop and year is shown in Tab. 1: the high coefficients of determination (r^2 ranged between 0.97 and 0.98) for the relative regression equations indicate the good fit of the model. Covariance analysis found no significant differences between the coefficients of Taylor's power law for cucumber at the two sites and years (Tab. 2): the regressions for cucumber were coincidental and a common regression was used to determine the relationship between mean and variance in this crop (Tabs. 1, 2; Fig. I). The regression slopes showed some variability for cucumber between 1992 and 1993, and the parallelism test showed a value of P = 0.09, which is near the level of significance and

Tab. 1 - Coefficients from Taylor's power law calculated for A. gossypii per crop and year.

Crop	Location	Years	n	log a (±s.e.)	b (±s.e.)	r^2	P
Cucumber	Granarolo (BO)	1992	17	1.63 (0.06)	1.82 (0.06)	0.98	< 0.001
Cucumber	Cesena (FO)	1992	17	1.75 (0.08)	1.78 (0.07)	0.97	< 0.001
Cucumber	Granarolo (BO)	1993	23	1.72 (0.06)	1.63 (0.04)	0.98	< 0.001
Cucumber	Granarolo (BO)	1992	57	1.70 (0.04)	1.71 (0.03)	0.97	< 0.001
(common)	Cesena (FO)	1993					
Melon	S.Pietro in Casale (BO)	1993	22	1.94 (0.06)	1.66 (0.04)	0.98	< 0.001

Tab. 2 - Comparison of coefficients of Taylor's power law by analysis of covariance for A. gossypii for cucumber per years and locations.

Location and year	n	Intercept (log a)	F	P	Slope (b)	F	P
BO - 1992 FO - 1992 BO - 1993	17 17 23	1.63 1.75 1.72	1.52	0.228	1.82 1.78 1.63	2.54	0.088

Tab. 3 - Comparison of coefficients of Taylor's power law by analysis of covariance for A. gossypii in cucumber (data pooled for all sites and years) and melon.

Location and year	n	Intercept (log a)	F	Р	Slope (b)	F	P
Cucumber (common)	57	1.70		35	1.71		
			9.36	0.003		0.73	0.39
Melon	22	1.94			1.66		

Tab. 4 - Parameters from the regression of $\ln(m)$ on $\ln[-\ln(po)]$ for cucumber and melon to estimate aphid density by incidence of infestation (for the symbols, see the test).

Crop	α (±s.e.)	β ($\pm {\rm s.e.}$)	r^2	P	$avglnln(p_0)$	mse	$s\beta^2$	N
Cucumber Melon	()	1.45 (0.08) 1.49 (0.11)		< 0.001 < 0.001	- 2.218 - 2.810	0.994 1.14	$0.006 \\ 0.012$	58 22

Cucumber (common)

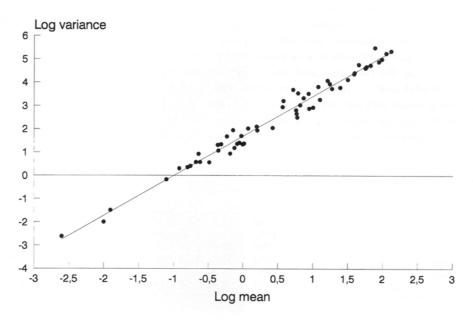


Fig. I - Taylor's power law for $A.\ gossypii$ on cucumber (data pooled).

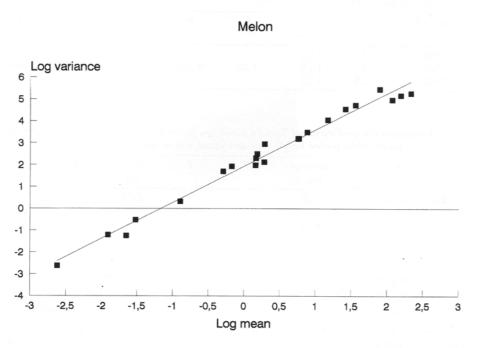


Fig. II - Taylor's power law applied to A. gossypii infestation of melon in Bologna Province (1993).

Cucumber

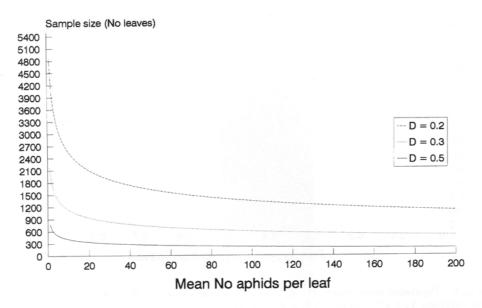


Fig. III - Numerical sample size curves for *A. gossypii* on cucumber at three confidence levels (D = $0.2, 0.3, 0.5, \alpha = 0.05$).

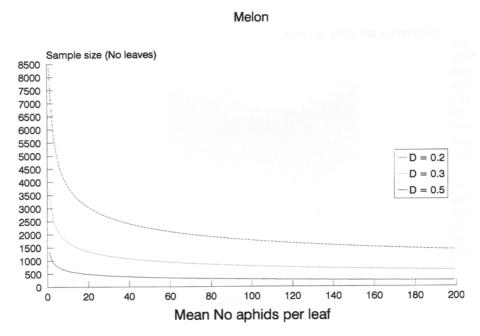


Fig. IV - Numerical sample size curves for *A. gossypii* on melon at three confidence levels (D = 0.2, 0.3, 0.5, $\alpha = 0.05$).

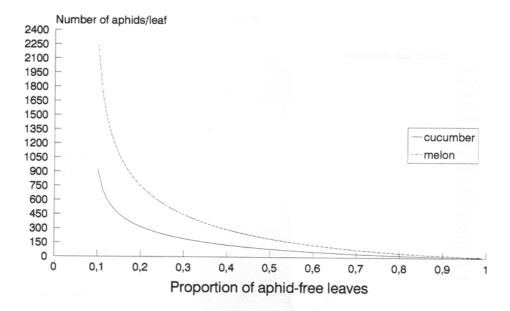


Fig. V - Population mean density (Y) of A. gossypii on cucumber (Y1) and melon (Y2) as estimated by equations Y1 = $e^{4.6}$ (- $\ln p_0$)1.45; Y2 = $e^{5.43}$ (- $\ln p_0$)1.49.

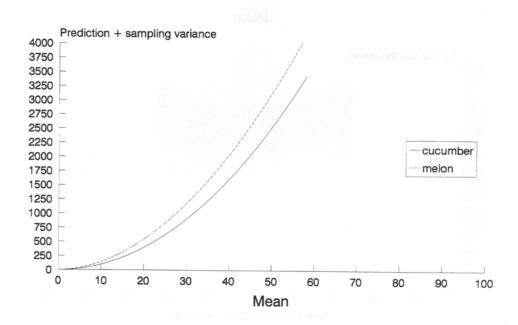


Fig. VI - Variances for A. gossypii density estimates from a sample of 400 leaves. For the variances, see Eqs. (5) and (6).

may be attributable to a certain variability in the type of infestation exhibited by *A. gossypii*. Ants that are symbionic with this aphid play an important role in its type of infestation, and hence in the variation in the data. Two species of ants, *Formica cunicularia* Latr. and *F. cinerea* Mayr were present near the aphid colonies throughout the sampling periods.

The comparison of slope coefficients of Taylor's power law between cucumber (pooled data) and melon showed no statistical difference (Tabs. 1, 2, 3; Figs. I, II). This lack of a difference is in accordance with Taylor's theory which postulates that b is a constant for a species. The value of b for both plant species exhibits a high degree of aggregation for *A. gossypii*. However, the two intercepts showed a statistical difference (Tab. 3). This difference is logical because a incorporates the effects of sample unit and host crop (Taylor, 1984). Thus, although from a biological viewpoint a single index of aggregation could be used as typical for the species so as to calculate the OSS in cucumber and melon, it is definitely more correct to use the parameters a and b of the separate equations (Tab. 1).

Guldemond (1993), using Taylor's law, calculated an angular coefficient of 1.43 for A. gossypii on cut chrysanthemums in greenhouse, which is a lower value for b than reported in the present study. It would be interesting to determine whether this difference results from different strains of A. gossypii in Northern Europe and the Mediterranean region. Though b can frequently be considered a species-specific constant (Taylor, 1961), it can vary markedly for some aphid species (Elliott & Kieckhefer, 1987) and can be influenced by genetic differences, influencing life-history traits (Taylor, 1984).

The curves plotted for the numerical sample size as calculated by Eq. (3) are shown in Figs. III and IV. When a higher accuracy is required, more samples have to be taken. Yet for a level of accuracy of D = 0.2, the number of samples of leaves proved to be too high and not applicable in actual practice. Feng & Nowierski (1992) advise that for scouting purposes both extension agents and growers use an accuracy of D = 0.5, whereas D should be at least 0.3 for research purposes. In our case, for example, estimating a population at 50, 100, 150 aphids per leaf requires, respectively, 723, 596 and 532 leaves for cucumber and 989, 783 and 682 for melon at D = 0.3, whereas the number of leaves required for the same population levels in a practical situation, i.e. for an accuracy of D = 0.5, are 260, 214 and 191 for cucumber and 356, 281 and 245 for melon.

Fig. V shows the regressions calculated by Eq.(3), which estimates aphid density via the incidence of uninfested leaves, and Table 4 lists the matching parameters. To correct for the bias introduced by transforming estimates of ln(m) back to original values, half of the residual mean square from the regression was added to the intercept (Hepworth & MacFarlane, 1992). Thus, in going from the logarithms to the original values and keeping this fact in mind, the aphid population can be estimated by the frequency of uninfested leaves as per Eq.(4) (see Fig. V), the variance of m being calculated with Eqs.(5) and (6) (Fig. VI) (Nyrop & Binns, 1991). However, because of the low intervention thresholds for cucurbit crops, the use of binomial sampling for A. gossypii on cucurbits could be somewhat problematic and dangerous, compared to other crop-pest systems (Ekbom, 1987; Binns & Bostonian, 1991; Nyrop & Binns, 1991; Feng & Nowierski, 1992; Hepworth & Mac Farlane, 1992).

The biological control techniques now under study, such as releases of the aphid parasitoid *Aphidius colemani* Viereck or of the predator *Aphidoletes aphidimiza* Rond., require that the introductions of these beneficials either be timed to the onset of aphid attack (van Steenis, 1992) or even precede natural infestation through the use of *banker plants* (Bennison, 1992) or the AHPAIF method (Stary, 1993). There is also a certain variability in the data, especially if the density of the aphids is estimated on the basis of low infestation rates (low number of infested leaves). The estimate of the aphid's population density is useful, however, in certain cases, *e.g.* to assess the results of IPM or biological control trials and, in general, whenever an estimate of infestation level is needed and one is short of both time and labour.

KEY WORDS: Aphis gossypii, cucumber, melon, spatial distribution, binomial sampling

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SUMMARY

Taylor's power law was employed to study the spatial distribution of *Aphis gossypii* in tunnel-grown cucumber and melon in northern Italy's Emilia-Romagna in order to improve sampling procedures. The aggregation index (b) in cucumber showed no significant differences between years (1992 and 1993), sites (Bologna and Cesena) or crop (b = 1.66 for melon and 1.71 for cucumber). Binomial sampling, which correlates infestation severity to infested leaf frequency, is also reported and discussed, along with problems inherent in sampling and in control methods for *A. gossypii* that are linked to the peculiar aggregate type of distribution of this aphid in the studied crops and the rapid development of infestations in field.

Distribuzione spaziale e campionamento binomiale di *Aphis gossypii* Glover (Homoptera: Aphididae) infestante cetriolo e melone in coltura protetta in Emilia-Romagna.

RIASSUNTO

È stata utilizzata la legge di Taylor nello studio della distribuzione spaziale di *Aphis gossypii* Glover in tunnel di cetriolo e melone in Emilia-Romagna. L'indice di aggregazione (b) su cetriolo non mostrò differenze significative riguardo sia alle due annate di studio (1992-1993) che alle due diverse località (Bologna-Cesena); non furono inoltre evidenziate differenze significative nel tipo di aggregazione fra cetriolo (b = 1.71) e melone (b = 1.66). Viene presentato un campionamento di tipo binomiale, che permette di stimare la densità delle popolazioni dell'afide, dalla frequenza delle foglie infestate. Sono messe in rilievo le problematiche del campionamento e della lotta contro tale fitofa-

go, legate alla sua distribuzione spaziale particolarmente aggregata, al rapido sviluppo in coltura protetta, ed alla bassa soglia economica.

REFERENCES CITED

- Anscombe F.J., 1949. The statistical analysis of insect counts based on the negative binomial distribution. - Biometrics, 5: 165-173.
- Bennison J.A., 1992. Biological control of aphids on cucumbers use of open rearing systems "banker plants" to aid establishment of *Aphidius matricariae* and *Aphidoletes aphidimyza*. *Med. Fac. Landbouww. Univ. Gent*, 57/2b: 457-466.
- BINNS M.R., BOSTONIAN N.J., 1990. Robustness in empirically based binomial decision rules for integrated pest management. - J. Econ. Entomol., 83: 420-427.
- BLISS C.I., OWEN A.R.G., 1958. Negative binomial distributions with a common k. *Biometrika*, 45: 37-58.
- CELLI G., BENUZZI M., MAINI S., MANZAROLI G., ANTONIACCI L., NICOLI G., 1991. Biological and integrated pest control in protected crops of Northern Italy's Po Valley: overview and outlook. -Bull. SROP/WPRS, 14 (5): 2-12.
- ELLIOTT N.C., KIECKHEFER R.W., 1987. Spatial distribution of cereal aphids (Homoptera: Aphididae) in winter wheat and spring oats in South Dakota. Environ. Entomol., 16: 896-901.
- EKBOM B.S., 1987 Incidence counts for estimating densities of Rhopalosiphum padi (Homoptera: Aphididae). J. Econ. Entomol., 80: 933-935.
- FENG MING-GUAN, NOWIERSKI R.M., 1992. Spatial distribution and sampling plans for four species of cereal aphids (Homoptera: Aphididae) infesting spring wheat in Southwestern Idaho. - J. Econ. Entomol., 85: 830-837.
- Gerrard D.J., Chiang H.C., 1970. Density estimation of corn rootworm egg populations based upon frequency of occurrence. *Ecology*, 51: 237-245.
- GULDEMOND J.A., 1993. Preliminary results on density and incidence counts of aphids in cut chrysanthemums in the greenhouse. *Bull. SROP/WPRS*, 16 (8): 92-97.
- HEPWORTH G., MACFARLANE J.R., 1992. Systematic presence-absence sampling method applied to twospotted spider mite (Acari: Tetranychidae) on strawberries in Victoria, Australia. - J. Econ. Entomol., 85: 2234-2239.
- HUTCHISON W.D., HOGG D.B., 1984. Demographic statistic for the pea aphid (Homoptera: Aphididae) in Wisconsin and a comparison with other populations. *Environ. Entomol.*, 13: 1173-1181.
- IWAO S., 1968. A new regression method for analyzing the aggregation pattern of animal populations. Res. Popul. Ecol., 10: 1-20.
- LENTEREN VAN J.C., WOETS J., 1988. Biological and integrated pest control in greenhouse. Ann. Rev. Entomol., 33: 239-269.
- LLOYD M., 1967. 'Mean crowding'. J. Anim. Ecol., 36: 1-30.
- NYROP J.P., BINNS M., 1991. Quantitative methods for designing and analysing sampling programs for use in pest management. In: Pimentel D.B.R. (ed.), CRC handbook of pest management in agriculture: 67-132. CRC, Boca Raton, Fla.
- KARANDINOS M.G., 1976. Optimum sample size and comments on some published formulae. Bull. Ent. Soc. Am., 22: 417-421.
- Schotzko D.J., Knudsen G.R., 1992. Use of geostatistics to evaluate a spatial simulation of Russian, wheat aphid (Homoptera: Aphididae) movement behavior on preferred and nonpreferred hosts. Environ. Entomol., 21 (6): 1271-1282.
- STARY P., 1993. Alternative host and parasitoid in first method in aphid pest management in glasshouses. J. Appl. Ent., 116: 187-191.
- STEENIS VAN M.J., 1992. Biological control of the cotton aphid, Aphis gossypii Glover (Hom., Aphididae): pre-introduction evaluation of natural enemies. J. Appl. Ent., 114: 326-380.
- TAYLOR L.R., 1961. Aggregation, variance and the mean. Nature, 189: 732-735.
- TAYLOR L.R., 1984. Assessing and interpreting the spatial distributions of insect populations. -Ann. Rev. Entomol., 29: 321-327.
- XU RUMEI, CHAO CHUO, VAN LENTEREN J.C., 1993. The parasite-host relationship between Encarsia formosa Gahan (Hym., Aphelinidae) and Trialeurodes vaporariorum (Westwood) (Hom., Aleyrodidae). XXIII. Application of different sampling methods on spatially stabilized whitefly adult populations. J Appl. Ent., 116: 199-211.