Spatial Distribution of Nymphs of *Scaphoideus titanus* (Homoptera: Cicadellidae) in Grapes, and Evaluation of Sequential Sampling Plans

FEDERICO LESSIO AND ALBERTO ALMA

Di. Va. P. R. A., Entomologia e Zoologia applicate all'Ambiente "C. Vidano," University of Turin via L. da Vinci 44, 10095 Grugliasco (TO), Italy

J. Econ. Entomol. 99(2): 578–582 (2006)

ABSTRACT The spatial distribution of the nymphs of *Scaphoideus titanus* Ball (Homoptera Cicadellidae), the vector of grapevine flavescence dorée (*Candidatus* Phytoplasma vitis, 16Sr-V), was studied by applying Taylor's power law. Studies were conducted from 2002 to 2005, in organic and conventional vineyards of Piedmont, northern Italy. Minimum sample size and fixed precision level stop lines were calculated to develop appropriate sampling plans. Model validation was performed, using independent field data, by means of Resampling Validation of Sample Plans (RVSP) resampling software. The nymphal distribution, analyzed via Taylor's power law, was aggregated, with $b = 1.49$. A sample of 32 plants was adequate at low pest densities with a precision level of $D_0 = 0.30$; but for a more accurate estimate ($D_0 = 0.10$), the required sample size needs to be 292 plants. Green's fixed precision level stop lines seem to be more suitable for field sampling: RVSP simulations of this sampling plan showed precision levels very close to the desired levels. However, at a prefixed precision level of 0.10, sampling would become too time-consuming, whereas a precision level of 0.25 is easily achievable. How these results could influence the correct application of the compulsory control of *S. titanus* and Flavescence dorée in Italy is discussed.

KEY WORDS grape, leafhopper, vector, Taylor's power law, field sampling

The leafhopper *Scaphoideus titanus* Ball is known to be the vector of the phytoplasma agent of Flavescence dorée (FD) (*Candidatus* Phytoplasma vitis, 16Sr-V), an economically important persistent disease of grapevine (Boudon-Padieu 2000). This species, native of the Nearctic region, was first found in Europe in the 1950s (Boudon-Padieu 2000) and is now widespread in France (including Corsica), Spain, Switzerland, Italy, former Yugoslavia, and Portugal (Alma 2004). Because eggs hibernate beneath the bark of 2-yr-old wood, the introduction of this pest via international marketing of cuttings and rootstocks, is feared in other grapevine-growing countries such as Australia, New Zealand, Chile, and South Africa (Alma 2004).

S. titanus feeds only on the vine and completes one generation per year; in northern Italy, nymphs occur in mid-May and are found until the end of June. They colonize the lower page of the leaves and generally do not walk far, but they can jump quickly if disturbed. They spend almost all their time feeding; the nymphs suck mainly the sap from smaller venations, whereas fourth and fifth instars (and adults) can feed on the midribs and also on green shoots and stems (Vidano 1964). Adults occur from mid-July to mid-October; they do not normally move far away from their host plant, and flight occurs mainly between the evening and the early morning (Lessio and Alma 2004a, b). Acquisition of phytoplasmas is performed by third instars, by feeding on infected plants: after a latency period lasting from 28 to 35 d, adults are able to transmit the disease to unaffected plants (Schvester et al. 1969). Given its economic importance, compulsory measures are applied against this pest: in Italy (besides the removal of infected plants) at least one application of insecticide is required. Active ingredients include chitin depressors (buprofezin and flufenoxuron), active against nymphs, and neurotoxic products (chlorpyrifos, fenitrothion, etofenprox, and indoxacarb) to control nymphs and adults (Bosio et al. 2004). In organic farming, pyrethrum and rotenone may be used (Caobelli and Carcereri 1995, Caruso and Mazio 2004). The first application, to avoid the acquisition of phytoplasmas, must be made 30 d after the first instars are detected.

Because field sampling is often very time-consuming, knowledge of the spatial distribution and minimum sample size required is the first step for resource optimization. The purpose of this article was to investigate the spatial distribution of *S. titanus* nymphs in the vineyard and to develop sampling plans that will be useful for integrated pest management (IPM).

Materials and Methods

Study Area. Studies were conducted in 2002–2004 in 13 vineyards (vin.) located in different grapevine-

0022-0493/06/0578–0582$04.00/0

Table 1. Sampling of nymphs of *S. titanus* on vine plants from different experimental sites

S	EXP	Yr	Period	N	NYM	
					Mean	SE
1	1	2004	Mid-June	50	0.17	0.11
2	2	2004	Late May	50	0.40	0.18
3	2	2004	Mid-June	50	0.22	0.12
4	3	2004	Mid-June	50	0.34	0.20
5	4	2002	Late May	40	2.78	0.83
6	4	2003	Late May	40	7.80	2.20
7	4	2002	Mid-June	40	5.58	2.21
8	4	2003	Mid-June	40	3.08	0.76
9	5	2003	Mid-June	40	2.23	0.79
10	5	2002	Mid-June	18	0.44	0.48
11	6	2004	Mid-June	47	2.40	0.89
12	7	2004	Late May	50	0.50	0.25
13	7	2004	Mid-June	50	0.50	0.26
14	8	2004	Mid-June	32	1.41	0.68
15	9	2004	Late May	50	0.60	0.28
16	9	2004	Mid-June	50	0.28	0.20
17	10	2004	Mid-June	50	0.90	0.53
18	11	2003	Late May	20	0.6	0.39
19	12	2002	Late May	40	1.42	0.69
20	13	2003	Mid-June	20	0.25	0.24

S, sampling; EXP, experimental vineyard; *N*, number of plants observed (five leaves per plant, close to the rootstock, and NYM, number of nymphs per plant.

growing areas of Piedmont: Langhe (44° 41′ N, 8° 01′ E) (vin. 1–6), Monferrato (44° 54′ N, 8° 12′ E) (vin. 7–12), and Tortonese (44° 53′ N, 8° 51′ E) (vin. 13). Some of the vineyards were subject to IPM practices, whereas others designated as organic (ORG) were not. The same vineyard sometimes received different pest management in different years; the most common IPM practices (or insecticide sprays) during each year included two treatments with different active ingredients chosen among Buprofezin, Fenitrotion, Clorpirifos, and Etofenprox (Muccinelli 2002).

Field Sampling. An experimental plot was traced out in each vineyard. Plots were 18 rows in width (27–30 m) and ≈100 m in length, therefore having an area of 2,700–3,000 m^2. *S. titanus* nymphs were counted on 20–50 randomly distributed plants per plot, choosing five leaves per plant close to the rootstock. Counts were done twice a year, at the beginning and at the middle of June, before any insecticide was sprayed. Sampling generally was carried out early in the morning, when nymphs were less active, and never after a heavy rain. Specimens were not removed from plants during sampling.

Statistical Analysis. Data were analyzed using SPSS 12.0 statistical software (SPSS Inc., Chicago, IL; http://www.spss.com/). Each plant was considered as a single replication for analysis. To study the spatial distribution of nymphs, we used Taylor's power law (Taylor 1961, 1984):

$$\mathrm{Log}_{10}(S^2) = \mathrm{Log}_{10}(a) + b\log_{10}(m) \qquad [1]$$

where S^2 is the sample variance, m is the sample mean, coefficient a depends upon the sampling method and coefficient b indicates the distribution of the population ($b < 1$, uniform; $b = 1$, random; and $b > 1$, aggregated). The coefficients a and b were calculated with the regression of variance on the mean of each plot, during different years and sampling dates: that is, a set of 20 samplings was available, after excluding those where no nymphs were detected. Different linear regressions were tested for equality of slopes (Sokal and Rohlf 1995) by performing an analysis of covariance on the data obtained from different times of the season and during different years.

Minimum sample size (Karandinos 1976) was estimated with the following equation:

$$N = Z/D^2 a m^{(b-2)} \qquad [2]$$

where N is the number of samples needed, $Z = 1.96$ for $\alpha = 0.05$, a and b are Taylor's power law coefficients, m is the population mean, and D is a fixed precision level which was fixed at 0.10, 0.20, and 0.30.

Green's formula (Green 1970) was used to establish fixed precision-level stop lines for sequential sampling of nymphal counts per plant:

$$\ln(T) = (\ln D^2)/(b-2) + [(b-1)/(b-2)]\ln(n) \qquad [3]$$

where n is the number of counted plants, T is the number of insects observed, b is the coefficient of Taylor's power law, and D is the precision level, which was fixed at 0.10 and 0.25.

Model Validation. To assess the reliability of Green's sequential sampling plan, 44 additional independent data sets were collected in 2005 from 31 vineyards (including the previous vineyards). Nymphs were sampled as described previously, but on 100 plants per plot. Validation was performed following the resampling approach, by means of Resampling Validation of Sample Plans (RVSP) software (Naranjo and Hutchison 1997). Simulations were conducted using 500 without replacement, with a minimum sam-

Table 2. Data of Taylor's power law linear regression obtained for nymphs of *S. titanus*, concerning different years and different periods of the season

Source of variation	R^2	ANOVA			Coefficients		
		$F_{(df)}$	P		B	t	P
Different yr	0.98	$204.25_{(2,17)}$	0.000	Intercept	0.415	3.482	0.003
				Slope	1.466	16.122	0.000
				Yr	−0.022	−0.426	0.675
Different periods	0.96	$222.19_{(2,17)}$	0.000	Intercept	0.218	1.791	0.091
				Slope	1.504	20.938	0.000
				Period	0.091	1.277	0.219

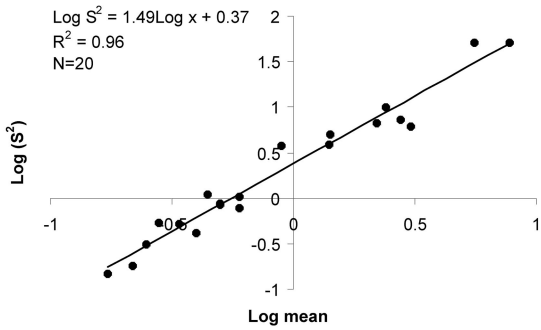

Fig. 1. Taylor's power law regression for nymphs of *S. titanus* on grapevine plants, from mean and variance pairs of all experimental sites.

ple size of 10, for prefixed precision levels (D_0) of 0.25 and 0.10. For $D_0 = 0.25$, 17 data sets of 44 were not used because of sample size exceeding 90% of the observations available; simulations were subsequently performed for 27 data sets. Because at $D_0 = 0.10$ the resampling without replacement failed to execute, we tested this level of precision by using a resampling with replacement (Naranjo and Hutchison 1997).

Results

The mean number of *S. titanus* nymphs per plant ranged from 0.17 to 7.80 (Table 1). No significant differences were found between slopes for the different periods, or for different years (Table 2). Therefore, common regression was used to predict the log (s^2) versus log (m) relationship. The slope was significantly >1.0 ($b = 1.49$, $R^2 = 0.96$; $t = 20.68$, df = 1, 18; $P < 0.001$), indicating a clumped distribution (Fig. 1).

Numerical sample size curves (Karandinos 1976) of *S. titanus* were obtained from Taylor's power law coefficients: a mean density of 0.20 nymphs per plant requires a sample size of 32 plants for $D = 0.30$ and of 72 plants for $D = 0.20$. Given the same pest density, 292 plants must be counted to achieve a precision level D = 0.10 (Fig. 2). The sequential sampling plan, estimated by Green's equation, indicated that, for the same mean value, sampling could stop when seven nymphs have been observed, with fixed precision levels of 0.25 (Fig. 3), whereas with a precision level of 0.10, sampling should be stopped after having observed 46 nymphs, the process, therefore, becoming too time-consuming (Fig. 4).

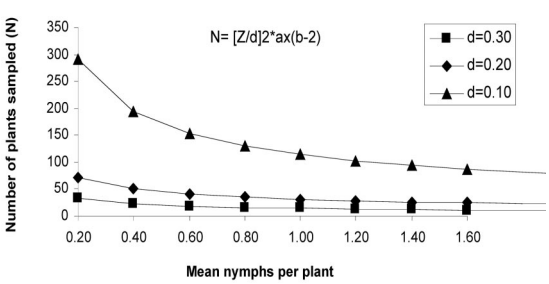

Fig. 2. Minimum sample size calculated for nymphs of *S. titanus*, at $D =$ of 0.10, 0.20, and 0.30.

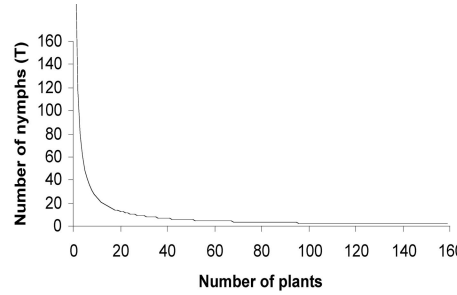

Fig. 3. Green's stop lines for sampling nymphs of *S. titanus*, at $D = 0.25$.

The simulations performed with RVSP software produced an average precision (D) of 0.24, very close to the desired precision of 0.25. On average, the sequential sampling model performed better than expected, and only in few cases was the precision achieved poorer than the preset value (Fig. 5). The average sample number (ASN) was 50 plants, with a range of 24–90 (Fig. 6).

When running simulation with $D_0 = 0.10$, we obtained the same average precision ($D = 10$), although in many cases the model performed poorer than expected (Fig. 7). However, this in understandable because resampling was performed with replacement for this prefixed precision level. Also, for this high level of precision, the ASN was 398 plants (Fig. 8).

Discussion

The distribution of *S. titanus* nymphs is aggregated: this could be, first, the consequence of the aggregated pattern of adults (Bosco et al. 1997) and hence of females laying eggs in aggregated sites. However, the aggregated pattern continues throughout June, suggesting that *S. titanus* nymphs do not move far away from the leaves where they settle at first. A similar distribution also was observed for nymphs of other leafhoppers such as *Empoasca vitis* Goethe (Maixner

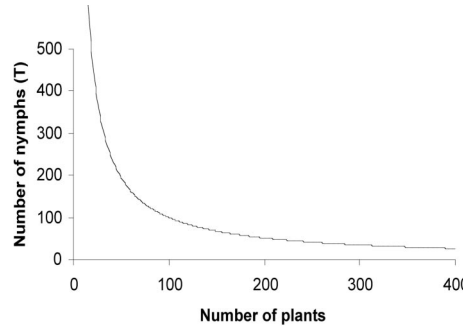

Fig. 4. Green's stop lines for sampling nymphs of *S. titanus*, at $D = 0.10$.

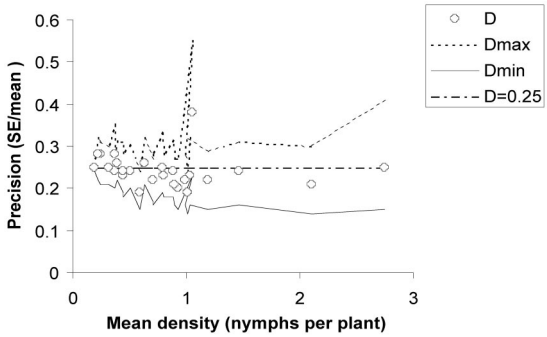

Fig. 5. Summary of resampling validation analysis showing actual precision levels obtained for Green's sequential sampling plan over a range of *S. titanus* nymphal densities. Green's parameters: $D_0 = 0.25$, $a = 2.34$, and $b = 1.49$.

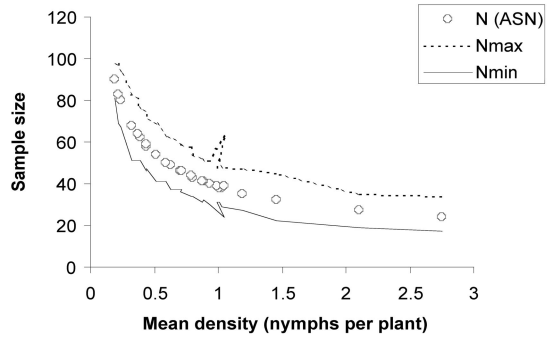

Fig. 7. Summary of resampling validation analysis showing actual ASN for Green's sequential sampling plan over a range of *S. titanus* nymphal densities. Green's parameters: $D_0 = 0.25$, $a = 2.34$, and $b = 1.49$.

2003), *Jacobiasca lybica* (Berg. et Zan.) (Delrio et al. 2001), and *Perkinsiella saccharicida* Kirkaldy (Allisop and Bull 1990), although slightly less aggregated than *S. titanus.* However, a similar aggregation index was calculated for *Typhlocyba pomaria* McAtee (Beers and Jones 2004).

As a consequence of its aggregated pattern, the numerical sample size for the correct estimate of *S. titanus*'s nymphal density can be very high in case of low population levels. Therefore, Green's stop lines could become a suitable method for estimating the population density of *S. titanus* nymphs. The proposed model performed as expected on the average: simulations were many times more accurate than predicted from Green's equation, having preset a precision level of 0.25. The ASN in this case was 50 plants, which can be easily covered in ≈30 min by a single data collector (personal observation). Because for IPM purposes this level of precision is acceptable (Southwood 1978), we recommend the use of Green's model with $D_0 = 0.25$ for sampling the nymphs of *S. titanus* on grapevine. However, to achieve a precision level of 0.10 would require an ASN of 350 plants, which is too time-consuming.

Correct sampling plans should have a positive impact on *S. titanus* pest management, if an economic threshold for this pest can be developed (Kogan 1998). Different thresholds could also be determined depending on the presence or the absence of the disease within a specific area, especially considering the low efficiency in disease transmission by *S. titanus* (Girolami 2000). Moreover, the percentage of FD-positive vectors is generally low at the beginning of the season (Lessio et al. 2003). Therefore, because aggregation of nymphs does not depend upon the season, a slight delay in applying insecticides could be reconsidered, to wait for more eggs to hatch. In fact, progressive hatching of nymphs during summer (Vidano 1964) is one of the primary problems for IPM of *S. titanus.* More information is also needed about the impact of generalist predators, such as spiders, on the dispersal of *S. titanus* nymphs, and hence on their spatial distribution; such a relationship was demonstrated for *Prokelisia crocea* Van Duree (Homoptera: Delphacidae) (Cronin et al. 2004).

Acknowledgments

We thank Daniela Tornato, Paolo Chiusano, Fabio Nicola, and Sara Delpiano, students of "Laurea di I Livello in Viticoltura ed Enologia," for cooperation in field sampling. We are grateful to Piero Caciagli (IVV-CNR, Turin) and to

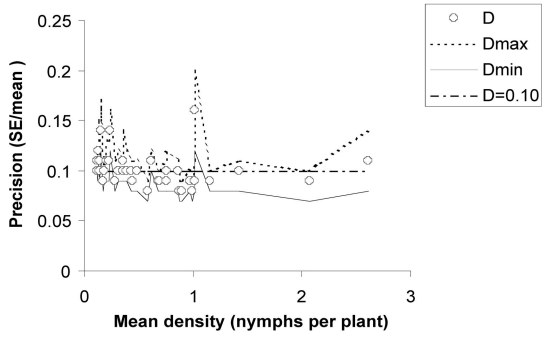

Fig. 6. Summary of resampling validation analysis showing actual precision levels obtained for Green's sequential sampling plan over a range of *S. titanus* nymphal densities. Green's parameters: $D_0 = 0.10$, $a = 2.34$, and $b = 1.49$.

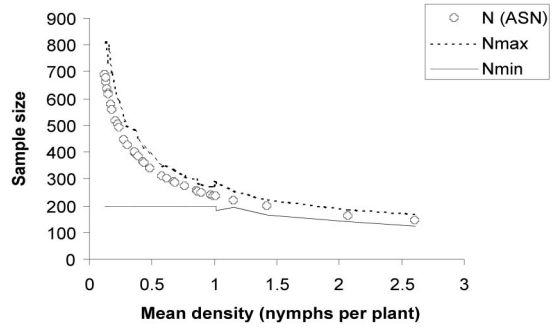

Fig. 8. Summary of resampling validation analysis showing actual ASN for Green's sequential sampling plan over a range of *S. titanus* nymphs' density. Green's parameters: $D_0 = 0.10$, $a = 2.34$, and $b = 1.49$.

Giovanni Burgio (DiSTA-area Entomologia, University of Bologna) for advice in data analysis. We thank Lorenzo Calorio (Coldiretti Cuneo), Piero Bosca (Coldiretti Asti), Anna Guercio (CIA Asti), Elena Bassignana (Agri. Bio. Piemonte), and Marco Boveri (CADIR Lab. SRL) for help in choosing suitable experimental sites. Funding for this research came from "Regione Piemonte-Assessorato Agricoltura, Programma Regionale dei Servizi di Sviluppo Agricolo," "Regione Autonoma Valle d'Aosta-Assessorato Agricoltura, Risorse Naturali e Protezione Civile," and "Martini & Rossi–Bacardi & Martini Group."

References Cited

Allisop P. G., and R. M. Bull. 1990. Sampling distribution and sequential sampling plans for *Perkinsiella saccharicida* Kirkaldy (Hemiptera: Delphacidae) and *Tytthus* spp. (Hemiptera: Miridae) on sugarcane. J. Econ. Entomol. 83: 2284–2289.

Alma A. 2004. The genus *Scaphoideus* in the world. The diffusion of *S. titanus* in Europe, pp. 3–5. *In* I. M. Kerzhner [ed.], Third European Hemiptera Congress, 8–11 June 2004, St. Petersburg, Russia.

Beers, E. H., and V. P. Jones. 2004. Fixed precision sampling plans for white apple leafhopper (Homoptera: Cicadellidae) on apple. J. Econ. Entomol. 97: 1752–1755.

Bosco, D., A. Alma, and A. Arzone. 1997. Studies on population dynamics and spatial distribution of leafhoppers in vineyards (Homoptera: Cicadellidae). Ann. Appl. Biol. 130: 1–11.

Bosio, G., M. C. Martinez, P. Occhetti, I. Rovetto, D. Della Valle, L. Laiolo, and G. Valota. 2004. Efficacy evaluation of synthetic and natural insecticides against *Scaphoideus titanus* Ball young stages on grapevine. Atti Giornate Fitopatol. 1: 95–102.

Boudon-Padieu, E. 2000. Recent advances on grapevine yellows: detection, etiology, epidemiology and control strategies, pp. 87–88. *In* Proceedings, 13th ICVG Conference, 12–17 March 2000, Adelaide, Australia. University of Adelaide and CSIRO, Adelaide, Australia.

Caobelli, R., and G. Carcereri. 1995. Lotta biologica alla cicalina della vite. Inf. Agr. 33: 75–77.

Caruso, S., and P. Mazio. 2004. Control trials on *Scaphoideus titanus* Ball in organic vineyards. Atti Giornate Fitopatol. 1: 109–110.

Cronin, J. T., K. J. Haynes, and F. Dillemuth. 2004. Spider effects on planthopper mortality, dispersal, and spatial population dynamics. Ecology 85: 2134–2143.

Delrio, G., A. Lentini, and G. Serra. 2001. Spatial distribution and sampling of *Jacobiasca lybica* on grapevine. Bull. OILB/SROP 24: 211–216.

Girolami V. 2000. Vettori e problemi aperti sui giallumi della vite. *In* Atti "Incontro nazionale sulle malattie da Fitoplasmi. Stato attuale delle conoscenze", Udine, 21–22 settembre 1999. Petria 10: 167–170.

Green, R. H. 1970. On fixed precision level sequential sampling. Res. Popul. Ecol. 12: 249–251.

Karandinos, M. G. 1976. Optimum sample size and comments on some published formulae. Bull. Entomol. Soc. Am. 22: 417–421.

Kogan, M. 1998. Integrated pest management: historical perspectives and contemporary developments. Annu. Rev. Entomol. 43: 243–270.

Lessio, F., and A. Alma. 2004a. Dispersal patterns and chromatic response of *Scaphoideus titanus* Ball (Homoptera: Cicadellidae), vector of the phytoplasma agent of grapevine flavescence dorée. Agric. For. Entomol. 6: 121–127.

Lessio, F., and A. Alma. 2004b. Seasonal and daily movement of *Scaphoideus titanus* Ball (Homoptera: Cicadellidae). Environ. Entomol. 33: 1689–1694.

Lessio, F., S. Palermo, R. Tedeschi, and A. Alma. 2003. Presence of grapevine yellows phytoplasmas vectors (Homoptera, Auchenorrhyncha) in northwestern Italy, pp. 75–76. *In* G. P. Martelli [ed.], Proceedings, 14th ICVG Conference, 12–17 September 2003, Locorotondo (BA), Italy.

Maixner, M. 2003. A sequential sampling procedure for *Empoasca vitis* Goethe (Homoptera: Auchenorrhyncha). Bull. OILB/SROP 26: 209–215.

Muccinelli M. 2002. Prontuario dei fitofarmaci, 9th ed. Edagricole, Bologna, Italy.

Naranjo, S. E., and W. D. Hutchison. 1997. Validation of arthropod sampling plans using a resampling approach: software and analysis. Am. Entomol. 43: 48–57.

Schvester, D., P. Carle, and G. Moutous. 1969. Nouvelles données sur la transmission de la flavescence dorée de la vigne par *Scaphoideus littoralis* Ball. Ann. Zool. Écol. Anim. 1969: 445–465.

Sokal, R. R., and F. J. Rohlf. 1995. Linear regression, pp. 451–554. *In* Biometry: the principles and practice of statistics in biological research, 3rd ed. W. H. Freeman, New York.

Southwood, T.R.E. 1978. Ecological methods with particular reference to the study of insect populations. Chapman & Hall, London, United Kingdom.

Taylor, L. R. 1961. Aggregation, variance and the mean. Nature (Lond.) 189: 732–735.

Taylor, L. R. 1984. Assessing and interpreting the spatial distribution of insect populations. Annu. Rev. Entomol. 29: 321–357.

Vidano, C. 1964. Scoperta in Italia dello *Scaphoideus littoralis* Ball cicalina americana collegata alla "Flavescence dorée" della vite. L'Italia Agric. 101: 1031–1049.

Received 11 April 2005; accepted 17 November 2005.