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BRIEF ARTICLE

A new method to assess air pollution using lichens as bioindicators

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Abstract: Lichens are increasingly used worldwide as air quality biomonitors because they are efficient, easy and cheap, but validation studies of the methodology are scarce. Three foliose lichen biomonitoring methods were compared by field tests (in the tropical urban habitat of San José, Costa Rica) and laboratory simulations: (1) the 100 uniform squares template traditionally used in North America, (2) the European 200 uniform points template and (3) a new computer-generated random points template (10 X 20 cm) in two versions: 100 points and 50 points. Repeated measurement by the same observer causes a variation of 2-14% and the templates' error is 0.2-11%. We recommend the 100 random point template (applied to four sides of trunk) for ecological studies and the 50 random points template (applied to side with greatest lichen cover) for biomonitoring because it reduces time and costs by nearly 50% but still has acceptable reliability values.

Key words: Bioindication, lichens, air pollution, methodology.

Lichens are increasingly used as air quality biomonitors (Bartoli *et al.* 1997, Malysheva 1998) because they have several advantages over electronic monitors. These are expensive and their use and maintenance are not simple or cheap. Even industrialized nations are unable to afford extensive networks of electronic monitors. They are limited to a few elements or chemical compounds and have no intrinsic relationship with the biological effect of the contaminants (Rodrigo *et al.* 1999).

In contrast, biomonitors are available for free and there are millions of them already functioning throughout the world (Ockenden *et al.* 1998). They integrally reflect the environmental influence over organisms and can be understood and used by the common citizen with minimal training. Even though almost all biological species can be considered biomonitors to some extent, the above advantages apply particularly well to lichens when air pollution is considered (van Dobben and Ter-Braak 1999).

In tropical regions, poor knowledge of lichen taxonomy does not affect basic biomonitoring because this method does not require species identifications (Carreras *et al.* 1998, Monge-Nájera *et al.* 2001). Air biomonitoring is particularly developed in Europe (Faltynowicz 1997), where the lichen *Hypogimnia physodes* is used as a standard species (Grüninger and Monge-Nájera 1988) and much research is done about the involved biochemistry (Hyvarinen and Critenden 1998), but to our knowledge, no new simple field methods have been developed for at least three decades. This paper compares the reliability and cost of two traditional methods and describes a new computer-generated method that proved reliable and reduced costs by 50%. The phorophytes were selected because they were available in representative parts of the city (Méndez and Fournier 1980), met the requirements for this type of study (Méndez and Fournier 1980), and because they had been under observation for two decades (Monge-Nájera *et al.* this issue).

MATERIALS AND METHODS

Template types: The following methods were compared: (1) the 100 uniform squares (10X10 cm) template used in North America and applied to the four cardinal sides of tree trunks (Méndez and Fournier 1980), (2) the European 200 uniform points (10 x 20 cm) template applied to the tree trunk side that is visually identified as having the highest lichen cover (Grüninger *et al.* 1980) and (3) a new random points template (10 x 20 cm) applied to the four cardinal sides of tree trunks. The random point positions were generated with standard random generator computer software and the 2 mm diameter points were applied with white paint on a translucent plastic sheet. The reliability of the three templates was examined under experimental conditions and, additionally, methods (2) and (3) were field tested in 1987 in the city of San José, Costa Rica and are compared here among themselves and with a parallel study with method (1) (Monge-Nájera *et al.* 2001). For details about the reference site see Méndez and Fournier (1980).

Reliability experiments: The differences produced by template design were measured by asking sixteen biology students to measure cover on the same section of a single trunk with the square, uniform point and random point templates. Real cover was measured by tracing the lichens on paper of homogeneous thickness and weighing the clippings to the nearest 0.0001 g.

To assess the effect of repeated sampling, the same students estimated lichen cover by blindly placing a template ten times over a drawing of bark with one dominant and one scarce lichen species (N = 160 measurements,

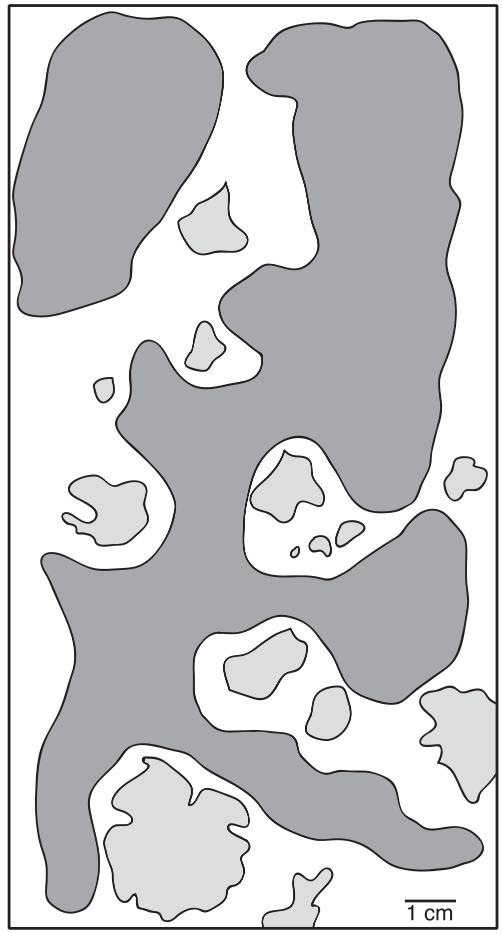

Fig. 1. Drawing of bark with one dominant and one scarce lichen species used to test template effect.

Fig. 1). Real cover was measured by clipping the drawings on paper of homogeneous thickness and weighing to the nearest 0.0001 g. This experiment was done with the uniform point template and with the 100 random point template. Additionally, the effect of sample size was examined in the point templates by calculating twice: with all data and with a random subsample of half the data (*i.e.* 50 points).

RESULTS

Field test: In one half of the stations, the uniform point method (Table 1) produced sig-

TABLE 1

*Comparison of uniform point and the random point method results in the stations (San José, Costa Rica, 1987) and F test significance values**

Station number	Uniform point method	Random point method, mean values	F significance	Random point method, maximum values	F significance
2	8.70	1.10	0.0005	2.9	0.1546
3	15.00	3.50	0.0366	8.2	0.8744
4	6.50	4.60	0.8245	12.0	0.0122
5	1.90	0.10	0.0000	0.5	0.0019
6	22.70	19.80	0.8010	36.8	0.0903
7	12.90	9.50	0.5264	23.5	0.1686
8	29.60	8.70	0.0025	33.5	0.5450
9	47.50	18.20	0.0837	51.4	0.2791
10	1.50	0.60	0.0388	2.6	0.0664
11	17.75	43.52	0.0028	70.0	0.0004

* These methods were applied to measure lichen cover in the city of San José, Costa Rica in 1987 as part of a long term study and the results are published somewhere else (Monge-Nájera *et al.* 2001).The uniform point method always uses the tree side with highest lichen cover; the random point method used the four "tree sides" in this case only for comparison: it is fit for use on the side with the highest cover. For comparison, the random point method used both the highest and the mean values for each tree. There were no lichens in Station 1.

nificantly higher estimates than the random points method applied to the four sides. When both methods were applied to the side with the highest cover, they produced statistically equivalent results.

Reliability experiments: Repeated measurements by the same observer causes a variation range (standard deviation) of 2-14% (Table 2). Relative lichen cover estimated when several observers measured the same tree with three templates were:

100 squares method: real cover 6%, estimated cover 17% (17; 1-79), N = 22.

200 uniform points method: real 51%, estimated 44% (17; 22-82), N = 21.

100 random points method: real 51%, estimated 59% (18; 30-95), N = 22.

The squares method overestimated by 11%. The uniform points method underestimated by 7%, and the random points method overestimated by 8%.

In the laboratory simulation, results differed significantly among templates (Kruskal-Wallis ANOVA and Tukey Test, $p < 0.01$) but errors are not large (0.2-11%). In comparison with using all the points, templates of the same size and shape but with only half the points are only about 5% less reliable (range 0.3-6.9%) than the full versions (Table 2).

DISCUSSION

The spatial microdistribution of lichens is associated with atmospheric pollutants in a complex way because pollutant impact depends on substrate texture, shape and inclination, wind, light, humidity, organism activity and other factors (van Dobben and Ter-Braak 1999). Furthermore, the association is highly variable (*e.g.* Monge-Nájera *et al.*); for this reason we recommend the 100 random point template (applied to four sides of trunk) for ecological studies, which requires a precise description of spatial distribution. The physiology of the lichen-environment interaction was studied among others by Malhotra and Khan (1983). When lichens are used as bioindicators for air pollution monitoring (Carreras *et al.* 1998, Hyvarinen and Crittenden 1998), we

TABLE 2

The effect of repeated measurement with two template types and two sample sizes on estimation of cover by a dominant and a minoritary lichen species (laboratory simulation). Each row: N = 160

Lichen cover (%) estimated by each method	Mean	Standard deviation	Minimum	Maximum
Dominant species				
Random template				
Subsample	55.4	12.4	20	90
Total sample	51.4	11.4	25	70
Uniform template				
Subsample	40.4	13.6	15	59
Total sample	52.3	1.9	48	58
Real value	51.2			
Minoritary species				
Random template				
Subsample	16.9	12.7	0	110
Total sample	12.4	6.5	0	30
Uniform template				
Subsample	9.7	3.6	2	20
Total sample	11.5	3.1	5	25
Real value	10.0			

recommend the 50 random points template (applied to the side with greatest lichen cover) because in comparison with the other methods it reduces time and costs by nearly 50% but still has acceptable reliability values (4.2% error, 12.4% standard deviation).

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RESUMEN

Se comparó tres métodos para la medición de cobertura de líquenes en el campo (ciudad de San José, Costa Rica) y en simulaciones de laboratorio: (1) la plantilla de 100 cuadrados uniformes usada en América del Norte, (2) la plantilla europea de 200 puntos distribuidos uniformemente y (3) una nueva plantilla generada por computadora que consta de puntos aleatoriamente ubicados en un rectángulo de 10 X 20 cm, en dos versiones: 100 puntos y 50 puntos. Los errores debidos al observador o a la plantilla son de 0.2-14%. Para estudios ecológicos se recomienda por su precisión la plantilla de 100 puntos aleatorios aplicada a los cuatro puntos cardinales del tronco y para monitoreo de contaminación, la de 50 puntos aleatorios aplicada al lado con mayor cobertura porque produce resultados semejantes a los métodos tradicionales pero reduce tiempo y costos a la mitad.

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