

**An investigation into the use of fluctuating asymmetry
as a measure of low level air pollution stress in plants.**



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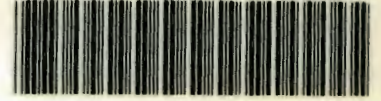
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Abstract

Monitoring the effects of point source pollutants on the vegetation surrounding them has been a difficult task (Lechowicz, 1987). This is largely due to the varied responses of plant species to pollution stress. A new measure of pollution stress is proposed, that of deviations from the symmetrical phenotypic optimum (that are normal in distribution with a mean of zero, fluctuating asymmetry). Asymmetry of phyllodes of *A. cyclops* shrubs surrounding an oil refinery on the south-west coast of the Cape Peninsula were analysed to investigate the correlation of fluctuating asymmetry with pollution stress. Three variables were assumed to affect the pollution stress imposed by the refinery, distance from the refinery, distance from the urban-industrial area of Cape Town and the frequency with which the site is fumigated. Fluctuating asymmetry correlated with both distance variables but not with fumigation frequency. It is assumed that the fumigation frequencies of the sample sites are not variable enough to differentially affect the exposure of the sites to the pollution from the refinery. Distance from the pollution source is therefore the variable of overriding importance in determining the pollution stress of the site. Fluctuating asymmetry correlated negatively with both distance variables and therefore proved to be a reliable and accurate measure of the effects of pollution stress on vegetation. This study should pave the way for further investigations into the use of fluctuating asymmetry as a measure of the effects of exogenous stress on vegetation and should stimulate further interest in the use of fluctuating asymmetry in botanical research.

Introduction

Developmental stability can be defined as the ability of an organism to withstand genetic and environmental disturbances encountered during development so as to produce a predetermined optimum phenotype (Clarke, 1993). Under favorable conditions, developmental stability is greatest and organisms produce phenotypes tending towards the ideal. Under suboptimal conditions however, developmental stability is low and many deviations from the ideal phenotype are produced (Freeman *et al.*, 1993). Suboptimal conditions may include abnormal temperatures, nutrient deprivation and exposure to pollutants (Parsons, 1989). Similar decreases in developmental stability occur as a result of endogenous stress brought about by the disruption of gene complexes as occurs in hybridization between disparate taxa or during inbreeding (Leary *et al.*, 1983; Bush *et al.*, 1987; Strauss, 1987). The basic assumption concerning the mechanisms producing developmental instability is that exogenous stress disrupts the physiology of the organism, altering its developmental pathway. Similarly, the disruption of gene complexes alters the regulation of physiological processes and thus the development of the phenotype (McKenzie and O'Farrell, 1993).

Given that stress is expected to cause deviations in developmental patterns, the consequences of developmental instability can be used to monitor the effects of stressors on organisms. This could prove useful, for example in monitoring the effects of anthropogenic pollution stress on vegetation. To date, the monitoring of such pollution effects on plants has proved difficult (Lechowicz, 1987). This is largely due to the varied reactions of plant species to pollution stress. These reactions include inconsistent resource allocation patterns among roots, reproductive organs and shoots. The pin oak (*Quercus palustris*) for example shows increased leaf allocation when fumigated with sulfur dioxide whereas other species such as the cucumber (*Cucumis sativus*) do not follow this trend (Lechowicz, 1987). In addition, there is no general trend in plant lifetime

resource accumulation under pollution stress, with the response largely being dependent on the type of pollution stress imposed (Crittenden and Read, 1978).

In order to utilise developmental instability as a monitor of the effects of pollution stress on plants, it must be measurable. The notion of symmetry, largely borrowed from mathematics and physics, has played a dominant role in the study and measurement of developmental instability (Graham *et al.*, 1993). It assumes the symmetrical state of a character to be the ideal solution; in the terminology of chaos theory, the point attractor. Freeman *et al.* (1993) have suggested that this optimal phenotypic solution represents a broad basin of attraction rather than a single point solution. This suggestion is more realistic in terms of the mechanisms guiding development as it allows some variation in phenotype to be expected, even under ideal conditions. Deviations from the ideal symmetrical state represent developmental instability. There are three types of deviations from symmetry; fluctuating asymmetry, directional asymmetry and antisymmetry. Each of these types of asymmetry are characterised by a different combination of the mean and variance of the distribution of the asymmetry measurements. Fluctuating asymmetry occurs when the deviations from symmetry show a normal distribution, with the mean of the distribution not differing significantly from zero (Palmer and Strobeck, 1986) (figure 1a). Directional asymmetry on the other hand represents a constant bias of a character to one plane of symmetry, for example the mammalian heart which shows greater development of it's left side (Graham *et al.*, 1993). Directionally asymmetrical traits such as the mammalian heart exhibit normally distributed asymmetry measurements with a mean that is significantly greater or less than zero (Palmer and Stroebeck, 1986) (figure 1b). Antisymmetry on the other hand occurs where asymmetry is present but is variable as to which side has the greater development. For example in the male fiddler crabs (Genus *Uca*) the oversized signaling claw, larger than the opposing one, occurs with approximately equal frequency on both the right and left sides in nearly all

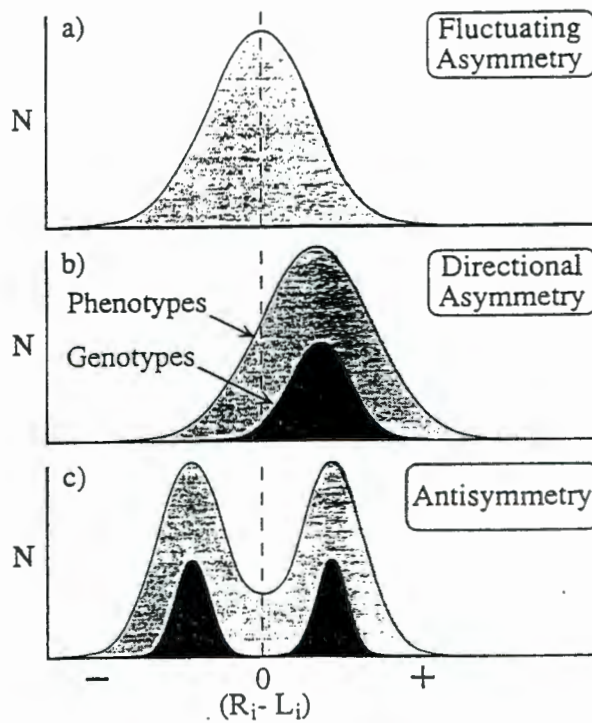


Figure 1. (a) Fluctuating asymmetry, (b) Directional asymmetry and (c) Antisymmetry shown as the number of individuals within a species (N) against the asymmetry measurement (Left-minus-right). Contribution of genetic factors to the three forms of asymmetry is also indicated. Black-fill frequency distributions symbolise genetically induced variation whereas gray-fill frequency distributions symbolise environmentally induced variation.

species (Andersson, 1986). Antisymmetry is distinguished by a bimodal distribution of asymmetry measurements about a mean of zero (Palmer and Strobeck, 1986) (figure 1c). Both directional and antisymmetry have an unknown genetic component and cannot therefore be used as a measure of exogenous environmental stress on developmental stability (Palmer and Strobeck, 1992). Fluctuating asymmetry on the other hand has no unknown genetic component and, assuming that the plants under consideration are genetically uniform, can be used as a measure of developmental stability and hence as a measure of the effects of environmental stressors such as pollution on vegetation.

Historically, the study of developmental stability has focused largely on animals. Freeman *et al.* (1993) note that of the hundreds of papers dealing with developmental stability, fewer than one dozen deal with plants. The reason for this zoological bias is twofold (Freeman *et al.*, 1993). Firstly, animal development is inextricably linked to the movement and behaviour of cells. Cell division in animals produces independent cell entities which migrate within the developing embryo to establish a given shape or arrangement of tissues. The disruption of this cell movement may lead to major disruptions in phenotype. Exposure to stressors, exogenous or endogenous, therefore results in conspicuous developmental instability. Secondly, the external body form of most animals is bilaterally symmetrical, lending itself to the measurement of developmental stability through analysis of deviations from the bilaterally symmetrical optimum. Plants, on the other hand, grow by accretion and show little reformation of structures. There is no significant cell migration during development and plants are therefore less prone to the developmental aberrations engendered by the disruption of cell movement in animals. In addition, whole plants rarely show bilateral symmetry, making the measurement of developmental stability differ from its measurement in animals. These characteristics of plants do not however render them incompatible with

developmental stability studies. Graham *et al.* (1993) have argued that all symmetries represent potential developmental invariants and may be used in a manner analogous to that of bilateral symmetry. These include radial symmetry (as in actinomorphic flowers), translatory symmetry (with scaling of leaves up a stem) and self-similarity (typical of fractal structures). In addition, growth by accretion has its own advantage in developmental stability studies as it represents a record of instability over time, with deviations from symmetry being magnified over time.

The potential of the concepts of developmental stability and fluctuating asymmetry in botanical studies is only beginning to be recognised and promises to reveal much about the effects of stress on plants. In particular, fluctuating asymmetry measurement may hold the key to understanding and monitoring the effects of pollutants on surrounding vegetation. Little work has been carried out investigating the use of fluctuating asymmetry as a quick and reliable method of monitoring pollution effects (Freeman *et al.*, 1993). This study aims to investigate whether fluctuating asymmetry can be used a reliable method of monitoring the effects of low level air pollution on plants and hopes to stimulate further interest in the use of fluctuating asymmetry in botanical studies.

Materials and Methods

Study site

The site chosen for investigation is situated in the south-western Cape Province, stretching as far north as Paternoster and to Scarborough in the south (figure 2). This site contains an oil refinery and an adjacent fertilizer plant that together release chemical pollutants including sulfur dioxide, hydrogen sulfide, nitrous oxide, lead and fluoride into the atmosphere (Botha *et al.*, 1989) (figure 3). These industries therefore impose pollution stress on the vegetation surrounding

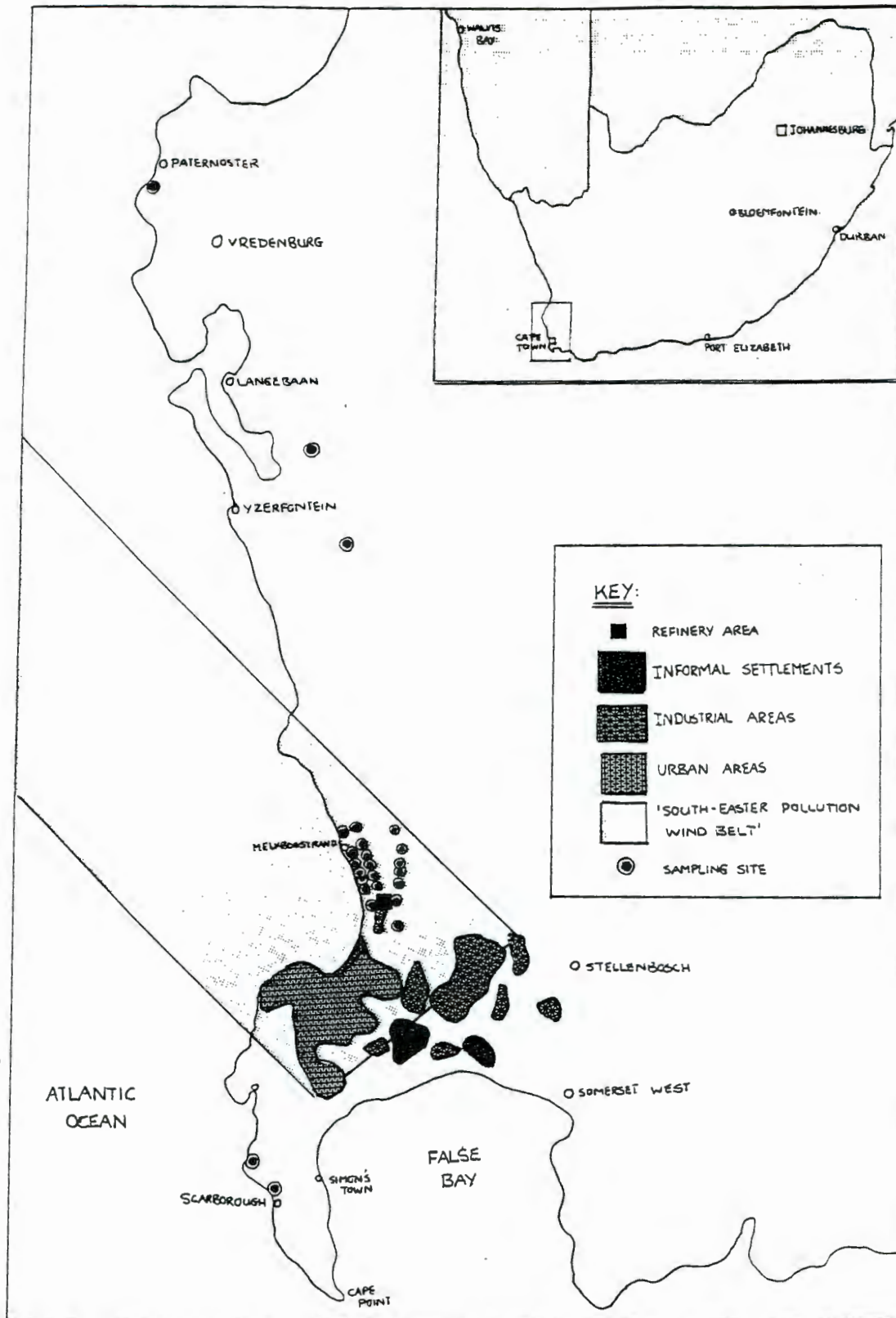


Figure 2. The location of the study site in the south-western Cape Province, South Africa. Included are the sampling sites, the position of the refinery area and the south-east wind-belt from the urban and industrial areas of Cape Town.



Figure 3. View of Caltex oil refinery releasing pollutants into the atmosphere from raised stacks.

them. The refinery and fertilizer plant are not the only sources of air pollution in the study site. A proportion of the site is situated in the wind-belt of the urban and industrial areas of Cape Town (figure 2). The predominant wind direction across Cape Town and its surrounding areas in summer (December to February) is from the south-east (Botha *et al.*, 1989) and the study site is therefore in the pollution cloud of Cape Town for most of the summer. The air pollutants produced by the urban and industrial areas of Cape Town include hydrocarbons, nitrous oxide, ozone, peroxyacyl nitrate (PAN) and lead, largely from automobile exhaust fumes (Benedict and Olson, 1971). For the purpose of this study therefore, the site is divided into two areas; "urban-industrial" which includes sites situated in the urban-industrial wind-belt, and "rural" which includes sites removed from urban or industrial influence. The oil refinery and fertilizer plant are situated in the "urban-industrial" area of the study site. To investigate the effects of these industries on the surrounding vegetation, only sites within the "urban-industrial" area were sampled. Sites in the "rural" area of the study site were sampled to reflect background levels of pollutants and plant asymmetry, without the influence of the urban and industrial areas or the refinery and its adjacent fertilizer plant.

The pollutants produced by the refinery and the fertilizer plant are rapidly carried away by the continual and relatively constant winds in the area (average wind speeds range from 5.3 to 7.7 m/s). The wind directions around the refinery area, unlike the wind speeds, vary considerably during the year. Figure 4 shows the area surrounding the refinery and includes a wind rose depicting the average annual refinery plume frequencies (per thousand). The plume directions were calculated from wind direction data collected for each of the eight main directions over 33 years at the D.F. Malan weather station. The directions have been modified to suit the study area (Dr. Mark Jury, oceanography department, University of Cape Town, pers. comm.).

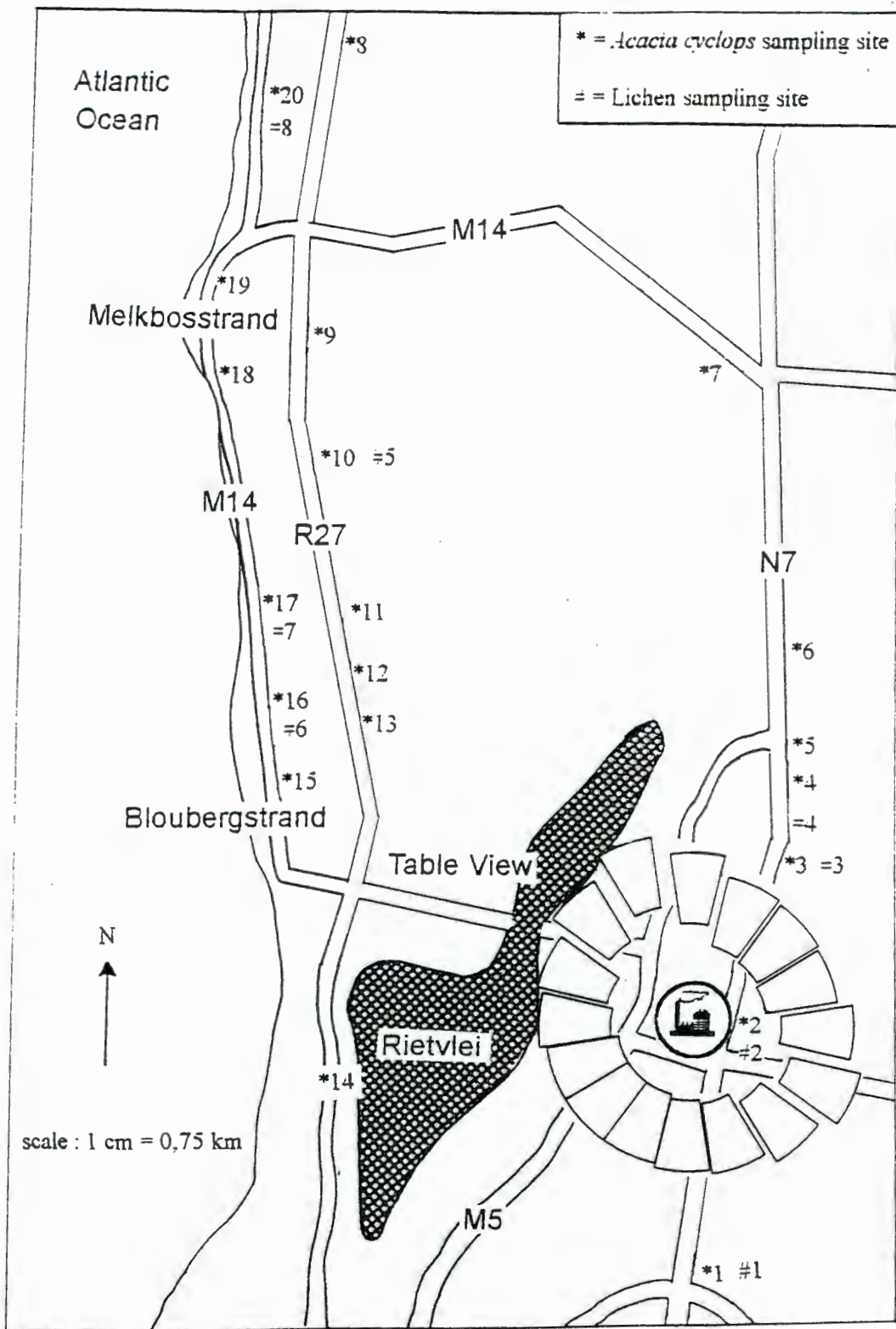


Figure 4. The location of the sampling sites within the urban-industrial wind belt. The windrose surrounding the refinery area depicts the average annual plume directions from the refinery (per thousand).

Plant species

The plant chosen for investigation is the shrub *Acacia cyclops* A. Gunn ex G. Don, listed as invasive in the fynbos biome (Stirton, 1978) (figure 5). This plant was chosen for study because of its ubiquitous nature, the shrub being found in most habitats included in the study site, including coastal areas and marginal habitats fragmented by urbanisation. The species is characterised by phyllodes (swollen petioles) rather than leaves, some of which exhibit curvature away from the symmetrical optimum. These deviations from symmetry are conspicuous and can be easily measured, making this plant species ideal for fluctuating asymmetry studies.

Phyllode measurement: Two phyllode morphs are present on the *A. cyclops* shrubs. Phyllode morph 1 shows curvature in one direction only, whereas phyllode morph 2 shows curvature to both the left and the right (figures 6 a and b respectively). It is assumed for this study that the curvatures of morph 2 are additive and represent high asymmetry, rather than canceling one another and tending towards symmetry. With this assumption, one measurement of asymmetry could be used for both phyllode morphs. The measurement entails drawing a line, X, from the tip to the base of the phyllode. This value represents the phyllode length measurement. This length is divided into three and at each point a line is drawn to the center of the phyllode blade (this accounts for varying phyllode widths). These lines are labeled Y and Z respectively (figure 6a and b). Absolute asymmetry is calculated according to equation 1, and relative asymmetry (taking character length effects into account) is calculated according to equation 2.

Absolute asymmetry = $Y+Z$ equation 1

Relative asymmetry = $(Y+Z)/X$ equation 2

(a)



(b)



(c)

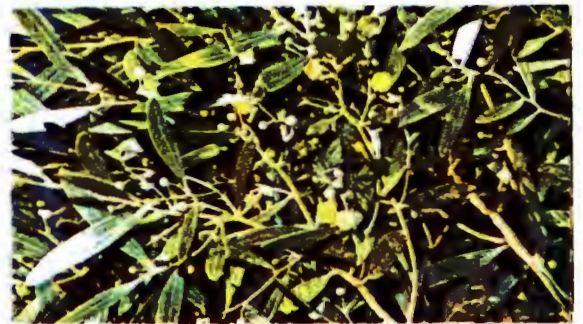


Figure 5. (a) Dense road-side infestation of the invasive *A. Cyclops*, commonly known as “Rooikrans” (b) a middle-aged bush about 2m tall, these shrubs may reach over 6m tall (c) cluster of flattened “swollen petioles” called phyllodes.

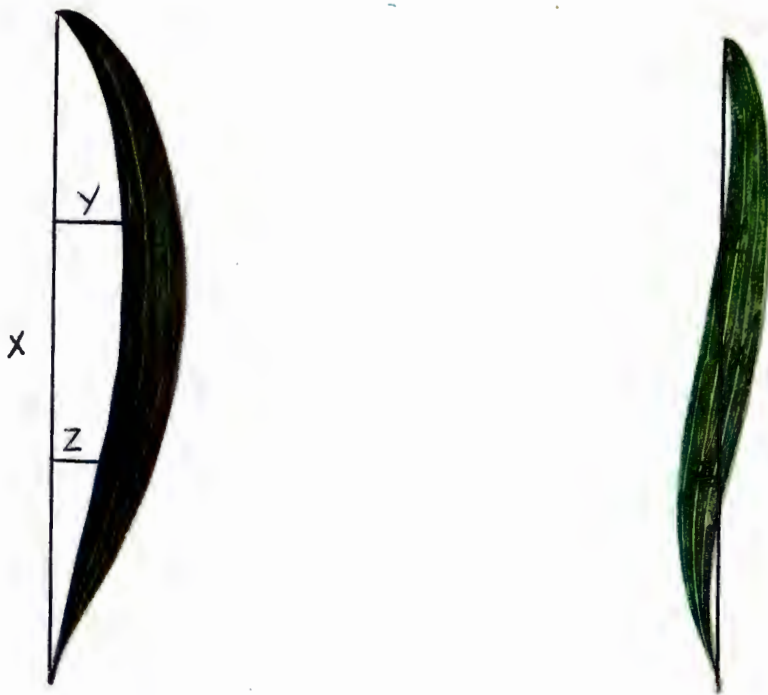


Figure 6. (a) Phyllode morph 1 showing curvature in one direction only; X, Y and Z are the measurements used in the calculation of the asymmetry index (b) Phyllode morph 2 showing curvature in two directions; X, Y and Z are used in the same way as for morph 1 to calculate the asymmetry index.

Sampling

All samples were taken from *A. cyclops* shrubs growing along road verges. This controlled for the effects of additional pollutants emitted by cars utilising the roads in the refinery area. One *A. cyclops* was sampled at each site. Collection of phyllodes were from mature shrubs only; with one branch being removed from the top of the shrub, one from the middle and one from the bottom of the shrub at each site. These branches were removed from the refinery side of the shrub. From each branch, 25 phyllodes were removed from the top of the branch down. There were therefore 75 phyllodes collected per sample site. These were then Photostatted at 100% and measured.

Twenty samples were taken from the “urban-industrial” area. These samples were taken along the three roads that run through the area; the N7, the R27 and the coast road (M14) (see figure 4, *A. cyclops* sampling sites 1-20). Five samples were taken from the “rural area” (figure 2).

Lichen

Lichens are dual organisms formed by the symbiotic association of two plants, a fungus and an alga (De Wit, 1976). They are generally able to withstand harsh conditions, yet their poikilohydrous nature makes them sensitive to air pollutants. As lichens take in moisture and nutrients from the atmosphere, pollutants are accumulated and concentrated into their thalli. This characteristic makes them ideal pollution monitors in terms of the identification of pollutants in the air and in quantifying pollution loads (Hawksworth and Rose, 1976). Lichens were used in this study to detect and quantify the presence of air pollutants within the study site. *Xanthoria* species were chosen for sampling as lichens of this genus are the most tolerant to pollution, occurring in all areas of the study site (M. Muophe, Honours thesis, 1994).

Xanthoria were collected from 8 sites within the “urban-industrial” area (figure 2, Lichen sampling sites 1-8) and from Paternoster to investigate background pollution levels (outside of the urban-industrial influence). At each sample site lichens of the *Xanthoria* genus were identified and carefully removed from their substrate. The lichens were placed into paper bags and returned to the laboratory where they were oven-dried for two days at 80°C and then finely ground using a mortar and pestle. The ground lichen samples were analysed by Dr. P. Benincasa of the organic chemistry department, University of Cape Town. The analyses were carried out for total sulfur and nitrogen contents using a Fisons CHNS Analyser (Totals).

Data analysis

Asymmetry distribution: The distribution of the 1875 relative asymmetry values obtained from measurement of the phyllodes of all sampling sites was plotted. This distribution plot depicts the type of asymmetry occurring in the *A. cyclops* phyllodes; fluctuating, directional or anti-symmetry. Both directional and antisymmetry have an unknown genetic component and cannot therefore be investigated as a measure of exogenous environmental stress on developmental stability (Palmer and Strobeck, 1992). Therefore, only fluctuating asymmetry will be able to be investigated as a measure of developmental stability and hence as a measure of the effects of environmental stressors such as pollution on the vegetation surrounding the refinery.

Age dependence: Absolute asymmetry was plotted against phyllode length (for 500 of the phyllodes sampled) to investigate the possibility of age-dependence in the asymmetry values. The comments of Sullivan *et al.* (1993) on the statistical errors commonly associated with asymmetry analyses were noted and absolute asymmetry, rather than relative asymmetry, used as the asymmetry index to analyse the relationship between character length and asymmetry.

Background levels: The 75 relative asymmetry values obtained for each sample site (25 top, 25 middle and 25 bottom of shrub) were averaged to represent a single asymmetry value per sample site. These average asymmetry values were plotted against area class (i.e. “urban-industrial” or “rural”). This plot revealed natural variation levels of asymmetry in the *A. cyclops* shrubs (under little or no urban or industrial influence). Similarly the total sulfur and nitrogen values of the lichens sampled were plotted against area class to reveal background levels of these air pollutants.

Pollution stress and it's component variables: Four major variables were identified that could possibly affect plant exposure to the pollution stress imposed by the refinery. These are distance from the refinery, distance from the urban-industrial center of Cape Town, the frequency with which the refinery plume affects the sample site and the wind speed at the sample site. At low wind speeds, mixing of the pollution plume with the vegetation layer is increased, resulting in increased exposure of the vegetation to air borne pollutants. Wind speeds in the study area are however consistently high and should not therefore be expected to differentially affect the exposure of the sample sites to pollution stress. Wind directions in the study area on the other hand are more variable and may have a differential effect on the exposures of each sampling site to the pollution stress. In theory, the higher the fumigation frequency of the sample site, the more exposed the sample site will be to the pollution stress. Distance from the refinery and from the urban-industrial center of Cape Town may also be important in determining the degree of exposure to the pollutants produced by the refinery; the further the sampling site from the pollution source, the less the pollution stress imposed on the vegetation at the site. The sampling sites represent a wide range of distances from the refinery and the urban-industrial center, allowing distance effects to be investigated.

Plots: The average relative asymmetry for each sample site was plotted against wind frequency, distance from the refinery and distance from the urban-industrial center. Distance from the urban-industrial center was measured as the shortest distance from the sample site to a line drawn through the center of the urban-industrial area (see figure 2). Regressions were performed on these plots. For both distance plots the axes were log transformed as the correlation appeared exponential and did not therefore meet the linear relationship assumption of the regression analysis. Note that all the data was normalised using a $\log(X+1)$ transformation to allow for the use of parametric statistics such as the linear regression analysis.

The average relative asymmetry measurements were then standardised for the distance variables and replotted against distance from the refinery and against distance from the urban-industrial center. The asymmetry value of each sample site was standardised for distance from the refinery by dividing the asymmetry value by the distance of the site from the refinery. This index (FA STD., dist. from refinery) was plotted against distance from the industrial center. The asymmetry of each site was similarly standardised for distance from the industrial center by dividing the relative asymmetry value of each site by the distance of the site from the industrial center (FA STD., dist from industrial center). This standardised asymmetry was then plotted against the distance of the site from the refinery. Regression analyses were performed on these standardised plots (axes log transformed).

Total sulfur and nitrogen: The total sulfur and nitrogen values obtained for each lichen sampling site were plotted against fumigation frequency, distance from the refinery and distance from the urban-industrial center of Cape Town. Linear regressions were carried out on the plots to indicate any correlations that may reveal a sulfur or nitrogen pollution gradient from the refinery, the urban-industrial center or both.

Results

Asymmetry distribution

Figure 7 shows the frequency distribution of the 1875 relative asymmetry values obtained through measurement of the phyllodes sampled. The distribution fits the definition of fluctuating asymmetry; it shows a clearly normal distribution with the mean of the distribution close to zero (Palmer and Strobeck, 1986). The phyllode deviations from symmetry therefore have no unknown genetic basis and can theoretically be used to measure developmental instability in response to the exogenous pollution stress imposed by the refinery.

Age dependence

Figure 8 shows 500 absolute asymmetry values plotted against phyllode length. The correlation is poor (Linear regression analysis, $r = 0.53$, $r^2 = 28.09\%$), indicating that the deviations from symmetry are not significantly correlated with phyllode age. There does however appear to be a general trend for higher asymmetry in old phyllodes (longer than about 90 centimeters). It is possible that, as plants grow by accretion, old phyllodes have accumulated the effects of the pollution stress over time which are revealed in the increased curvature of the phyllode phenotype.

Background levels

The average relative fluctuating asymmetry values are plotted against the area class for each site in figure 9. The fluctuating asymmetry values of the urban-industrial sites are significantly higher than those of the rural area (Two way analysis of variance; d.f. = 1, 23; F-ratio = 13.97; $p < 0.01$). The background level of fluctuating asymmetry has a mean of 3.5 and shows little variation (Coeff. of variation = 6.6%). The fluctuating asymmetry values of the urban-industrial area on the other hand are higher (mean average relative asymmetry value for urban-industrial site = 6.3) and show higher variation (Coeff. of

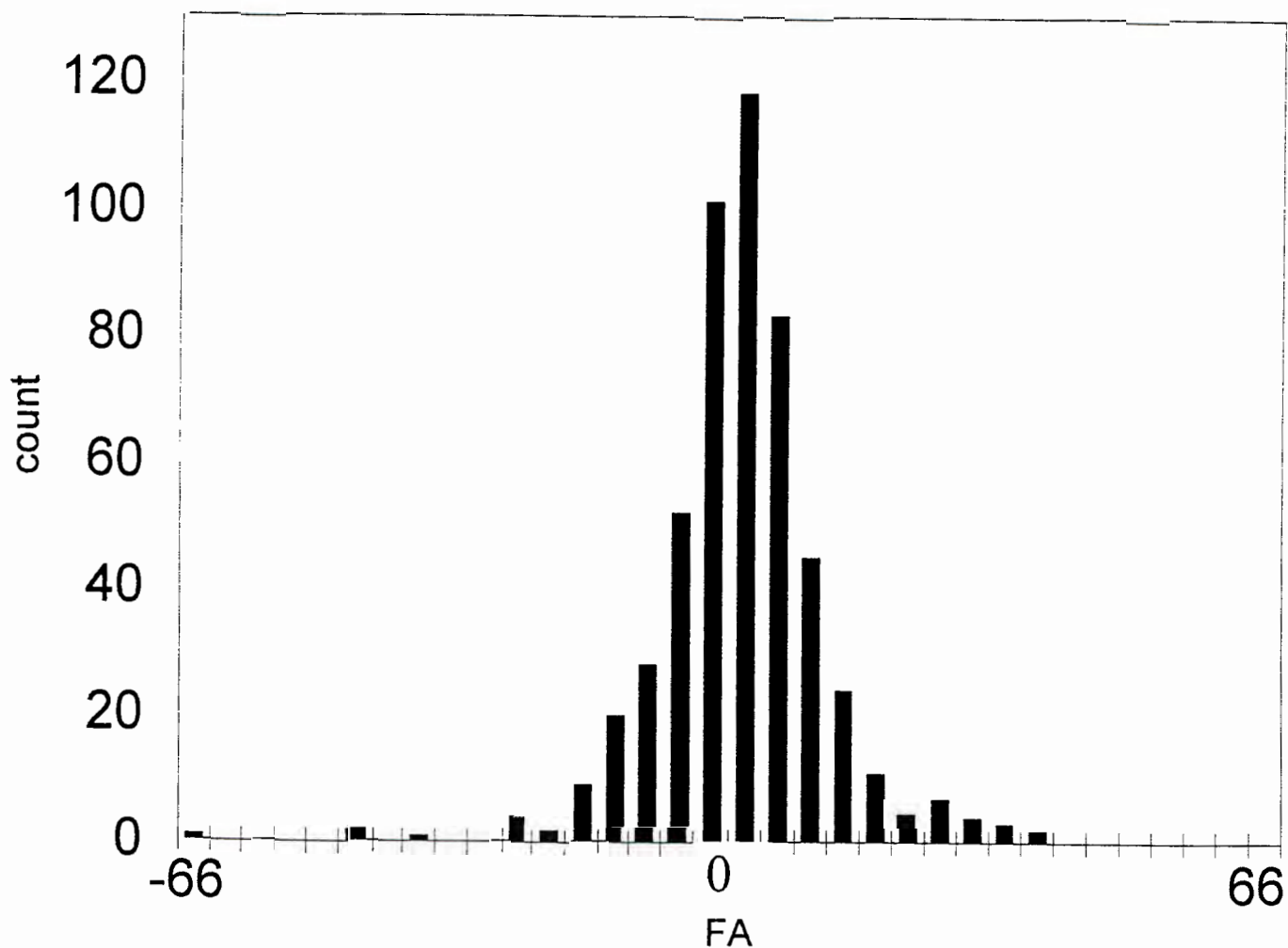


Figure 7. Frequency distribution of the 1875 relative asymmetry values obtained through measurement of the phyllodes sampled. The distribution is normal with a mean close to zero and therefore fits the definition of fluctuating asymmetry (Palmer and Stroebeck, 1986).

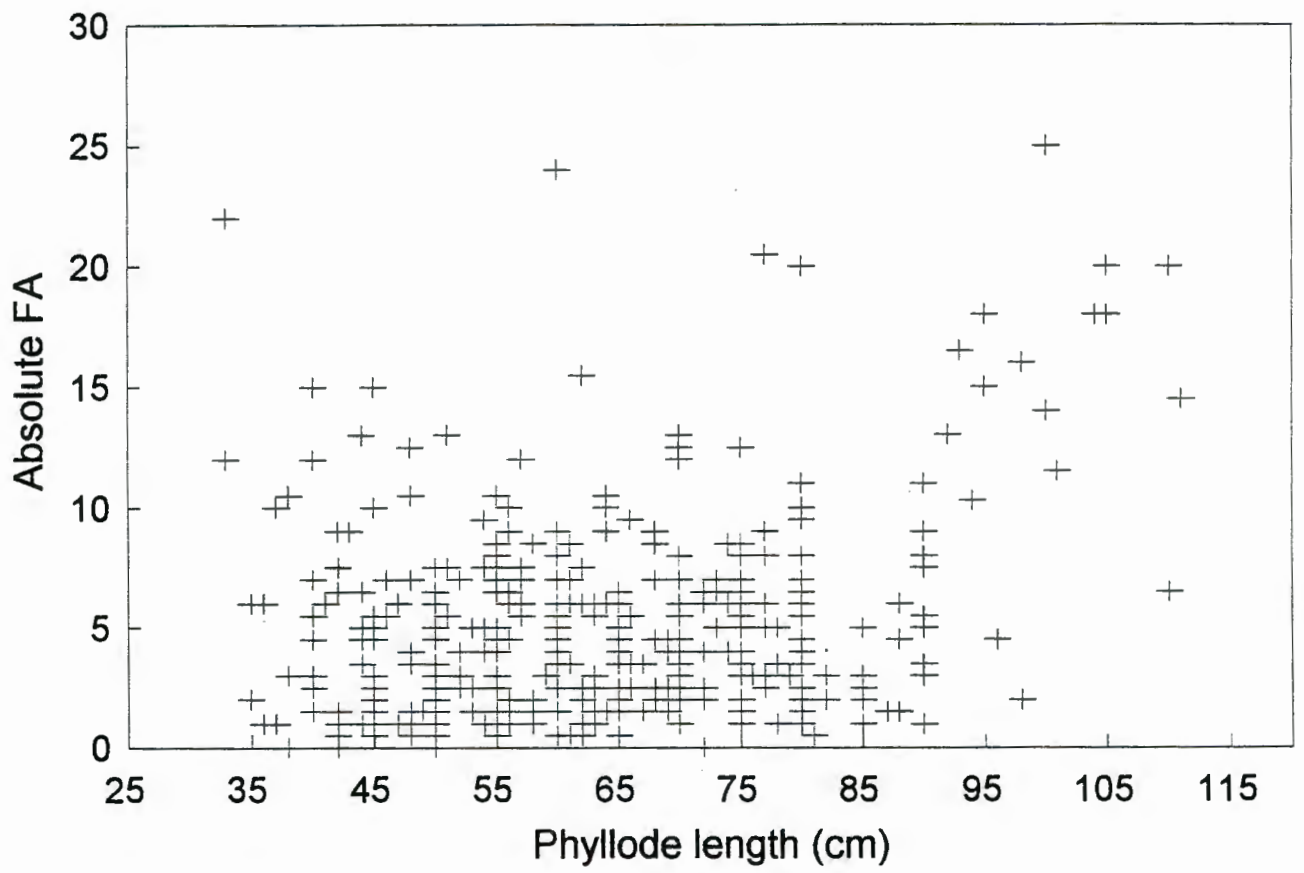


Figure 8. 500 absolute asymmetry values as a function of phyllode length. The correlation is poor ($r^2 = 28.1\%$) indicating that phyllode asymmetry is not dependent on leaf age.

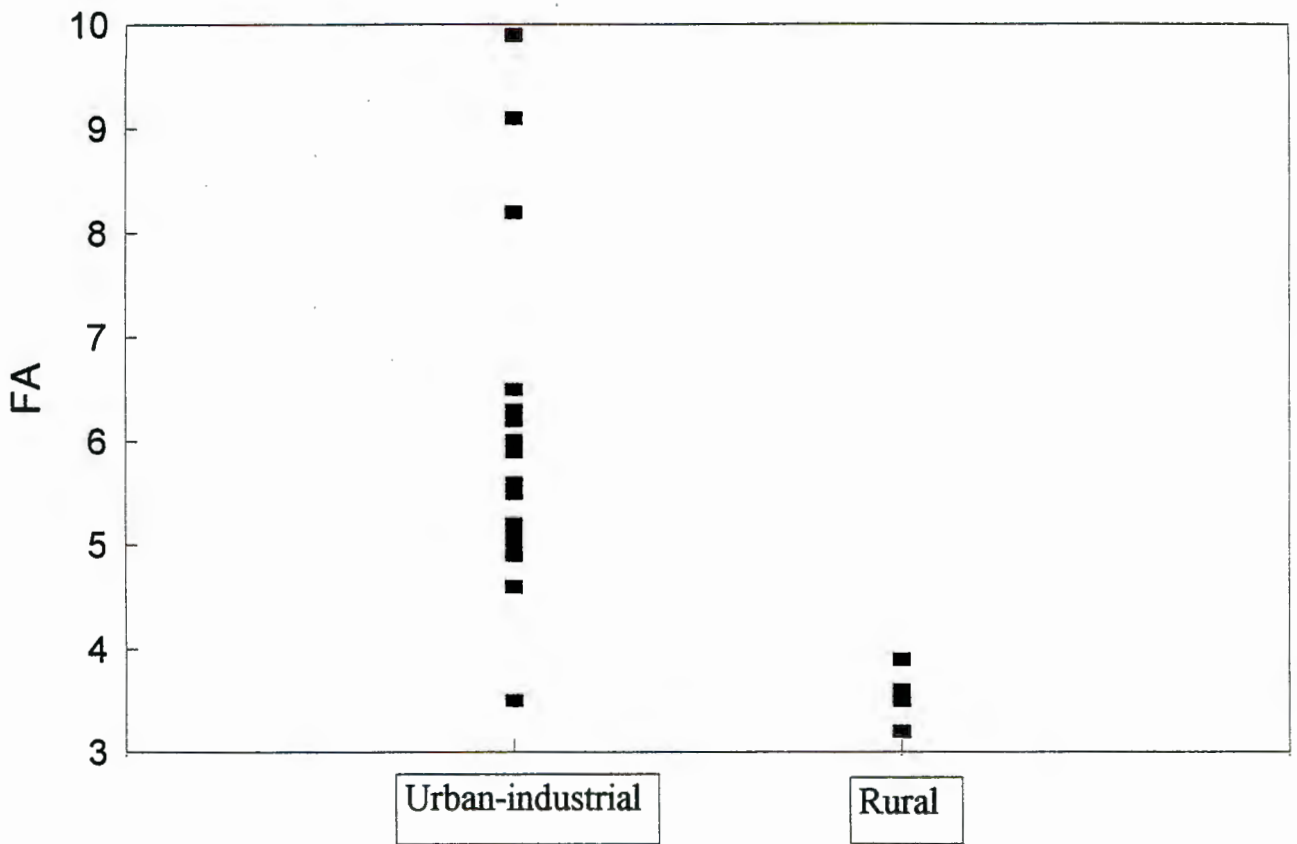


Figure 9. Average relative asymmetry (fluctuating asymmetry, FA) values for each sample site plotted against the area class of the site, “urban-industrial” sites occur in the wind-belt of the urban and industrial areas of Cape Town, “rural” sites are situated outside major urban or industrial influences.

variation = 28.3%). This high level of variation is possibly a result of the factors of distance from the refinery and from the urban-industrial center as well as differing fumigation frequencies incorporated into the fluctuating asymmetry values shown for the urban-industrial area class.

Similar results were obtained for the total sulfur and nitrogen contents of the lichens from the two area classes (figures 10 and 11 respectively). The background level of sulfur (rural area) was undetectable yet the urban-industrial levels were significantly higher and averaged at 0.42% (Two way analysis of variance; d.f. = 1,9; F-ratio = 21.4; $p < 0.01$). Again, the variation was high in the urban-industrial class (Coeff. of variation = 20.6%). The total nitrogen values showed less of a difference between the rural and the urban-industrial site (1.09% versus an average of 2.59%) yet the difference was significant (Two way analysis of variance; d.f. = 1, 9; F-ratio = 5.88; $p < 0.05$). The raised value of the total nitrogen from the rural site may be due to the presence of cars in the rural area releasing nitrous oxide in their exhaust fumes.

The component variables of the imposed pollution stress

Fumigation frequency: Figure 12 shows the average relative fluctuating asymmetry values (FA values) for each urban-industrial site plotted against fumigation frequency. The correlation is poor (Linear Regression analysis; $r = 0.57$, $r^2 = 32.4\%$, $p > 0.01$) indicating that the asymmetry values of each sample site are not linked to the fumigation frequency at the site.

Distance from the refinery: The average relative fluctuating asymmetry values for each urban-industrial site are plotted against the distance of the site from the oil-refinery (figure 13). The value for zero kilometers distance from the refinery is omitted from the linear regression analysis as this point is situated directly under the refinery stacks and does not therefore receive exposure to the pollution plume (figure 4, *A. cyclops* sampling site 2). With this point omitted, the curve

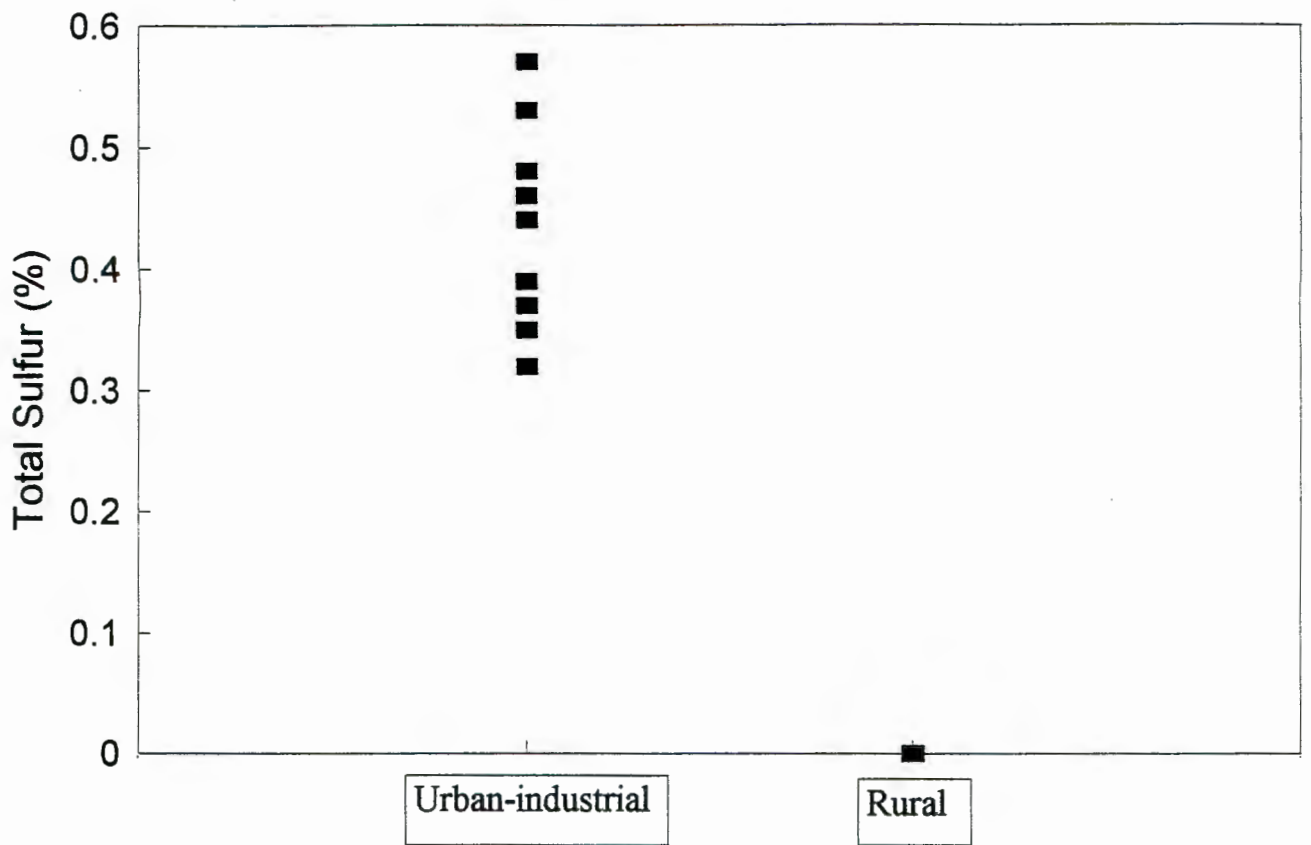


Figure 10. Total sulfur of the lichens sampled plotted against the area class of the lichen sampling site.

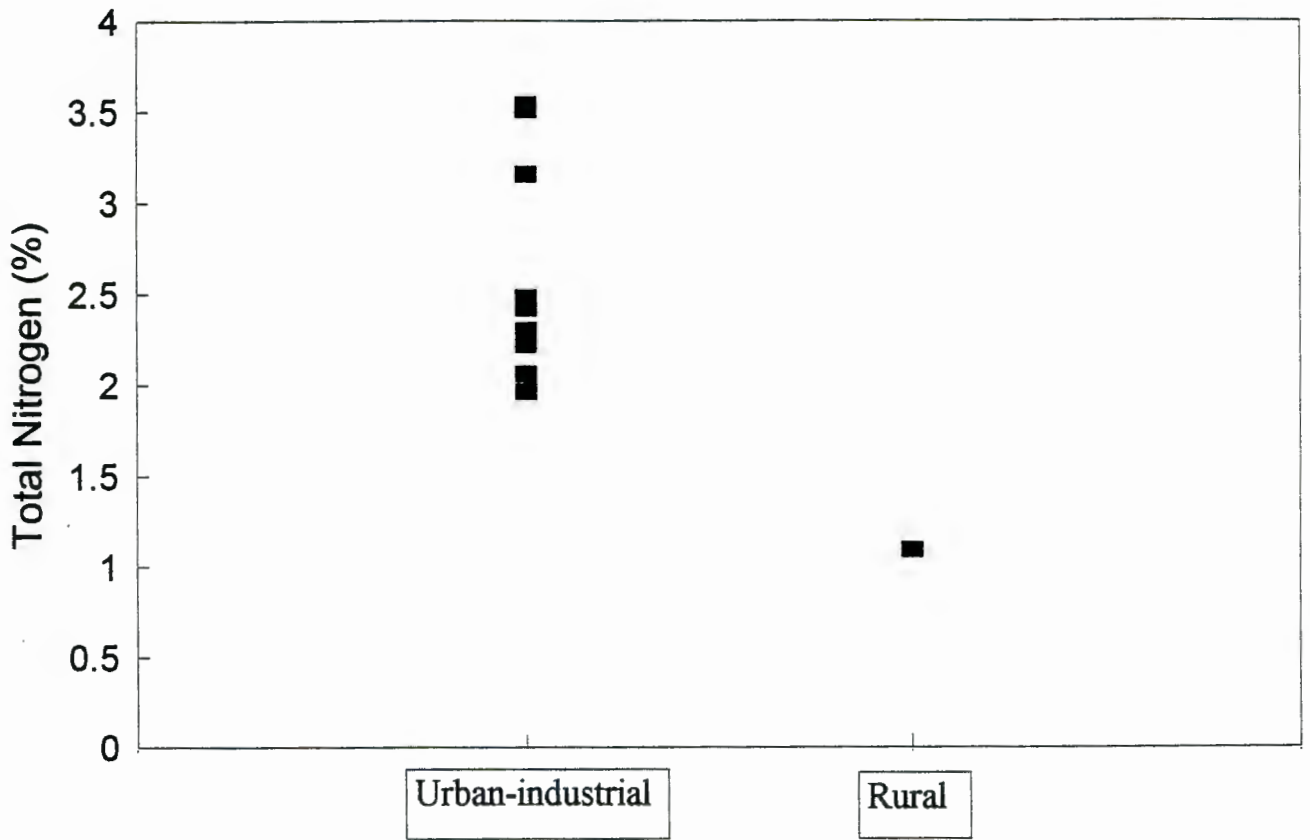


Figure 11. Total nitrogen of the lichens sampled plotted against the area class of the lichen sampling site.

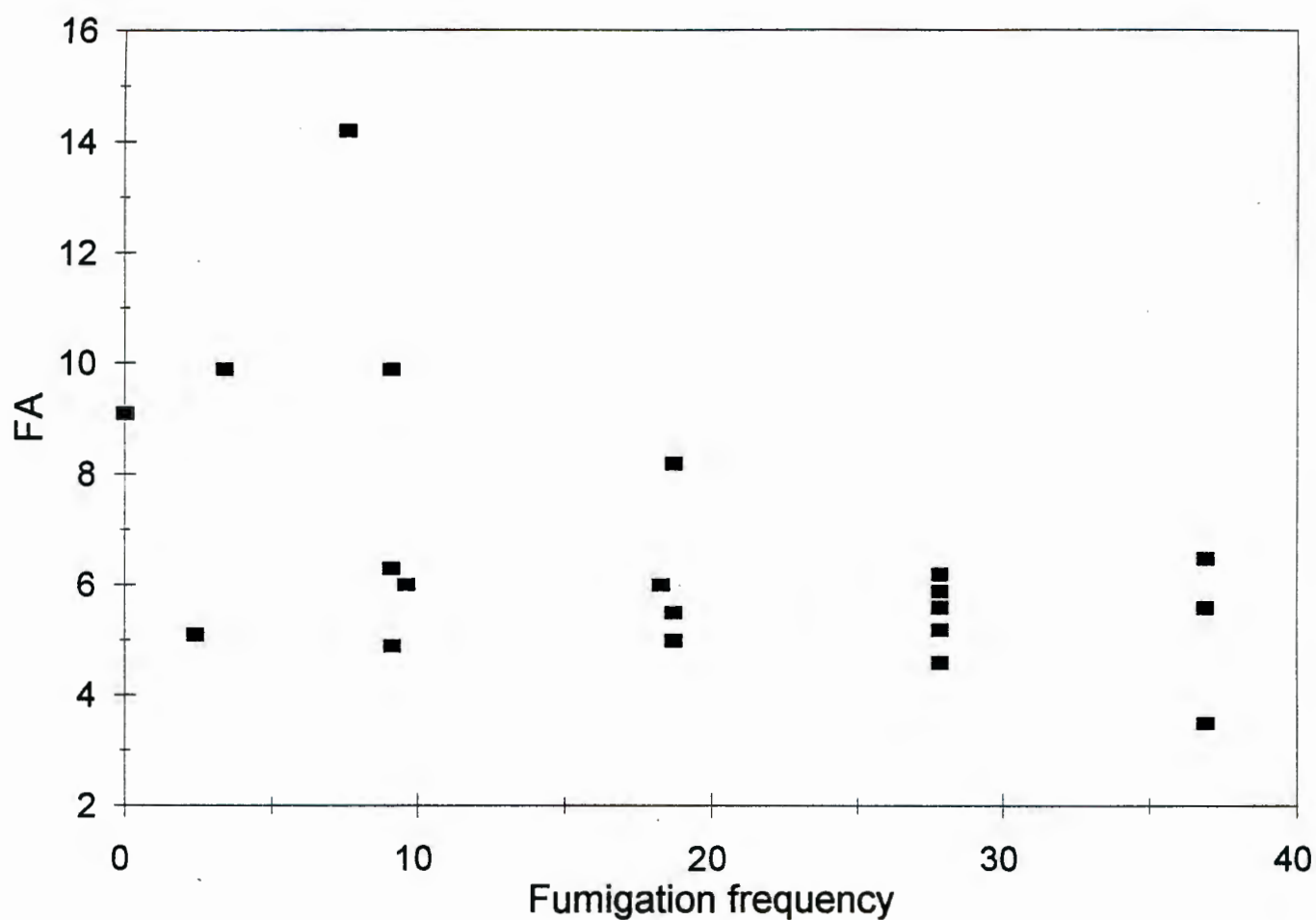


Figure 12. Average relative asymmetry (fluctuating asymmetry, FA) values plotted against the frequency with which the refinery plume fumigates the site (per thousand) ($r^2 = 32.4\%$).

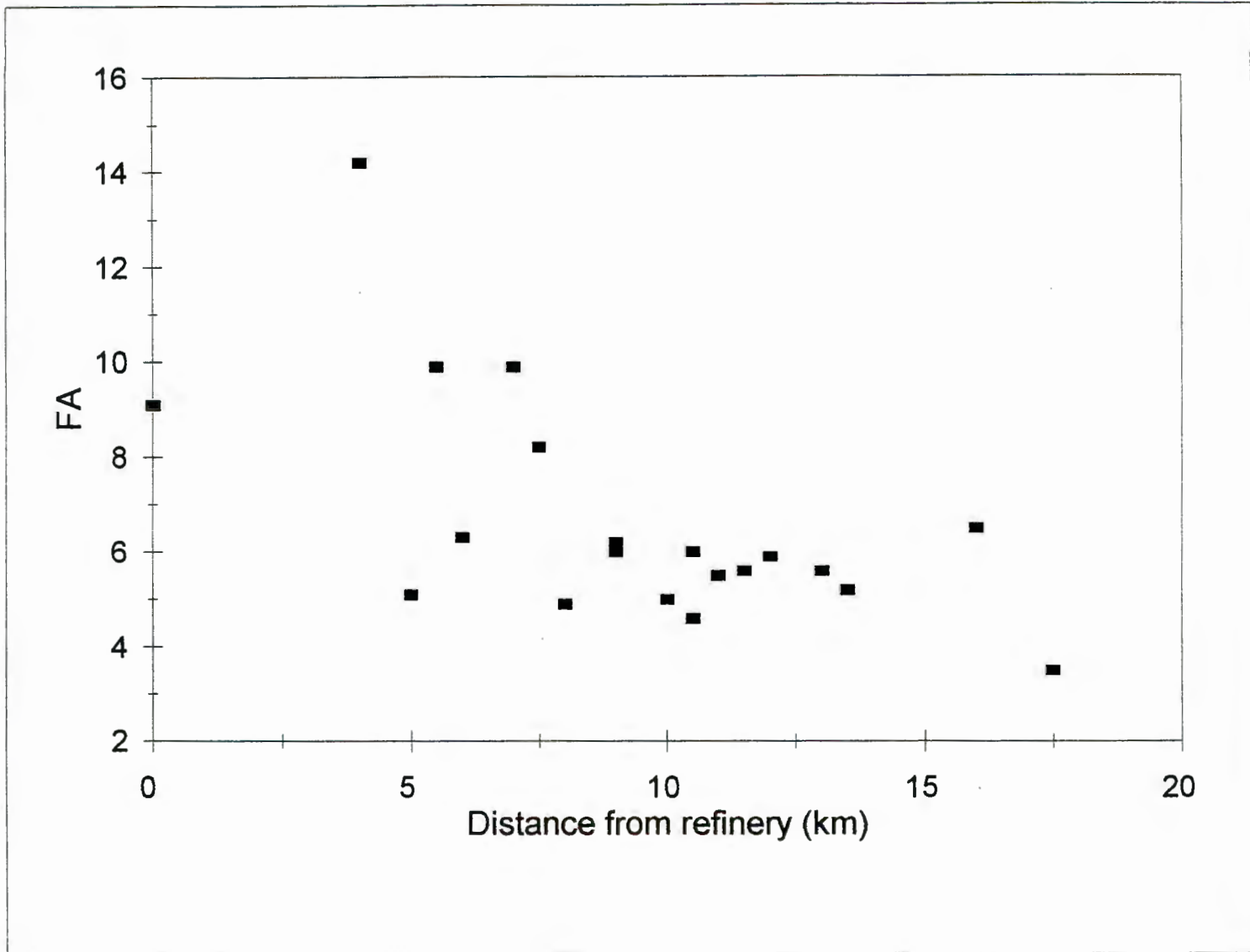


Figure 13. Average relative asymmetry (fluctuating asymmetry, FA) values plotted against the distance of each sampling site from the refinery ($r^2 = 59.3\%$, axes log transformed, excluding point representing zero distance from the refinery as this point receives no pollution load as it is situated under the stacks).

shows and strong exponential decrease in asymmetry values with distance from the refinery (Linear regression analysis on log transformed data; $r = -0.77$, $r^2 = 59.3\%$; $p < 0.01$).

Distance from the industrial center: The average relative fluctuating asymmetry values for each site are plotted against the distance of each site from the industrial center (figure 14). There is no statistically significant correlation shown, yet a negative exponential decrease in asymmetry with distance from the urban and industrial areas seems apparent from the plot (Linear regression analysis on log transformed data, $r = -0.59$; $r^2 = 34.8\%$, $p > 0.01$).

Standardised plots: The average fluctuating asymmetry values, standardised for distance from the urban-industrial center of Cape Town is plotted against distance from the refinery (figure 15). The correlation obtained (again omitting the value for the *A. cyclops* sampling site 2) is high (Linear regression analysis on log transformed data, $r = -0.80$; $r^2 = 62.5\%$; $p < 0.01$). There is an exponential decrease in asymmetry with increasing distance from the refinery. This may be due to the high mass of some of the air borne pollutants (e.g. sulfur atomic mass = 32.07, Gillespie *et al.*, (1989)) which possibly drop from the pollution plume within a short distance from the refinery.

The fluctuating asymmetry values for each site, standardised for their distance from the refinery, are plotted against their distance from the urban-industrial center of Cape Town (figure 16). A stronger correlation is obtained (Linear regression on log transformed data; $r = 0.63$; $r^2 = 39.6\%$, $p < 0.05$). There is an exponential decrease in phyllode asymmetry from the urban-industrial center of Cape Town, again possibly due to heavy pollution particles falling from the pollution plume close to the pollution source.

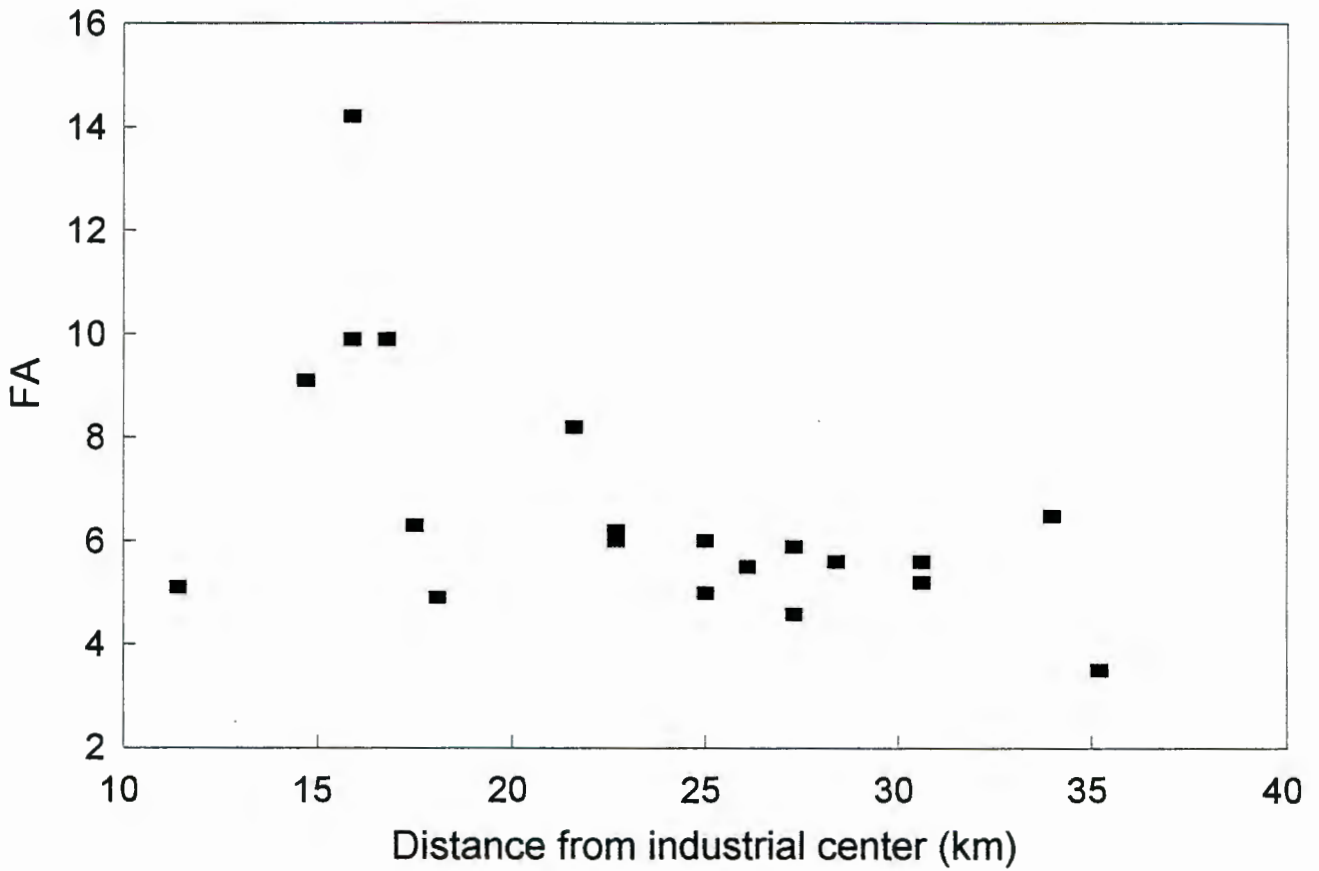


Figure 14. Average relative asymmetry (fluctuating asymmetry, FA) values plotted against the distance of each site from the industrial center of Cape Town. These distances were calculated as the shortest distance of the site to a line drawn through the center of the urban and industrial areas of Cape Town (see figure 2) ($r^2 = 34.8\%$, axes log transformed).

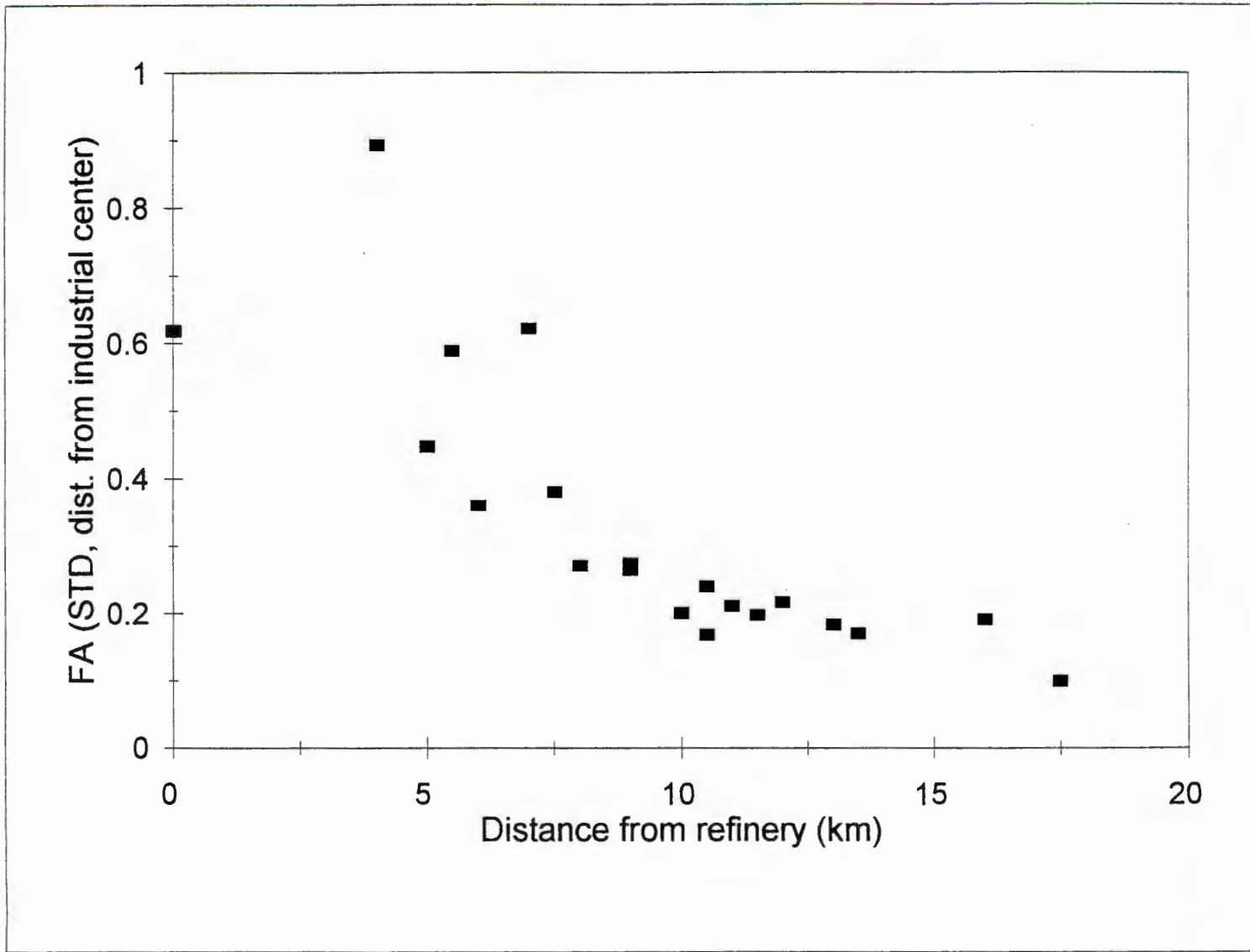


Figure 15. Average relative asymmetry (fluctuating asymmetry, FA) values standardised for distance from the urban-industrial center of Cape Town and plotted against distance from the refinery ($r^2 = 62.5\%$, log transformed axes, excluding point representing zero distance from the refinery).

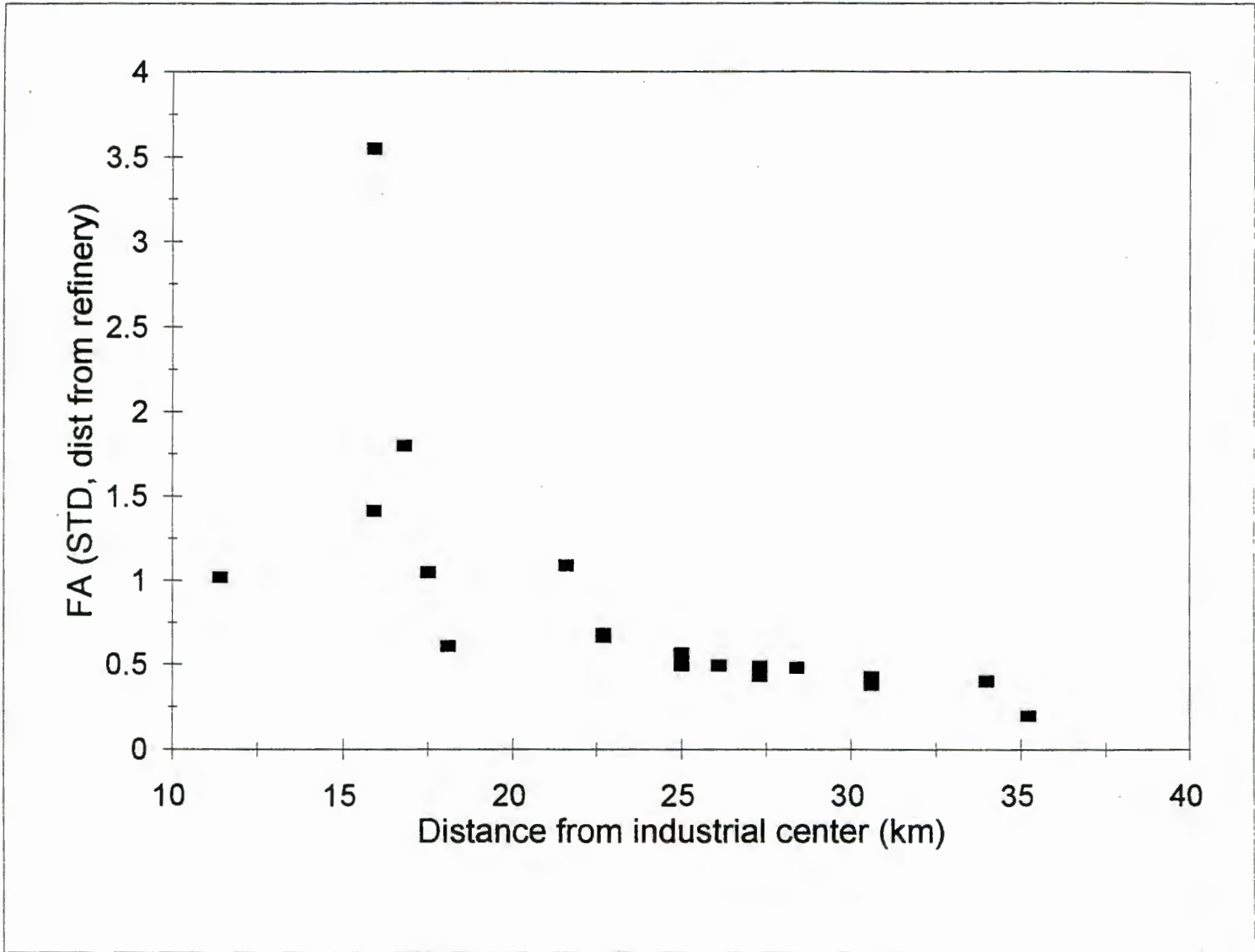


Figure 16. Average relative asymmetry (fluctuating asymmetry, FA) values standardised for distance from the refinery and plotted against distance from the urban-industrial center of Cape Town ($r^2 = 39.6\%$, axes log transformed).

Standardising the plot of average fluctuating asymmetry against fumigation frequency for either distance effects does not however produce a stronger correlation than that obtained in figure 12. Fluctuating asymmetry of the vegetation surrounding the refinery remains uncorrelated with the refinery plume fumigation frequency of the vegetation.

Total sulfur and nitrogen

Total sulfur and nitrogen are plotted against fumigation frequency of the sites, distance of the sites from the refinery and their distance from the urban-industrial center (figures 17, 18 and 19 respectively). No correlations were shown for any of these plots (see table 1). This may indicate that the refinery (together with the fertilizer plant) and the urban-industrial area of Cape Town are not the only sources of sulfur and nitrogen. Much of the nitrogen accumulated by the lichens in the area may, for example, be derived from the nitrous oxide released from car exhaust fumes within the study site rather than from the point source of the refinery or the urban-industrial area.

Table 1. Results of the regression analyses carried out for the plots of total sulfur and nitrogen against fumigation frequency, distance from the refinery and distance from the urban-industrial center (figures 17-19).

Plot: Total sulfur or nitrogen against:	Regression analysis					
	Total sulfur			Total nitrogen		
	r	r^2	sign.?	r	r^2	sign.?
1) Fumigation frequency	0.43	18.5%	N	0.51	26.0%	N
2) Distance from the refinery	0.42	17.6%	N	0.46	21.1%	N
3) Distance from the urban-industrial center	0.42	17.6%	N	0.47	22.1%	N

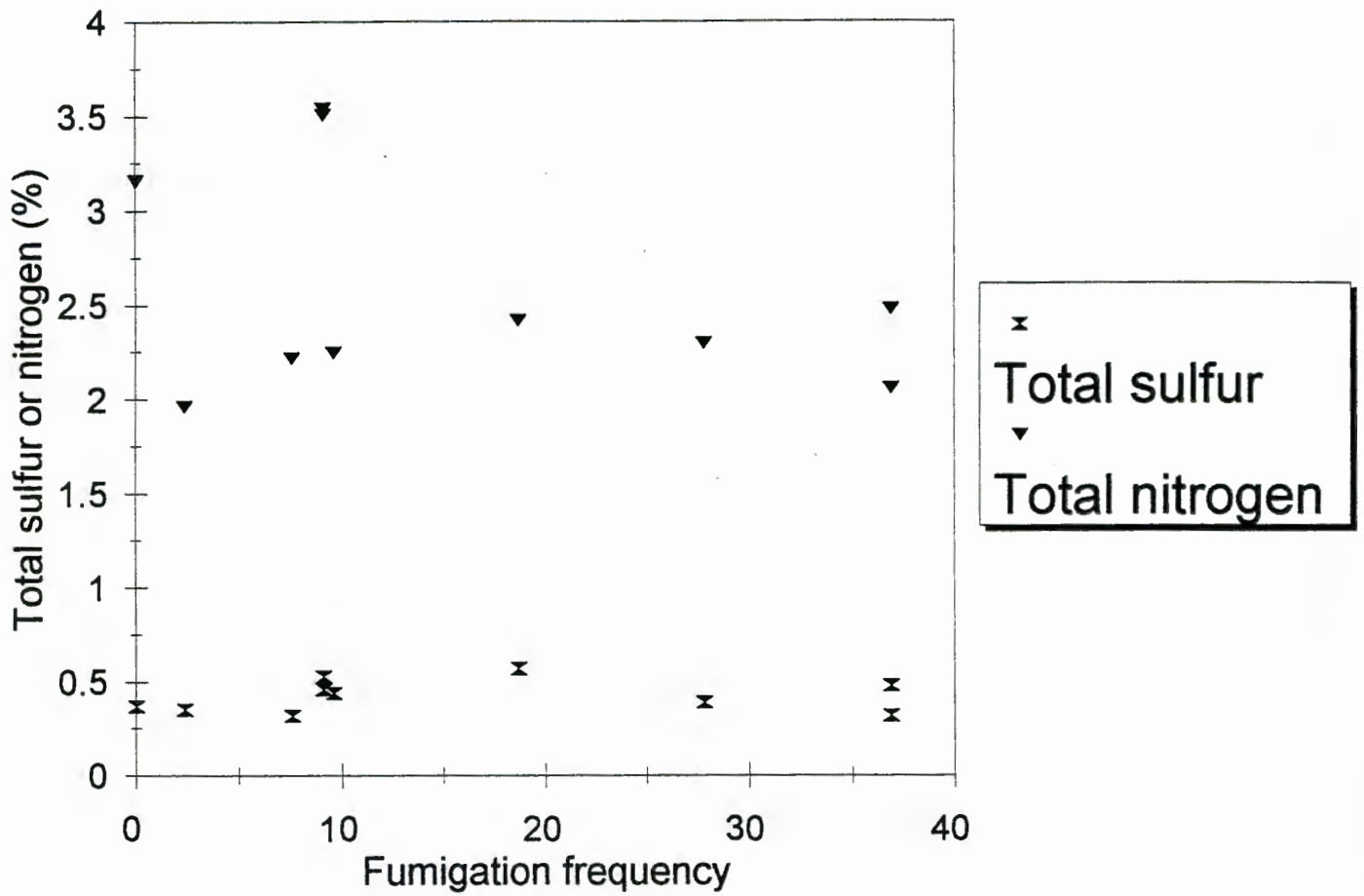


Figure 17. Total sulfur or nitrogen plotted as a function of the frequency of fumigation of each lichen sampling site with the refinery plume (sulfur $r^2 = 18.5\%$, nitrogen $r^2 = 26.0\%$).

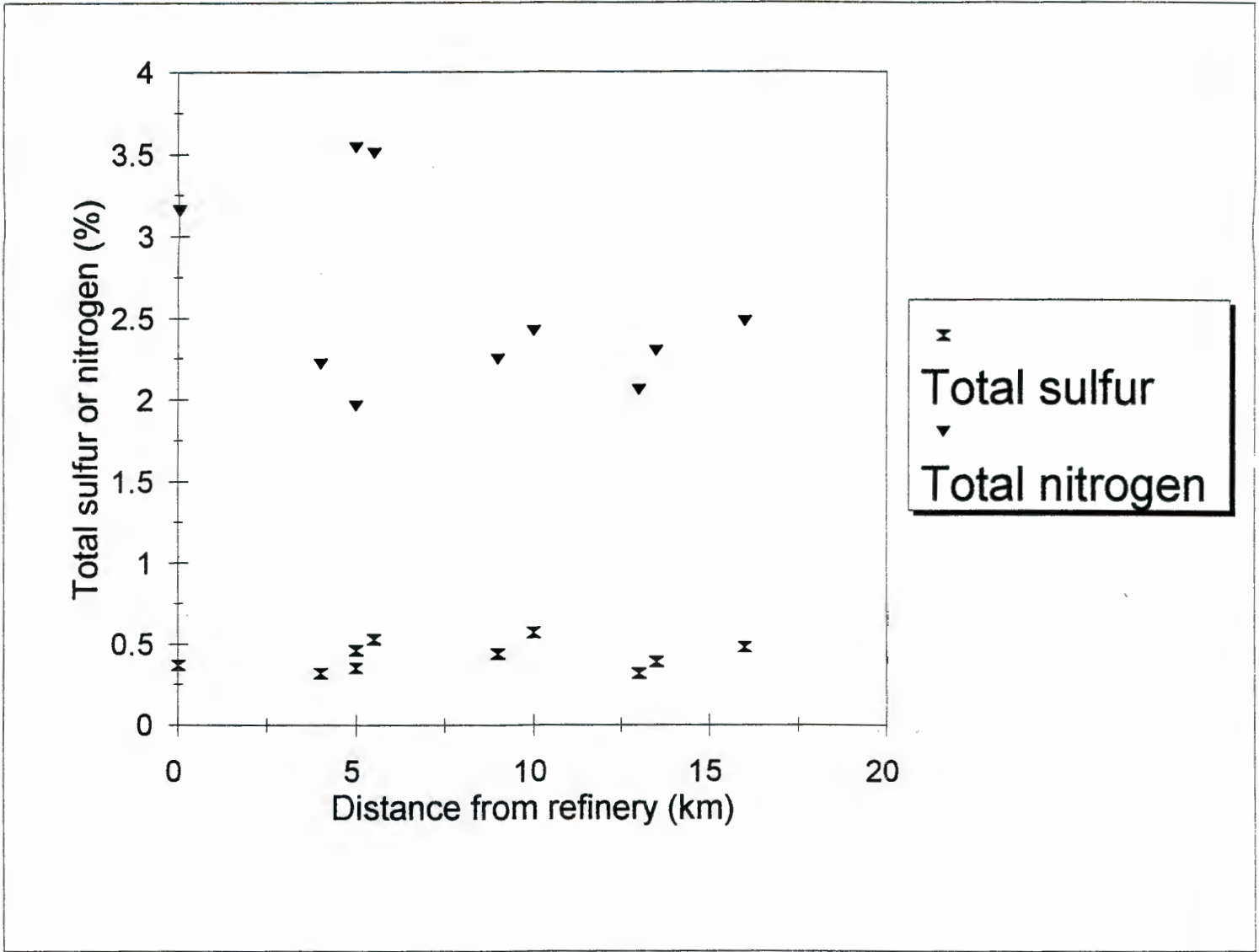


Figure 18. Total sulfur or nitrogen plotted as a function of the distance of the sampling sites from the refinery (sulfur $r^2 = 17.6\%$, nitrogen $r^2 = 21.1\%$).

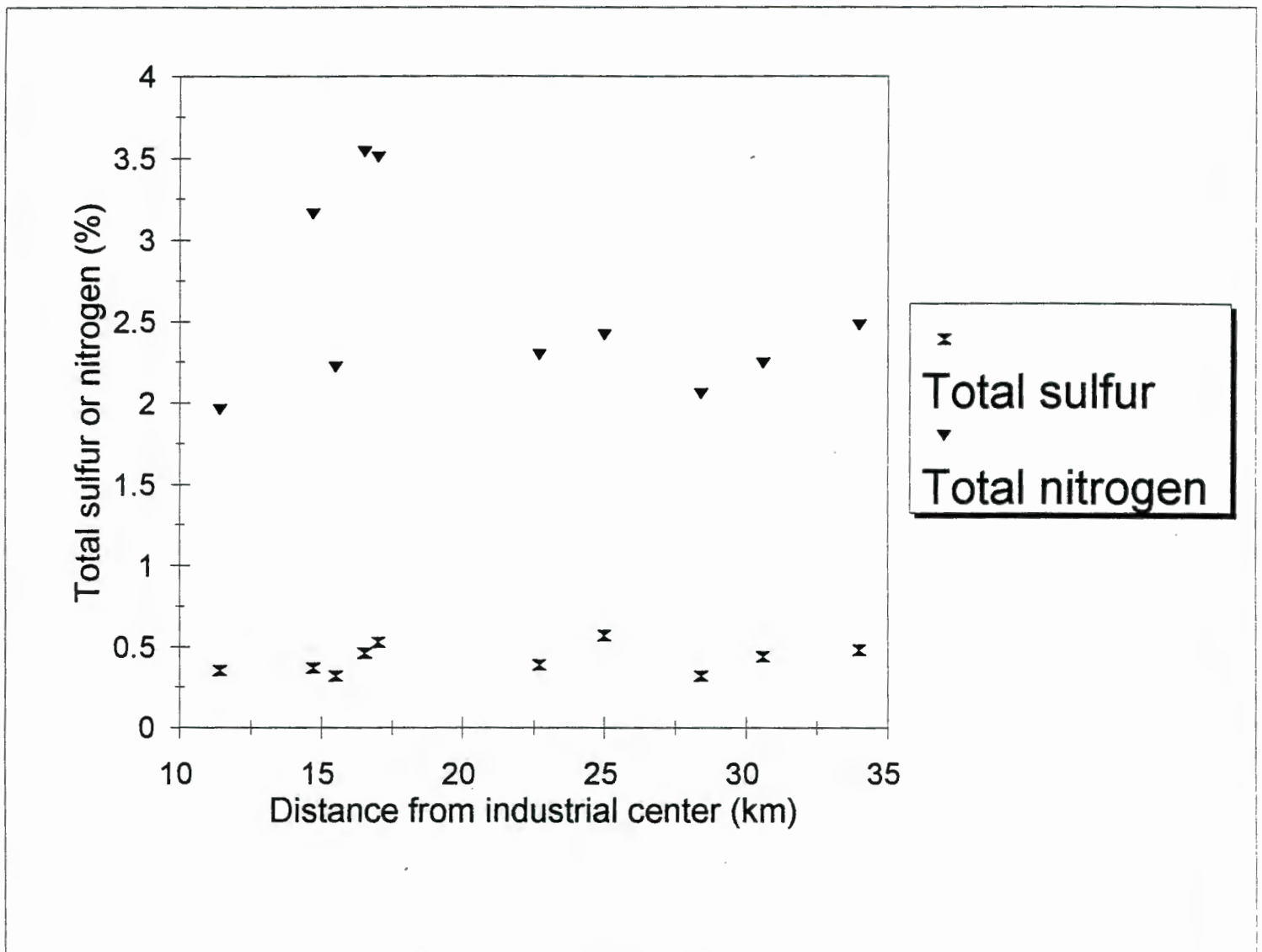


Figure 19. Total sulfur or nitrogen plotted as a function of the distance of the sampling sites from the urban and industrial areas of Cape Town (sulfur $r^2 = 17.6\%$, nitrogen $r^2 = 22.1\%$).

Discussion

To investigate the use of fluctuating asymmetry as a measure of developmental instability in plants (resulting from exogenous air pollution stress) the fluctuating asymmetry of the vegetation surrounding the pollution source should be correlated with the pollution stress imposed on the vegetation. A strong positive correlation will indicate the plausibility of using fluctuating asymmetry as a reliable measure of the effects of pollution stress on surrounding vegetation. The independent axis representing pollution stress is however often composed of more than one variable and the selection of one variable against which to correlate fluctuating asymmetry will lead to invalid conclusions concerning the reliability of fluctuating asymmetry as a measure of pollution stress. Freeman *et al.* (1993) for example correlated fluctuating asymmetry (in leaf width of *Epilobium angustifolium* L.) with distance from a chemical production facility in northern Russia. Distance was the only variable taken into account in this study and it was therefore assumed that the pollution stress imposed on the vegetation at each site was directly correlated with the distance of the site from the facility. Such assumptions may lead to spurious results and therefore, in this study three variables were taken into account when considering the exposure of the sample sites to pollution stress; distance from the refinery, distance from the urban and industrial centers of Cape Town and frequency of fumigation with the refinery plume. Correlations were found between the fluctuating asymmetry of the vegetation and both distance from the refinery and from the urban-industrial center of Cape Town. No correlation however was found between the fluctuating asymmetry of the vegetation surrounding the refinery and the frequency with which the vegetation was fumigated by the refinery plume.

The poor correlation between fluctuating asymmetry and fumigation frequency may indicate that fluctuating asymmetry is a poor measure of the developmental stability of the plants surrounding the refinery, and hence of the effects of the pollution stress imposed them. This conclusion however assumes fumigation

frequency to be an essential variable influencing the pollution stress imposed on the plants surrounding the refinery. The strong correlation of fluctuating asymmetry against distance from the refinery seems to negate this conclusion. It appears that distance from the refinery and from the urban-industrial areas are the overriding variables influencing the pollution stress imposed on the vegetation at each site. It is possible that the range of fumigation frequencies for the study site is not high enough to differentially affect the exposure of the sampling sites to the pollution stress imposed by the refinery. In addition, mixing of the wind plume due to high wind speeds in the area may further reduce the effect of frequency on pollution exposure. It is interesting to note however that frequency of fumigation influenced the variability of the fluctuating asymmetry values within the study site. For sampling sites exposed to low fumigation frequency (below 20 per thousand), the coefficient of variation was 17.8% ($n = 8$, mean = 5.38) whereas for sites exposed to a fumigation frequency of above 20 per thousand, the coefficient of variation was much higher at 28.7% ($n = 11$, mean = 6.9) (see figure 12). This may be a result of the differential response of vegetation to sporadic (low frequency) versus continual (high frequency) stress. The developmental stability of plants possibly responds to the continual influence of pollution stress with less variation than to sporadic fumigation events.

Assuming therefore that distance from the pollution source is the major variable affecting the pollution stress imposed at each sampling site, fluctuating asymmetry proves to be a reliable and accurate method of measuring the effects of pollution stress on the vegetation. Proximity of the vegetation to the pollution source influences the developmental stability of the vegetation and therefore the symmetry of its phenotype. The exact nature of the pollutant causing the observed developmental instability requires further investigation. Sulfur and nitrogen appear to be general pollutants in the urban-industrial area but do not reveal a gradient correlating with that of the vegetation asymmetry. Botha *et al.*

(1989) found fluoride damage of the leaves of *Eucalytus gomphocephala* DC. surrounding the refinery. The damage symptoms included leaf tip and margin necrosis. Such damage symptoms were apparent on many of the *A. cyclops* phyllodes surrounding the refinery (see figure 20) and it is therefore possible that fluoride may be the pollutant causing the observed increases in developmental instability and therefore fluctuating asymmetry towards the refinery. Other pollutants, such as lead and hydrogen sulfide, may also be involved, particularly towards the urban-industrial areas of Cape Town.

In conclusion, fluctuating asymmetry is a promising solution to the problem of monitoring the effects of point source pollutants on the surrounding vegetation. Surprisingly, little work has been carried out to determine the plausibility of this measure of pollution stress in plants (Freeman *et al.*, 1993). This study should pave the way for further investigation into the use of fluctuating asymmetry as a measure of the effects of exogenous stress on vegetation and should stimulate further interest in the use of fluctuating asymmetry in botanical research.

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(a)



(b)



Figure 20. (a) *A. cyclops* phyllodes collected from Langebaan area (rural), note their symmetry and entire phyllode form (b) *A. cyclops* phyllodes collected 2.5km from the refinery, note their asymmetry and damage to the margins and leaf tips, possibly a result of fluoride damage.

References

- Andersson, M. 1986. Evolution of condition-dependent sex ornaments and mating preferences: sexual selection based on viability differences. *Evolution* 40:804-820.
- Benedict, H.M., and Olson, R.E. 1971. *Economic Impacts of Air Pollutants on Plants*, volume 1, Coordinating Research Council New York, New York.
- Botha, A.T., Visser, J.H. and Moore, L.D. 1989. Evaluation of possible fluoride injury to vegetation in the vicinity of an industrial site near Cape Town. *South African Journal of Science* 85: 741-745.
- Bush, R.M., Smouse, P.E. and Ledig, F.T. 1987. The fitness consequences of multiple locus heterozygosity: the relationship between heterozygosity and growth rate in pitch pine (*Pinus rigida*). *Evolution* 41:787-798.
- Clarke, G.M. The genetic basis of developmental stability. I. Relationships between stability, heterozygosity and genomic coadaptation. *Genetica* 89: 15-24.
- Crittenden, P.D. and Read, D.J. 1978. The effects of air pollution on plant growth with special reference to sulfur dioxide. *New Phytologist* 80:49-62
- DeWit, T. 1976. *Epiphytic Lichens and Air Pollution in the Netherlands*. J. Crammer, Germany.
- Gillespie, R.J., Humphreys, D.A., Baird, N.C. and Robinson, E.A. *Chemistry* 2nd edition. Allyn and Bacon, Inc. Massachusetts.
- Graham, J.H., Freeman, D.C. and Emlen, J.M. 1993. Antisymmetry, directional symmetry and dynamic morphogenesis. *Genetica* 89: 121-138.
- Hawkesworth, D.L. and Rose, F. 1976. *Lichens as Pollution monitors*. Edward Annold, London.
- Freeman, D.C., Graham, J.H. and Emlen, J.M. 1993. Developmental stability in plants: symmetries, stress and epigenesis. *Genetica* 89: 97-119.
- Leary, R.F., Allendorf, F.W. and Knudsen, K.L. 1983. Developmental stability and enzyme heterozygosity in rainbow trout. *Nature* 301:71-72.

Lechowicz, M.J. 1987. Resource allocation by plants under air pollution stress: implications for plant-pest-pathogen interactions. *The Botanical Review* 53(3): 281-300.

McKenzie, J.A. and O'Farrell, K. 1993. Modification of developmental instability and fitness: malathion-resistance in the Australian sheep blowfly, *Lucilia cuprina*. *Genetica* 89: 67-76.

Mmboneni Muophe,. 1994. *Lichens as Air pollution Assays on the Western Cape Coast*. Honours thesis, Bolus Library, Botany Department, University of Cape Town.

Palmer, A.R. and Stroebeck, C. 1987. Fluctuating asymmetry measurement, analysis, patterns. *Annual Review of Ecology and Systematics* 17:391-421.

Palmer, A.R., Stroebeck, C. and Chippendale, A.K. 1993. Bilateral variation and the evolution of macroscopic asymmetries. *Genetica* 89: 201-218.

Parsons, P.A. 1990. Fluctuating asymmetry: an epigenetic measure of stress. *Biological Review* 65: 131-145.

Stirton, C.H. 1978. *Plant Invaders: Beautiful but Dangerous*. Department of Nature and Environmental Conservation of the Cape Provincial Administration, Cape town.

Strauss, S.H. 1987. Heterozygosity and developmental stability under inbreeding and cross-breeding in *Pinus attenuata*. *Evolution* 41: 331-339.

Sullivan, M.S., Robertson, P.A. and Aebischer, N.A. 1993. Fluctuating asymmetry measurement. *Nature* 361:409-410.