SUSCEPTIBILITY OF VARIOUS *GLADIOLUS* CULTIVARS TO FLUORIDE POLLUTION AND THEIR SUITABILITY FOR BIOINDICATION[1]

ANDREAS KLUMPP[2], IRENE F. MODESTO[3], MARISA DOMINGOS[4] and GABRIELE KLUMPP[5]

ABSTRACT - Ten Brazilian cultivars of *Gladiolus*, a widely used bioindicator plant of fluoride pollution, were exposed to ambient air at a severely fluoride-polluted site and a reference site near the industrial complex of Cubatão, SP, Brazil, in order to determine their sensitivity and to select cultivars with adequate bioindication characteristics. Assessment of pollution-induced effects included quantitative evaluation of leaf injuries, determination of leaf and flower dry weight as well as analysis of foliar fluoride concentrations. Plants exposed at the polluted site showed necroses at tips and margins of the leaves, typically caused by fluoride on this species. Intensity and distribution of lesions on the blades varied between the cultivars. Fluoride pollution resulted in accumulation of fluoride in leaves. Dry weight of leaves and flowers of plants exposed at the polluted site were significantly reduced when compared with the reference site. Considering sensitivity and evaluability of leaf injuries, the cultivars White Friendship and Eurovision are suggested for use as bioindicators of fluoride pollution in tropical/subtropical as well as in temperate regions.

Index terms: air pollution, bioindicator, leaf damage.

SENSIBILIDADE DE CULTIVARES DE *GLADIOLUS* À POLUIÇÃO POR FLUORETOS, E SUA APTIDÃO PARA A BIOINDICAÇÃO

RESUMO - Dez cultivares brasileiras de *Gladiolus*, planta amplamente utilizada como bioindicadora da poluição aérea por fluoretos, foram expostas ao ar ambiente em um local altamente poluído por esse elemento e em um local de referência, nas proximidades do complexo industrial de Cubatão, SP, visando verificar a sensibilidade de cada cultivar ao poluente e selecionar as que possuem características mais adequadas à bioindicação. A determinação dos efeitos induzidos pela poluição incluiu avaliação quantitativa dos danos foliares, do peso seco de folhas e flores e das concentrações foliares de fluoreto. Plantas expostas ao local poluído mostraram necroses nas pontas e margens das folhas, as quais são tipicamente causadas por fluoretos nessa espécie. A intensidade e distribuição das lesões na lâmina foliar variaram entre as cultivares. A poluição por fluoreto resultou na acumulação de flúor nas folhas. O peso seco das folhas e flores de plantas expostas no local poluído foi significativamente reduzido, em comparação ao de plantas expostas em local de referência. Considerando a sensibilidade e facilidade de avaliação de danos foliares, as cultivares White Friendship e Eurovision foram sugeridas para uso como bioindicadores da poluição por fluoretos em regiões tropicais/subtropicais e em regiões temperadas.

Termos para indexação: poluição aérea, bioindicador, danos foliares.

[1] Accepted for publication on September 25, 1996.

[2] Biologist, Dr., Institut für Landschafts- und Pflanzenökologie (320), Universität Hohenheim, D70593 Stuttgart, Germany.

[3] Biologist, Instituto de Botânica, Seção de Ecologia, Caixa Postal 4005, CEP 01061-970 São Paulo, SP, Brazil.

[4] Biologist, M.Sc., Instituto de Botânica, Seção de Ecologia.

[5] Biologist, Dr., FB 13, Universität Kassel, D34109 Kassel, Germany.

INTRODUCTION

Gaseous fluorides, mainly hydrogen fluoride, are extremely hazardous to plants, exceeding by far the phytotoxicity of most other air pollutants. Despite the knowledge of their potential risk, fluoride emissions from brick and glassworks, fertilizer industries, aluminum smelters and coal combustion processes still constitute a threat to vegetation on a local scale in many parts of the world (Weinstein & Hansen, 1988; Arndt & Schweizer, 1991).

Biomonitoring of fluoride effects on plants can be done by using accumulative indicator plants, i.e. plants that readily accumulate the toxic agent in their tissues often without showing visible injuries, or by using response indicators, i.e. sensitive plants that develop typical foliar lesions even at low fluoride concentrations in leaves. *Lolium multiflorum*, the so-called Standardised Grass Culture (Verein Deutscher Ingenieure, 1982), is probably the most frequently used accumulative indicator of fluoride. Many monocotyledonous species, such as tulips, day-lilies and gladioli, have been found to be extremely sensitive to fluorides and serve as response indicators of fluoride pollution (Steubing et al., 1976; Posthumus, 1983; Klumpp et al., 1995).

The high fluoride sensitivity of *Gladiolus* was studied in the United States as early as the 1940s and 1950s. Large differences in the sensitivity of various *Gladiolus* cultivars were observed. Some cultivars proved to be very sensitive, already showing typical tip and margin necroses ("tip burn") at low foliar fluoride concentrations, others turned out to be quite resistant, remaining free of injuries even with heavily increased leaf fluoride contents (Johnson et al., 1950; Hendrix & Hall, 1958; Hitchcock et al., 1962). Since then, *Gladiolus* has been used as a bioindicator of fluoride pollution in many studies throughout the world. Most investigations were carried out using the sensitive cultivar Snow Princess (Steubing et al., 1976; Naveh et al., 1979; Posthumus, 1983; Kostka-Rick, 1991).

As this cultivar is no longer available on the market today, the need of searching for other sensitive cultivars with adequate bioindication characteristics appeared. Few efforts have been made until now, including the exposure of some cultivars near brickworks in Germany (Kostka-Rick, 1991) and a biomonitoring study using the White Friendship cultivar in Brazil (Klumpp et al., 1994). The present work shows the results of a comparative study with ten *Gladiolus* cultivars in a severely fluoride-polluted region in the vicinity of the industrial complex of Cubatão, SP, Brazil. The paper includes results of the evaluation of visible symptoms, analysis of fluoride accumulation in the leaves and studies on plant growth. These data are used to determine the susceptibility to atmospheric fluoride and the suitability for bioindication of each cultivar thus contributing to the selection of a *Gladiolus* cultivar that may substitute Snow Princess.

MATERIAL AND METHODS

The study was conducted at two experimental sites at the coastal plain near the industrial complex of Cubatão in the State of São Paulo, Brazil (23°54' S, 46°25' W). The climate of the region is characterised by mean annual temperatures of 20 - 25°C, high relative humidity and annual precipitation of about 2500 mm. The site Mogi Valley where a strong degradation of the vegetation cover can be observed is known to have been a severely fluoride-affected area for decades. Main emission sources for gaseous and particulate fluorides are the superphosphate-producing industries located at the entrance of the valley, at a distance of about 1500 to 2500 m. In spite of the long history of air pollution, only few data on atmospheric fluoride concentrations are available. Measurements of the State Environmental Agency (CETESB) made in 1985 gave a mean value of about 4.5 $\mu g\ m^{-3}$ (Alonso & Godinho, 1992). In the meantime, due to mitigation measures, atmospheric fluoride concentrations dropped to average values of 0.5 - 0.9 $\mu g\ m^{-3}$ with a maximum of 5.5 $\mu g\ m^{-3}$ as could be shown by episodic measurements performed in 1991 and 1992 by CETESB (Secretaria de Estado do Meio Ambiente, 1993). Measurements of fluoride concentrations in rain and soil water (Klockow & Targa, 1993), repeated analyses of vegetation samples (Klumpp et al., 1996) and studies with other bioindicator plants (Klumpp et al., 1994, 1995) made in the course of an interdisciplinary research project in the Cubatão region clearly demonstrated the strong impact of atmospheric fluorides on the Atlantic Forest ecosystem at Mogi Valley. Due to the emissions from fertilizer plants and a steelmill, not only gaseous and particulate fluorides but also ammonia, sulphurous compounds and dust are occurring there at elevated atmospheric concentrations.

The site Pilões Valley exhibiting an apparently undamaged forest vegetation was considered a reference area as this site is not affected by fluoride pollution. During the 1991/1992 measuring campaigns, values at Pilões Valley remained below 0.1 $\mu g\ m^{-3}$ (Secretaria de Estado do Meio Ambiente, 1993). Both sites, with a distance of approximately 15 km, are located at altitudes of 20 - 40 m above sea-level and show similar climatic conditions. A more detailed description of the investigation area and of the state of natural vegetation is given by Secretaria de Estado do Meio Ambiente (1990) and Leitão Filho (1993).

Corms size no. 10/12 of ten *Gladiolus* cultivars were obtained from a local producer at Holambra, Brazil. The following cultivars were used: Fidelio, Gold Field, Traderhorn, Red Beauty, White Friendship, Peter Pears, Jacksonville Gold, Eurovision, White Goodes and Yester. The corms were planted in 3-litre plastic pots containing standardised substrate (Eucatex Plantmax SFA: vermiculite 3:1 v/v) and grown in a greenhouse at the Instituto de Botânica (IBt). Water supply during cultivation and later field exposure was guaranteed by nylon wicks, fertilization was done with NPK solution (Klumpp et al., 1994).

For exposure in the field, aluminum racks with plastic boxes as water reservoirs were used. Plants were exposed at 1 m above ground for a period of four weeks, then they were substituted by another set. At each site, three pots of each cultivar with two plants per pot were exposed. Experimental periods were 28/3 - 25/4/1994 and 25/4 - 23/5/1994.

Based on the studies of Steubing et al. (1976) and Coulter et al. (1985), which demonstrated a close correlation between leaf area and leaf length of *Gladiolus*, leaf injuries were evaluated by measuring the lengths of whole leaves and of necroses. The length of tip necroses and the length of margin necroses, multiplied by a factor between 0.1 and 0.9 considering the width of necroses (Kostka-Rick & Arndt, 1989), were added and put in relation to the total leaf length. Average injury degrees per plant and per cultivar and site were calculated. The dry weight of leaves, flowers and corms was determined. For fluoride analysis, the leaf tips (15 cm) of the two plants of each pot were joined. Concentrations were measured by means of an ion-sensitive electrode (Orion) according to Association of Official Analytical Chemists (1975).

Data were compared through analysis of variance (procedures ANOVA and ONEWAY). Significant differences between cultivars were determined by the Tukey multiple comparison test (Zar, 1984). Linear regression analysis was made for the relation between leaf injury and top dry weight and between leaf injury and foliar fluoride concentration.

RESULTS

Symptomatology

Soon after the first days of exposure at the polluted site Mogi Valley, *Gladiolus* plants developed foliar lesions very characteristic of fluoride impact on monocotyledonous plants and have been extensively described in the literature. The injuries started as dull-green discolouration at the leaf tips and, to a smaller extent, at the margins of the leaves. These injuries extended from the tips downward, soon turning into tan or brown necroses ("tip burn"). Healthy tissue frequently remained separated from the necrotic areas by a reddish-brown line. Besides these typical symptoms, irregular necrotic flecks on the blade were observed at some of the cultivars. Most severe damage normally occurred at fully-expanded middle-aged leaves, whereas older ones were less affected, and young, still expanding leaves only occasionally showed some tip necroses.

The results of the quantitative evaluation of leaf injuries are presented in Table 1. As differences between the first and the second experiments were quite small, data of both experiments were joined to calculate a mean value. Analysis of variance revealed highly significant ($p<0.001$) effects of the site and cultivar factors as well as the interactions between both factors. Therefore, for all parameters, the statistical differences among cultivars were separately studied in both exposure sites, accepting the $p=0.05$ as the maximum limit of significance. Whereas at the reference area Pilões Valley tip necroses were observed only ocasionally with mean values of 0.6% to 1.9%, depending on the cultivar, at the fluoride-affected Mogi Valley necroses occupied between 12 and 20% of the whole leaf length. Individual plants reached injury degrees of up to 27%. Based on the average injury index of both experiments, the following sequence of sensitivity is suggested: White Friendship > Eurovision > Peter Pears > Jacksonville Gold > Gold Field > Yester > White Goodes > Traderhorn > Red Beauty > Fidelio. However, the differences between cultivars occupying neighbouring positions in this sequence were not statistically significant. Multiple range analysis by Tukey-test showed significant differences ($p<0.05$) between the most sensitive cultivars White Friendship and Eurovision and the group of five less sensitive cultivars. For discussion of this kind of classification, it should be considered that Red Beauty was affected by disease in one experiment, and Fidelio by insect attack in the other, which caused the loss of leaves and whole plants. Therefore, the sensitivity of these two cultivars might have been somewhat underestimated.

TABLE 1. Percentage of leaf injury (average for the first and second exposure experiments), leaf fluoride concentrations for the first exposure experiment and for the second exposure experiment, and top dry weight (sum of leaf and flower dry weight - average for the first and second exposure experiments) of ten *Gladiolus* cultivars exposed at the reference site Pilões Valley and the polluted site Mogi Valley[1].

Cultivar	Leaf injury (%)		Fluoride -1st expos. (μg.g-1 d.w.)		Fluoride -2nd expos. (μg.g-1 d.w.)		Top dry weight (g)	
	Pilões	Mogi	Pilões	Mogi	Pilões	Mogi	Pilões	Mogi
Fidelio	1.1ab	12.7a	10.1b	91.8cd	6.7a	189.7abc	6.7abc	4.7ab
Gold Field	0.8a	15.5ab	5.4a	58.3ab	6.5a	230.0cd	7.0abc	3.6a
Traderhorn	1.2ab	14.0a	8.6ab	50.8ab	6.0a	171.3abc	8.5cd	4.7ab
Red Beauty	1.9b	13.0a	8.6ab	27.8a	8.6a	202.5bcd	6.3ab	3.9a
W. Friendship	0.6a	20.3b	8.4ab	101.0d	6.6a	223.7cd	7.8bc	4.6ab
Peter Pears	1.3ab	18.3ab	8.0ab	65.6bc	6.1a	266.5d	7.4abc	3.8a
J. Gold	1.1ab	18.2ab	8.9ab	76.7bcd	8.0a	175.6abc	7.9bcd	3.9a
Eurovision	1.4ab	19.7b	8.4ab	96.8cd	4.7a	181.4abc	9.8d	5.6b
White Goodes	1.1ab	14.0a	5.5a	67.1bc	7.1a	147.5ab	6.1ab	4.1ab
Yester	0.7a	14.3a	7.1ab	76.4bcd	7.2a	121.2a	5.8a	4.9ab

[1] Values followed by the same letter in the same column don't differ among themselves (Tukey test, $p<0.05$).

Fluoride concentrations of leaf tips

As opposed to the results of the symptomatological evaluation, fluoride concentrations in leaf tips of *Gladiolus* cultivars exposed during the first and second experiments differed considerably. Therefore, the results are presented separately (Table 1). Foliar fluoride values of plants exposed at the reference area Pilões Valley did not exceed 12 μg g^{-1} dw (dry weight), i.e. they remained within the range of normal background values of plants not affected by fluoride pollution (Weinstein, 1977). In the first experiment, 'Fidelio' showed the highest mean value of 10.2 μg g^{-1} dw and 'Gold Field' and 'White Goodes' the lowest values with approximately 5.5 μg g^{-1} dw (significantly different from each other with $p<0.05$). In the second experiment, no differences in fluoride values of plants from Pilões Valley were observed. The results obtained attest the characterisation of this area as a reference site, at least with respect to atmospheric fluorides.

By contrast, *Gladiolus* plants exposed at Mogi Valley revealed a strong accumulation of fluorides in their leaf tips. During the first experiment, White Friendship, Eurovision and Fidelio were the cultivars that accumulated most fluoride, reaching mean values of up to 100 μg g^{-1} dw. In the second experiment, mean fluoride values varied between 121 and 266 μg g^{-1} dw, Peter Pears, Gold Field, and White Friendship being the cultivars that presented the highest leaf fluoride contents. Although the injury index of both experiments was in the same range and leaf fluoride concentrations were not, a significant linear relationship exists between injury degree and fluoride accumulation for each one of the two experiments (Table 2). In general, the most sensitive cultivars showed the highest accumulation rates.

Growth parameters

Air pollution at Mogi Valley caused a significant reduction in plant growth. The dry weight of leaves was diminished by up to 50% compared to plants from the reference area. The most affected cultivars were Jacksonville Gold, Gold Field, and Peter Pears, whereas leaf dry weights of Yester and Fidelio were only slightly reduced.

Pollution-induced growth reduction appeared to be even more pronounced when the dry weight of all above-ground plant parts, the top weight, was considered (Table 1). At the Mogi Valley, only the cultivars Eurovision and White Friendship, and during the first experiment Peter Pears, developed flowers, which did not open during the course of the exposure experiment; the other cultivars produced only few buds until harvest. Considering the top weight, the most sensitive cultivars showed the most accentuated decrease, reaching reductions of up to 51%. By contrast, the total dry weight of Yester was reduced by only 16%. Regression analysis was made for the relation between injury index and top dry weight (Table 3). Significant linear relationship between both parameters were found for most of the cultivars studied; only Red Beauty and Yester showed no linear relationship between leaf injury and dry weight. In general, higher R values were obtained for the most sensitive cultivars.

The reduction of top dry weight may be explained partly by the loss of necrotic leaf parts, mainly leaf tips. The observed inhibition of bud and flower formation as well as a significantly smaller average leaf length ($p<0.001$) and a reduced number of leaves ($p<0.001$) of plants from Mogi Valley compared to reference plants indicate that a pollution-induced growth retardation occurred. Contrarily to these findings, corm weight was not affected by pollution. In view of the relatively short exposure duration, which did not permit plants to conclude their life-cycle, reduction of corm weight had not been expected.

TABLE 2. Relationship between foliar fluoride concentrations and leaf injury of *Gladiolus* cultivars exposed at Mogi Valley. Results of linear regression analysis using % leaf injury as dependent variable.

Experiment	Regression equation	R-value	Significance level
1	y = 5.58 + 0.13x	0.67	p<0.001
2	y = 7.23 + 0.05x	0.66	p<0.001

TABLE 3. Relationship between leaf injury and top dry weight of *Gladiolus* cultivars exposed at Pilões Valley and at Mogi Valley. Results of linear regression analysis using top dry weight as dependent variable.

Cultivar	Regression equation	R-value	Significance level
Fidelio	y = 7.32 - 0.16x	0.65	p<0.01
Gold Field	y = 6.55 - 0.16x	0.60	p<0.01
Traderhorn	y = 8.59 - 0.26x	0.72	p<0.001
Red Beauty	y = 5.92 - 0.09x	0.34	not significant
White Friendship	y = 7.76 - 0.15x	0.80	p<0.001
Peter Pears	y = 7.20 - 0.16x	0.75	p<0.001
Jacksonville Gold	y = 8.09 - 0.22x	0.87	p<0.001
Eurovision	y = 10.06 - 0.22x	0.86	p<0.001
White Goodes	y = 6.06 - 0.12x	0.54	p<0.01
Yester	y = 5.71 - 0.05x	0.32	not significant

DISCUSSION

The differences in fluoride sensitivity of the ten cultivars investigated in the present study were quite small when compared with similar experiments. Leaf injury of 72 cultivars studied by Johnson et al. (1950) near an aluminum reduction factory varied between 5% and 60%. Hendrix & Hall (1958) tested 110 cultivars and found an injury degree of 4% to 83% in plants exposed at a polluted site. More recently, Kostka-Rick (1991) exposed seven cultivars in the vicinity of a brickwork in Germany and found the total injury length of the most sensitive cv. Oscar to be about eight times longer than that of Flowersong, the most resistant one. In the same study, Eurovision proved to be rather more sensitive than Jacksonville Gold, whereas in the exposure experiment in Brazil both cultivars hardly differed in their fluoride sensitivity. Due to changing commercial popularity of *Gladiolus* cultivars, the cultivars used were different from those evaluated by Johnson et al. (1950) and Hendrix & Hall (1958), so difficulting direct varietal comparisons with these former studies. Differences in plant response of diverse studies may be due to different pollution characteristics (type of pollutant, concentration, exposure duration) and to climatic factors that may influence symptom expression.

From a practical point of view, not only the intensity of plant response, in this case extension of leaf necroses, but also the type of injury and its distribution on the leaf blade are important for a decision on the usefulness of a given cultivar as a response indicator. Indeed easily evaluated responses are a prerequisite for routine use in monitoring networks. Striking differences in symptom expression were found in the present study. Some cultivars showed well delimited necroses on the tips and margins of leaves, thus facilitating the estimation of injury degree. Others developed typical tip necroses and in addition to that necrotic flecks scattered on the blade and alternating with areas of healthy tissue. The evaluation of this type of injury proved to be much more difficult, implying a higher probability for errors. Considering the type of injury and the easiness of estimating the injured leaf portion, the cultivars were separated into four classes:

I - Very easy estimation: well delimited tip and margin necroses - White Friendship, Eurovision.

II - Easy estimation: tip and margin necroses, few necrotic flecks - Gold Field, Jacksonville Gold, Yester.

III - Intermediate estimation: tip necroses, frequent necrotic flecks - Fidelio, Red Beauty, White Goodes.

IV - Difficult estimation: scattered and striped necroses on the whole leaf blade - Traderhorn, Peter Pears.

Except for Peter Pears, the most sensitive cultivars proved to be also the cultivars with the most distinct injury type, thus facilitating injury evaluation.

In a recently performed greenhouse experiment using the same cultivars, fumigation with 1.5 to 2 $\mu g\ m^{-3}$ HF produced largely the same injury symptoms as observed in the field and in general confirmed our results regarding to sensitivity and evaluability of leaf injuries. Like in the field experiments, White Friendship turned out to be the most sensitive cultivar and Traderhorn and Peter Pears developed lesions that could hardly be estimated quantitatively. In contrast to these results, Eurovision was less sensitive in the fumigation experiment (Franz-Gerstein et al., 1995).

The most striking result of leaf fluoride analysis was the marked difference between the first and the second experiment despite similar injury indices. Unfortunately, no data on the atmospheric concentrations of gaseous and particulate fluorides are available for the experimental period. Therefore, it cannot be excluded that different proportions of gaseous and particulate fractions of fluoride pollution may have occurred during the two experimental periods. Particulate fluorides have only little effect on plant metabolism and thus on injury development, but contribute to elevated leaf fluoride levels when leaves are not washed before analysis (Garrec & Passera, 1980) as in the present study. The main reasons for the observed differences, however, are probably the different weather conditions in the course of the two experiments. Rainfall during the first period was about ten times higher than during the second. Studies of several authors have shown that climatic parameters such as temperature, humidity and precipitation, may exert strong influence on plant sensitivity to pollution and on accumulation capacity (MacLean & Schneider, 1971; MacLean et al., 1973). High precipitation rates not only reduce atmospheric pollutant concentrations but also diminish leaf fluoride values by washing off deposited fluoride and leaching from leaves, particularly from necrotic tissues (Steubing et al., 1976; Garrec, 1982). The intense rainfall during the first exposure period probably reduced the amount of accumulated fluoride in the *Gladiolus* leaves.

Comparison with results of other exposure studies, mainly from temperate climates, is complicated due to the differences in several exposure parameters, such as duration, plant age, cultivation methods and climatic conditions. In general, fluoride concentrations in leaf tips of *Gladiolus* were rather lower than in the present study. Only indicator plants exposed at a very short distance to the emission source exhibited fluoride contents of more than 100 $\mu g\ g^{-1}$ dw (Steubing et al., 1976; Naveh et al., 1979; Posthumus, 1983). Therefore, our results prove again the severe fluoride impact at Mogi Valley near Cubatão.

In the comparative studies with several *Gladiolus* cultivars of Johnson et al. (1950) and Hitchcock et al. (1962), a negative linear relation between leaf injury and fluoride accumulation was observed, i.e. sensitive cultivars normally accumulated less fluoride than resistant ones. By contrast, the present study as well as the work performed by Steubing et al. (1976) and Posthumus (1983) came to a positive relationship of both parameters. This finding is in agreement with Weinstein (1977), who suggested a negative correlation between injury and fluoride accumulation for different species rather than for different cultivars of a given plant species.

With respect to fluoride effects on plant growth, contradictory findings are reported in the literature. Stimulation of leaf length growth of various plant species after exposure to low HF concentrations has been described by several authors (Matsushima & Brewer, 1972; MacLean et al., 1976; Bunce, 1985). In fumigation experiments with *Gladiolus* 'Snow Princess', Kostka-Rick (1988) found stimulation or inhibition of leaf length growth to depend on plant age, whereas plants exposed to ambient fluoride pollution did not show growth alterations. A clear inverse relationship between leaf injury and top dry weight of *Gladiolus* was observed by Brewer et al. (1966). The experiments conducted by Hitchcock et al. (1962) showed drastic growth reduction of *Gladiolus* 'Snow Princess' in one case, while in other experiments the same authors did not find fluoride-induced growth suppression. Compared to these observations, the growth reduction of up to 50% found in the present study is quite impressing. However, even considering fluoride the most important phytotoxic component among the air pollutants emitted by the industries of Cubatão, it should be stressed that the growth reduction of *Gladiolus* plants observed there, cannot be traced back exclusively to fluoride impact, but to the whole complex air pollution affecting the area. Other factors such as soil or climatic conditions that could influence plant growth even more than pollution, by contrast, can be regarded less important in the present study, as the same substrate was used for all plants and the two sites are located at the same altitude and with similar climatic conditions.

As in the experiments of Hitchcock et al. (1962) and Brewer et al. (1966), who described a reduced number and weight of flower spikes of *Gladiolus* cultivars due to fluoride treatment, a suppression of flower development was observed in the present study. This result has important implications as it suggests that air pollution at Mogi Valley not only damages the individual plant by causing leaf injuries and growth reduction but may also result in an uncompleted or at least retarded life-cycle of sensitive plant species.

CONCLUSIONS

1. The cultivars White Friendship, Eurovision and, with some restrictions, Jacksonville Gold and Gold Field are useful as response indicators of fluoride impact in Brazil and regions with similar climate.

2. The White Friendship is appointed as the most promising cultivar for substituting the Snow Princess.

ACKNOWLEDGEMENTS

This project was executed within the scope of the Agreement on Co-operation in Scientific Research and Technological Development signed by the German and Brazilian governments, and within the Project Agreement of GKSS/Germany and SMA/Brazil. The donation of *Gladiolus* corms by Agrícola Schoenmaker & DeWit Ltda., Holambra, Brazil, is gratefully acknowledged.

REFERENCES

ALONSO, C.D.; GODINHO, R. A evolução da qualidade do ar em Cubatão. **Química Nova**, v.15, p.126-136, 1992.

ARNDT, U.; SCHWEIZER, B. The use of bioindicators for environmental monitoring in tropical and subtropical countries. In: ELLENBERG, H. (Ed.). **Biological monitoring - signals from the environment**. Braunschweig, Germany: Vieweg, 1991. p.199-260.

ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS. Fluoride: Potentiometric method - -official first action. **Association of Official Analytical Chemists Journal**, v.58, p.384-385, 1975.

BREWER, R.F.; GUILLEMET, F.B.; SUTHERLAND, F.H. The effects of atmospheric fluoride on *Gladiolus* growth, flowering, and corm production. **Proceedings of the American Society for Horticultural Science**, v.88, p.631-634, 1966.

BUNCE, H.W.F. Apparent stimulation of tree growth by low ambient air levels of fluoride in the atmosphere. **Journal of the Air Pollution Control Association**, v.35, p.46-48, 1985.

COULTER, C.T.; PACK, M.R.; SULZBACH, C.W. An evaluation of the dose-response relationship of fluoride injury to *Gladiolus*. **Atmospheric Environment**, v.19, p.1001-1007, 1985.

FRANZ-GERSTEIN, C.; FOMIN, A.; KLUMPP, A.; KLUMPP, G.; DOMINGOS, M.; ARNDT, U. Untersuchungen zur Eignung verschiedener Gladiolensorten als Bioindikatoren für Fluorwasserstoff. **Verhandlungen der Gesellschaft für Ökologie**, v. 23, p.293-296, 1995.

GARREC, J.P. Studies of fluoride pollution impact on coniferous forests. In: UNSWORTH, M.H.; ORMROD, D.P. (Eds.). **Effects of gaseous air pollution in agriculture and horticulture.** London: Butterworths Scientific, 1982. p.476-477.

GARREC, J.P.; PASSERA, N. Uptake of particulate fluorides from an aluminum smelter by plants. Effects of humidity of the air. **Fluoride**, v.13, p.105--117, 1980.

HENDRIX, J.W.; HALL, H.R. The relationship of certain leaf characteristics and flower color to atmospheric fluoride-sensitivity in *Gladiolus*. **Proceedings of the American Society for Horticultural Science**, v.72, p.503-510, 1958.

HITCHCOCK, A.E.; ZIMMERMAN, P.W.; COE, R.R. Results of ten years' work (1951-1960) on the effect of fluorides on *Gladiolus*. **Contributions from Boyce Thompson Institute**, v.21, p.303-344, 1962.

JOHNSON, F.; ALLMENDINGER, F.; MILLER, V.L.; GOULD, C.J. Leaf scorch of *Gladiolus* caused by atmospheric fluoric effluents. **Phytopathology**, v.40, p.239-246, 1950.

KLOCKOW, D.; TARGA, H. Air pollution and vegetation damage in the tropics. The German/Brazilian interdisciplinary project "Serra do Mar" in the industrial area of Cubatão/São Paulo, Brazil. In: JUNK, W.J.; BIANCHI, H.K. (Eds.). **Studies on human impact on forests and floodplains in the tropics.** Geesthacht, Germany: GKSS, 1993. p.13-18.

KLUMPP, A.; KLUMPP, G.; DOMINGOS, M. Plants as bioindicators of air pollution at the Serra do Mar near the industrial complex of Cubatão, Brazil. **Environmental Pollution**, Barking, v.85, p.109--116, 1994.

KLUMPP, A.; KLUMPP, G.; DOMINGOS, M.; SILVA, M.D. Fluoride impact on native tree species of the Atlantic Forest near Cubatão, Brazil. **Water, Air, and Soil Pollution**, Dordrecht, v.87, p.57-71, 1996.

KLUMPP, G.; KLUMPP, A.; DOMINGOS, M.; GUDERIAN, R. *Hemerocallis* as bioindicator of fluoride pollution in tropical countries. **Environmental Monitoring and Assessment**, v.35, p.27-42, 1995.

KOSTKA-RICK, R. Application of measurements of immission rates and of their biological effects for monitoring the success of emission-reducing measures at a brickworks. **Ziegelindustrie International**, v.6, p.312-319, 1991.

KOSTKA-RICK, R. **Untersuchungen zur Bioindikation fluorhaltiger Luftverunreinigungen mit *Gladiolus x gandavensis hort.* cv. 'Snow Princess'.** Stuttgart, Germany: Universität Hohenheim, 1988. 225p. Doctor Thesis.

KOSTKA-RICK, R.; ARNDT, U. Schädigungs--Zuwachsrate bei Gladiole - eine Wirkungsmessgrösse für fluorhaltige Luftverunreinigungen. **Angewandte Botanik**, v.63, p.17-31, 1989.

LEITÃO FILHO, H.F. (Ed.). **Ecologia da Mata Atlântica em Cubatão.** Campinas: UNESP/UNICAMP, 1993. 184p.

MACLEAN, D.C.; SCHNEIDER, R.E. Fluoride phytotoxicity: its alteration by temperature. In: INTERNATIONAL CLEAN AIR CONGRESS, 2., 1971. Washington. **Proceedings...**Washington: [s.n.], 1971. p.292-295.

MACLEAN, D.C.; SCHNEIDER, R.E.; MCCUNE, D.C. Fluoride phytotoxicity as affected by relative humidity. In: INTERNATIONAL CLEAN AIR CONGRESS, 3., 1973. Düsseldorf. **Proceedings...** Düsseldorf: [s.n.], 1973. p.A143-A145.

MACLEAN, D.C.; SCHNEIDER, R.E.; MCCUNE, D.C. Fluoride susceptibility of tomato plants as affected by magnesium nutrition. **Journal of the American Society of Horticultural Science**, v.101, p.347-352, 1976.

MATSUSHIMA, J.; BREWER, R.F. Influence of sulfur dioxide and hydrogen fluoride as a mix or reciprocal exposure on citrus growth and development. **Journal of the Air Pollution Control Association**, v.22, p.710-713, 1972.

NAVEH, Z.; STEINBERGER, E.H.; CHAIM, S. The use of bioindicators for monitoring of air pollution by fluor, ozone, and sulphur dioxide. In: CAIRNS JUNIOR, J.; PATIL, G.P.; WATERS, W.E. (Eds.). **Environmental biomonitoring, assessment, prediction, and management** - certain case studies and related quantitative issues. Fairland, USA: International Cooperative Publishing House, 1979. p.21-47.

POSTHUMUS, A.C. Higher plants as indicators and accumulators of gaseous air pollution. **Environmental Monitoring and Assessment**, v.3, p.263-272, 1983.

SECRETARIA DE ESTADO DO MEIO AMBIENTE (SÃO PAULO, SP). **Poluição atmosférica e danos à vegetação nos trópicos** - a Serra do Mar como um exemplo. São Paulo: Cooperação Brasil-Alemanha em Pesquisa e Tecnologia Ambiental, 1993. 181p.

SECRETARIA DE ESTADO DO MEIO AMBIENTE (SÃO PAULO, SP). **The rain forest of the Serra do Mar**: degradation and reconstitution. São Paulo, 1990. (Documents Series).

STEUBING, L.; KIRSCHBAUM, U.; GWINNER, M. Nachweis von Fluorimmissionen durch Bioindikatoren. **Angewandte Botanik**, v.50, p.169--185, 1976.

VEREIN DEUTSCHER INGENIEURE. **Messen der Immissions-Wirkdosis.** Blatt 2: Messen der Immissions-Wirkdosis von gas- und staubförmigem Fluorid in Pflanzen mit dem Verfahren der standardisierten Graskultur. Düsseldorf, Germany: VDI, 1982. (Handbuch Reinhaltung der Luft, Richtlinie, 3792).

WEINSTEIN, L.H. Fluoride and plant life. **Journal of Occupational Medicine**, v.19, p.49-78, 1977.

WEINSTEIN, L.H.; HANSEN, K.S. Relative susceptibilities of Brazilian vegetation to airborne fluoride. **Pesquisa Agropecuária Brasileira**, Brasília, v.23, n.10, p.1125-1137, 1988.

ZAR, J.H. **Biostatistical analysis.** Englewood Cliffs, USA: Prentice-Hall, 1984. 718p.