Ozone is not mutagenic in the Tradescantia and tobacco mutagenicity assays


GSF-Institut für Biochemische Pflanzenpathologie, D-8042 Neuherberg (Germany)

(Received 28 August 1991)
(Revision received 29 October 1991)
(Accepted 31 October 1991)

Keywords: Ozone; Tradescantia; Tobacco; Mutagenicity assays; Air pollutants

Summary

Ozone fumigation of a double heterozygous chlorophyll mutant Nicotiana tabacum var. xanthi n.c. with concentrations up to 300 nl/l and of a heterozygous Tradescantia clone 4430 with concentrations up to 800 nl/l did not increase the frequency of somatic mutations above the spontaneous levels. However, ozone fumigation at these concentrations led to distinct physiological damage to plant tissues.

Ozone (O$_3$) is one of the most widespread air pollutants. High ambient ozone concentrations contribute to the novel forest decline phenomena observed in Europe and North America (Forschungsbeirat, 1989) and to the decrease in productivity of crop plants (Heck et al., 1988). Data on the extensive physiological and biochemical effects of ozone on higher plants exist (Heath, 1988; Sandermann et al., 1989). However, few studies have evaluated the genotoxic potential of O$_3$ (Schairer and Sautkulis, 1982; Sandermann, 1988; Ma et al., 1984).

The objective of this investigation was to determine if O$_3$ can induce genetic damage in 2 plant mutagenicity assays, (i) in the Nicotiana tabacum var. xanthi n.c. leaf color reversion assay, and (ii) in the Tradescantia stamen hair assay. The effects of the pesticide and plant growth regulator maleic hydrazide (MH) are presented for comparison and as a positive control.

Material and methods

Ozone fumigation and pollutant-free air

Plexiglass exposure chambers (108 × 71 × 95 cm) were placed in a walk-in growth cabinet (10 m$^2$). Chamber conditions included 25/20 ± 0.5°C day/night temperatures, 11–12-h photoperiod, 100 μE/sm$^2$ light intensity and 70 ± 5% relative humidity.

Ozone was generated by electrical discharge in dry oxygen (500 M, Fisher, Cologne) and was added into the filtered air stream by computer-controlled mass flow-meters (MKS). O$_3$ concentrations were measured at the plant level with a CSI/3100 analyzer (Messer-Griesheim, Munich). A switching system was used to monitor air samples from the fumigation chambers. O$_3$-free air was obtained by passing ambient air through a filter system consisting of 5 units (particle, activated charcoal, KMnO$_4$-coated alumina granules...
(Purafil II), charcoal, particle). This system effectively removed \( \text{O}_3 \), \( \text{SO}_2 \), and nitrous oxides to concentrations below 5 nl/l (Langebartels et al., 1991). Gas exchange measurements were performed with a LCA-2 analyzer (Parkinson Leaf Chamber; air supply by mass flow-meters; ADC, GB-Hoddesdon).

**Mutagenicity assays**

**Tobacco assay.** Seedlings of a double heterozygous chlorophyll mutant \((a1+a1 a2^+/a2^+)\) of *Nicotiana tabacum*, var. xanthi n.c. (kindly provided by Dr. H. Dulieu, Dijon) were cultivated in plastic pots filled with a mixture of soil and perlite \( (2:1) \) placed in a controlled environment cabinet with a 12-h photoperiod and 25/20°C day/night temperatures. At the stage of 4–6 true leaves the plants were fumigated with \( \text{O}_3 \) for 5 h or 11 h daily for 1–18 days. After the ozone fumigation, the plants were returned to the growth chamber with filtered air for 2–6 weeks. The frequency of dark green sectors (reversions to the dominant phenotype), yellow sectors (reversions to the recessive phenotype) and dark green/yellow double sectors were evaluated on leaves 5–7. Means of mutation frequencies and their 95% confidence intervals were calculated from the total number of mutation events per evaluated leaf. For estimating the mutation rate (expressing not only the number, but also the size of the mutated sectors) the methods developed by Dulieu and Dalebroux (1975) and Fabries and Delpoux (1978) were used. The negative control consisted of plants that were exposed to filtered air only. The positive control used chronic exposure to 80 \( \mu \text{M} \) MH applied as reported by Briza et al. (1984).

**Tradescantia assay.** Stock plants of *Tradescantia*, clone 4430 (a hybrid of *T. subacaulis × T. hirsutiflora*) were cultivated in pots with a mixture of soil and perlite \( (2:1) \), and placed in a greenhouse. For each treatment, 30–40 cuttings (10–15 cm long) with inflorescences were placed in beakers with tap water and fumigated with ozone. The treatment times and the concentrations of ozone are presented in Table 2. After \( \text{O}_3 \) treatment the cuttings were placed in an environment chamber with a 14-h photoperiod and 20/18°C day/night temperatures. In the experiments in which intact potted Tradescantia plants were fumigated with \( \text{O}_3 \), the plants were kept in the fumigation chamber for 13 or 15 days.

For each treatment, 5–15 flowers were evaluated daily, starting on the 5th day after \( \text{O}_3 \) fumigation. The frequency of pink mutation events was scored according to the method of Underbrink et al. (1973). One or more contiguous pink cells were considered to have resulted from one mutation event. Means of mutation frequencies and their 95% confidence intervals were calculated from the data obtained on days 5–15 after the onset of \( \text{O}_3 \) treatment. The negative controls

### TABLE 1

**EFFECTS OF \( \text{O}_3 \) FUMIGATION ON THE FREQUENCY OF MUTATIONS IN THE DOUBLE HETEROZYGOUS CHLOROPHYLL MUTANT *Nicotiana tabacum*, var xanthi n.c.**

<table>
<thead>
<tr>
<th>Ozone fumigation (nl/l)</th>
<th>Leaves scored</th>
<th>Mutation events</th>
<th>Mutations per leaf ( ^a )</th>
<th>Mutation rate ( \times 10^{-5} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( 0 )</td>
<td>–</td>
<td>72</td>
<td>104 4 5 113</td>
<td>1.57 ± 0.32 10.7</td>
</tr>
<tr>
<td>( 100^b )</td>
<td>18</td>
<td>84</td>
<td>115 8 127</td>
<td>1.51 ± 0.32 6.0</td>
</tr>
<tr>
<td>( 150^b )</td>
<td>18</td>
<td>84</td>
<td>94 7 109</td>
<td>1.30 ± 0.24 7.7</td>
</tr>
<tr>
<td>( 300^c )</td>
<td>1</td>
<td>72</td>
<td>84 2 3 89</td>
<td>1.24 ± 0.25 7.0</td>
</tr>
<tr>
<td>MH ( d )</td>
<td>–</td>
<td>30</td>
<td>485 24 16 525</td>
<td>17.5 ± 2.68 63.6</td>
</tr>
</tbody>
</table>

Mutations were scored on leaves 5, 6 and 7.

\( G \), green; \( Y \), yellow; \( G/Y \), green/yellow double sectors.

\( ^a \) Means ± 95% confidence intervals.

\( ^b \) Seedlings fumigated for 5 h per day.

\( ^c \) Seedlings fumigated for 11 h.

\( ^d \) 25 ml of 0.08 mM maleic hydrazide administered 3 times a week for a period of 4 weeks. Mutations scored on leaves 6, 7 and 8.
for the Tradescantia cuttings or intact plants were exposed to filtered air only. The positive control consisted of a single exposure of 10 mM MH as reported by Gichner et al. (1982).

**Results**

The fumigation of the tester strain tobacco seedlings with 100–300 nl/l O₃ ozone for up to 18 days did not significantly increase the frequency of mutational events above the spontaneous control level. In comparison, the administration of MH increased the number of mutational events per leaf about 10 times and the mutational rate about 6 times above the negative control values (Table 1).

While the plants were tolerant to 100 nl/l O₃, leaf damage occurred after exposure to 150 nl/l O₃ for 6 days. White to pergament-like lesions were found on 10–20% of the leaf area of middle-aged leaves of the tester strain. Fumigation with 300 nl/l ozone for 3 h caused severe damage to these leaves while the younger leaves were not visibly affected. In comparison, the biomonitor plant, tobacco Bel W3 (Langebartels et al., 1991), showed about 60% necrotic leaf area after a 5-h treatment with 150 nl/l O₃. Following 5-h exposure to 150 nl/l O₃, the stomatal conductance was similar in control (40.8 ± 4.8 mmole/m²s) and treated plants (43.8 ± 3.9 mmole/m²s). No statistical differences between the treatments were found (P = 0.6854).

Ozone fumigation for 1–7 days of Tradescantia cuttings with inflorescences in concentrations up to 800 nl/l did not significantly enhance the frequency of pink mutations in stamen hair cells (Table 2). Similarly, fumigation of whole plants with inflorescences by ozone (150 and 300 nl/l) for a period of 15 or 13 days had no significant influence on the frequency of pink mutations, although the fumigation strongly inhibited the flowering and caused chlorotic and necrotic lesions beginning at the tips of the leaves. Application of MH on inflorescences increased the frequency of pink mutations more than 40 times compared to the control treatment.

**Discussion**

Data on testing the mutagenicity of ozone on higher plants were initially provided by Sparrow

### Table 2

<table>
<thead>
<tr>
<th>Ozone fumigation (nl/l)</th>
<th>Hairs scored</th>
<th>Pink mutations</th>
<th>Pink mutations per 100 hairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 b</td>
<td>29952</td>
<td>36</td>
<td>0.120 ± 0.039</td>
</tr>
<tr>
<td>150 b</td>
<td>33352</td>
<td>45</td>
<td>0.135 ± 0.039</td>
</tr>
<tr>
<td>300 b</td>
<td>38115</td>
<td>43</td>
<td>0.113 ± 0.034</td>
</tr>
<tr>
<td>800 b</td>
<td>37700</td>
<td>42</td>
<td>0.111 ± 0.034</td>
</tr>
<tr>
<td>0 b</td>
<td>22410</td>
<td>16</td>
<td>0.071 ± 0.035</td>
</tr>
<tr>
<td>800 b</td>
<td>28785</td>
<td>22</td>
<td>0.076 ± 0.032</td>
</tr>
<tr>
<td>800 b</td>
<td>34104</td>
<td>32</td>
<td>0.094 ± 0.032</td>
</tr>
<tr>
<td>800 b</td>
<td>30096</td>
<td>31</td>
<td>0.103 ± 0.036</td>
</tr>
<tr>
<td>0 c</td>
<td>26163</td>
<td>26</td>
<td>0.099 ± 0.038</td>
</tr>
<tr>
<td>150 c</td>
<td>38623</td>
<td>33</td>
<td>0.085 ± 0.025</td>
</tr>
<tr>
<td>300 c</td>
<td>17496</td>
<td>14</td>
<td>0.080 ± 0.042</td>
</tr>
<tr>
<td>MH d</td>
<td>30384</td>
<td>1336</td>
<td>4.397 ± 0.231</td>
</tr>
</tbody>
</table>

*a* Means ±95% confidence intervals.

*b* Cuttings with inflorescence fumigated.

*c* Whole plants with inflorescence fumigated.

*d* 20 μl of 10 mM maleic hydrazide administered per inflorescence.
and Schairer (1974) and Schairer et al. (1978). These authors used cuttings with inflorescences of Tradescantia and exposed them to 5000–50,000 nl/l ozone for 6 h. Although the frequency of mutations was lower than 0.3%, the authors concluded that ozone exhibits a weak mutagenicity. However, the minimum concentration used in their experiments (5000 nl/l; Schairer and Sautkulis, 1982) was 10–100 times higher than average environmental levels. The range of O₃ concentration during the summer months in Europe and North America is 30–60 nl/l (Forschungsbeirat, 1989). Peak values between 100 and 300 nl/l occur periodically. When similar O₃ concentrations were used for tobacco and Tradescantia plants in our studies, typical visible leaf damage occurred after 1–18 days of treatment. The stomatal conductance of tobacco plants was not affected by short-term exposure to 150 nl/l O₃. These observations indicated that the pollutant was able to penetrate into the leaves under treatment conditions. The same concentrations or 2–3 times exceeding the maximum environmental levels (800 nl/l) did not significantly increase the mutation rate above the background in both test plants.

In summary, it can be concluded that concentrations of O₃ that occur under real-world environmental conditions were not mutagenic in the short-term Tradescantia and tobacco mutagenicity assays. However, these O₃ concentrations caused severe injury to the leaves of the tester plants. These experiments do not address the question of possible genotoxic effects of long-term O₃ treatment on forest tree species (Forschungsbeirat, 1989), but at present no plant mutagenicity assay that could solve this problem is available.

Acknowledgement

This work was supported in part by the Fonds der Chemischen Industrie.

References


Sparrow, A.H., and L.A. Schairer (1974) The effects of chemical mutagens (EMS, DBE) and specific air pollutants (O₃, SO₂, NO₂, N₂O) on somatic mutation rates in Tradescantia, Brookhaven National Laboratory, Biology Department, 40 pp.


Communicated by J. Velemínský