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A Miniaturized Kit for Ozone Biomonitoring

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ABSTRACT

A new miniaturized kit based on very young supersensitive tobacco Bel-W3 plantlets, which can be easily used to detect phytotoxic levels of ozone in ambient air in large scale surveys, is described. It has been developed in laboratory as well as field studies. The optimal sampling time is 5–7 d. The advantages of the kit are its user-friendliness, low cost, and reliability. The kit may be integrated by a passive sampling tube set and may be also proposed for educational programs.

Index Entries: Air pollution; tropospheric ozone; biomonitoring; integrated monitoring; miniaturized kit; tobacco Bel-W3.

INTRODUCTION

Ozone, a major component of photochemical air pollution (smog), is one of the most toxic and abundant air contaminants. Its deleterious effects on human and animal respiration processes have been documented. Tropospheric ozone also adversely affects plants, manufacts, and visibility. Even if the precursor pollutants of ozone (i.e., nitrogen oxides and volatile hydrocarbons) are typically produced in urban and metropolitan areas, relevant ozone levels are frequent in rural and remote regions, owing to medium/long range transport of precursors and of ozone itself.

The knowledge of ozone distribution at ground level is therefore a priority in the environmental monitoring activities in order to prevent risks to human welfare. In spite of this, ozone monitoring has, until now,

been limited to a handful of urban cities (*1*). This is mainly because of the high costs of establishment and management of chemicophysical automatic analyzers.

Biological active monitoring of ambient ozone with the super-sensitive, highly specific, test plant tobacco cv Bel-W3 (*Nicotiana tabacum* L.) has been extensively performed in many countries all over the world since 1962 (*2*). Bel-W3 is a shade-grown cigar wrapper tobacco that exhibits typical bifacial lesions, rather than only on the adaxial leaf surface, which is typical of ozone injury on tobacco. In adult plants, ozone sensitivity is inversely related to leaf maturity: Recently mature are more sensitive than overmature or rapidly expanding younger leaves, which are nonreactive until their length is <6–7 cm. Lesions are roundish with well-defined margins, at first adaxial, a few millimeters in diameter, and grayish; complete bifacial-tissue collapse occurs in a couple of days, and is accompanied by tissue necrosis and the bleaching of lesions, which become light ivory in color. The threshold for visible injury is around 40–50 ppb (the maximum concentration in which ozone normally occurs naturally) for some 4 h of exposure.

It has been reported that ozone injures cotyledons and mature leaves similarly, and ozone injury has been reported on cotyledons of Bel-W3 tobacco seedlings (*3*) as well as other species, such as soybean (*4*); it is ironic that these findings have been evaluated for a quick screening for resistance but not for biomonitoring purposes.

The biomonitoring procedures with tobacco plants are well-standardized and applied in local, regional, and nationwide studies (*5*): The plants start in an ozone-free greenhouse and when they have five or six leaves they are transplanted into cultivation containers provided with water-dispensing devices and moved to the test sites. At regular intervals, usually once a week, leaf injury is assessed in terms of the percentage of leaf area damage on all the expanded leaves. Increases in the injury index from one week to the next one are then computed. Plants of a resistant tobacco cultivar, such as Bel-B, are usually included in the plots to confirm the ethiology of foliar lesions.

The biomonitoring of ozone with tobacco plants has several advantages: It allows a capillary distribution in the space of the monitoring sites and a satisfactory coverage of the territory, it does not require elevated technological know-how, it does not depend on electrical supply, and it causes people to become emotionally involved in air quality problems. However, the use of adult tobacco plants implies remarkable logistical difficulties in establishing a large number of plots and may represent a limiting factor to the full application of the methodology. A more manageable system for achieving the same results obtained with whole plants should be desirable.

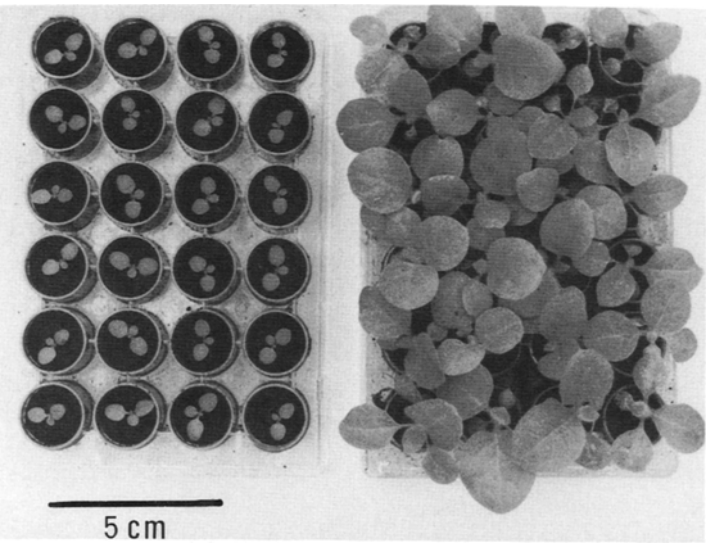

Fig. 1. A kit of tobacco Bel-W3 plantlets at the beginning of the ambient air exposure (left) and 7 d after (right).

DESCRIPTION OF THE KIT AND FINAL COMMENTS

A minibioassay kit, which utilizes tobacco Bel-W3 seedlings (10-d-old, one leaflet partially expanded), was developed. Preliminary assays, both in fumigation chambers and in the open air, have highlighted the suitability of very young plantlets for biomonitoring purposes. A commercial polystirol tissue culture plate (14 × 9 cm), with 24 round wells (16-mm diameter, 20-mm depth) is modified to allow the continuous watering of the wells, which are filled with an organic compost. The operational protocol is simple. Tobacco seeds are germinated in a separate tray in an ozone-free environment, and plantlets are singly transplanted in the plate wells (Fig. 1). A typical configuration is with 20 plantlets of Bel-W3 and of the ozone-resistant tobacco Bel-B, which act as control. The following day, the plates are exposed to the open air, under a shady structure. In the presence of the abovementioned ozone levels or higher ones, cotyledons and the first emerging leaflets of Bel-W3 rapidly exhibit typical flecking (Fig. 2). The plates stay in the study sites 5–7 d and after a further day of permanence in ozone-free air, visible injury is evaluated in terms of the percentage of leaf area (cotyledons and the first leaflet) covered by necrotic lesions. The kit, whose patent is pending, may be integrated by a passive sampling tube set, such as that described by Monn and Hangartner (*6*).

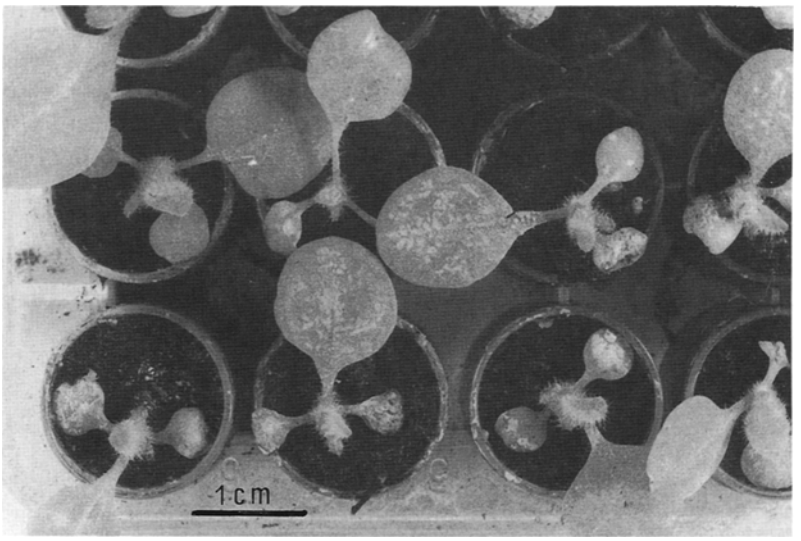

Fig. 2. A detail of a plate, with tobacco Bel-W3 plantlets showing typical foliar lesions induced by ozone after 1 wk of exposure to ambient air.

The kit, whose nickname is *KOMB* (for "Kit for Ozone Miniaturized Bioassay"), is reliable, sensitive, specific, and rapid, small and lightweight, cost efficient, simple to operate and to transfer (it may be shipped by mail), user-friendly, and does not require continuous assistance; it may represent a useful and practical tool for large-scale, nationwide, biomonitoring activities. Its educational value is noteworthy.

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