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Tradescantia Bioassays as Monitoring Systems for Environmental Mutagenesis: A Review

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ABSTRACT: Since the early studies on the genetic effects of chemical and physical agents, species and clones of *Tradescantia* have been used as experimental subjects, by virtue of a series of favorable genetic characteristics. Bearing just six pairs ($2n = 12$) of large, easily observable chromosomes, cells from almost every part of the plant, from the root tips to the developing pollen tube, yield excellent material for cytogenetic studies.

As a consequence of the intensive use of *Tradescantia* in genetic studies, a series of genetic characteristics have been found that offer opportunities for the detection of agents affecting the stability of the genome. At least five such characteristics have been selected as endpoints for the establishment of assays to evaluate mutagenesis. Three of these, root-tip mitosis, pollen-tube, and microspore mitosis are essentially chromosome aberration assays, wherein one observes and evaluates the visible damage in the chromosomes. A fourth, the stamen-hair mutation assay (Trad-SHM), is a point mutation mitotic assay based on the expression of a recessive gene for flower color in heterozygous plants. The fifth assay is a cytogenetic test based on the formation of micronuclei (Trad-MCN) that result from chromosome breakage in the meiotic pollen mother cells.

This article examines the characteristics and fundamentals of the Trad-MCN and the Trad-SHM assays and reviews the results obtained to date with these systems in the assessment of environmental mutagenesis.

KEY WORDS: *Tradescantia*, environmental mutagenesis, stamen hair mutation, micronuclei, bioindicator, genotoxicity.

I. TRAD-MCN: FUNDAMENTALS AND DEVELOPMENT OF THE SYSTEM

Studies of the *Tradescantia* genome began with the pioneering work of Sax and Edmonds (1933) on the development of the male gametophyte of *Tradescantia reflexa* Raf. Important observations were made in a study of the effects of X-rays on microspores of this species (Sax, 1938). First, it was noticed that meiotic chromosomes were more susceptible to breakage than mitotic chromo-

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somes; more importantly, dividing chromosomes were at least ten times more susceptible as resting ones. Second, breaks were not randomly distributed along the chromosomes. Loci positioned closer to the centromeres were more likely to suffer the splitting effects of radiation. These observations led to the conclusion that coiling of the chromosomes after replication, with the consequent mechanical strain involved, would strongly influence susceptibility to mutational events. These inferences were later confirmed in a study of the effects of ^{60}Co-gamma radiation on *T. paludosa* And. and Woods (Sparrow and Singleton, 1953). The concepts of temporality and sensitivity are important for the selection of bioindicators of mutagenicity, for synchrony in cell development, and timely recovery periods are two decisive factors in the performance of bioassays (Ma, 1979b).

The greater susceptibility of meiotic when compared with mitotic chromosomes was confirmed in a study of the influence of lack of oxygen on meiosis of *T. paludosa* (Steinitz, 1944). This research represented the first attempt to use micronuclei of the pollen mother cells as a direct indication of chromosome fragmentation. A spontaneous level of 0.87% cells with micronucleus was defined for *T. paludosa*, a rate that rose to 8.0% for cells under anaerobic conditions at the early stages of prophase. The results corroborated the earlier findings of Sax (1938), indicating that prophase, especially pachytene and diplotene, was the most susceptible stage of meiosis.

The pace of the meiotic stages was characterized further in a study of the differentiation of excised anthers of *T. paludosa* (Taylor, 1950). Approximately 24 h elapsed for the development of the meiotic nucleus from pachytene to early tetrad. This result proved valuable for the definition of the appropriate recovery period required between exposure of the prophase nucleus in the developing inflorescence of *Tradescantia* and fixation of the material for tetrad analysis.

The growing interest during the 1950s in the radiomimetic (principally genotoxic) capabilities of chemical substances suggested the utilization of *Tradescantia* as a bioindicator. A pollen tube mitosis assay was first used in a comparative study of simple chemical agents in *T. paludosa* (Smith and Lofty, 1954). Ethylene oxide, a known effective mutagen, ketene (a compound giving conflicting results), and methyl chloride (an alkylating agent of low potency) were compared for inducing chromatid breaks and chromosome erosions and contractions. The pollen tube assay proved effective in detecting genotoxicity, as the results revealed that the more active compounds, ethylene oxide and ketene, caused more extensive and numerous chromosome aberrations of all types. Possibly due to its postulated faster penetration into the cells, methylchloride was effective in causing chromosome breaks. The propitious selection of chemicals in this early research proved instrumental in demonstrating the sensitivity of *Tradescantia* and showing its capacity for precisely differentiating nearly comparable effects. A series of studies employing this pollen tube chromatid aberration assay (Ma, 1967; Ma, 1982) was later carried out regarding the effects of the atmospheric pollutant SO_2 (Ma, 1982; Ma et al., 1973; Ma and Khan, 1976), ultraviolet light (Ma et al., 1971), X-rays (Ma and Wolff, 1965), and hydroxylurea (Khan and Ma, 1974). A procedure involving the evaluation of sister chromatid exchanges (SCE) in the root tips of *Tradescantia* for the evaluation of mutagenic agents was also described (Peng and Ma, 1990).

In a series of papers concerning the role of selected nutrients on meiosis, the production of micronuclei in the microspores of *T. paludosa* was cited as indicative of chromosome breakage (Steffensen, 1953; Steffensen, 1954; Steffensen, 1955). When studying the effects of magnesium (Mg) deficiency on meiosis, the author noted the greater sensitivity of the microspores when compared with

root tips, supporting previous evidence of greater susceptibility of meiotic than mitotic cells. Micronuclei were more numerous in the microspores of plants deficient in Mg, calcium (Ca), and sulfur (S). It was pointed out that the first two nutrients were responsible for bonding with macromolecules in the nucleus, contributing to the stability of proteins and DNA. This was demonstrated further by increased susceptibility to X-rays of plants grown on Ca-deficient media because of the relationship of Ca with sulfhydryl groups in nuclear division, particularly in spindle formation. A spontaneous micronuclei frequency of 0.84% was recorded and increased to 3.89% in plants grown under suboptimal Ca supply (Steffensen, 1955). This corroborated earlier observations on micronuclei production in *T. paludosa* (Steinitz, 1944).

More than 30 years after Steinitz's observation of micronuclei for the detection of chromosome damage in meiosis, Ma and coworkers (1978) at Brookhaven National Laboratory devised the Micronucleus-in-Tetrad Assay for Environmental Mutagenesis (later referred to as the Trad-MCN assay) (Knasmuller and Ma, 1992; Lower et al., 1984; Ma, 1981b; Ma, 1981c; Underbrink et al., 1984). Employing the hybrid clone 4430 (*T. hirsutiflora* Bush x *T. subacaulis* Bush) they compared the production of micronuclei in the pollen mother cells with the mutation for pink cells in the stamen hairs of *Tradescantia* exposed to the known mutagen 1,2-dibromoethane (DBE).

By that time, the stamen hair mutation assay (Trad-SHM) (Underbrink et al., 1973b) had been applied extensively and was a well-recognized test for radiobiological and chemical mutagenesis. The micronucleus assay, however, exhibited an efficiency approximately 36 times as great. This extraordinary sensitivity was credited to the much smaller specificity of the damage needed to produce a micronucleus when compared with a pink mutation. Indeed, it could be assumed that numerous sites in any of the 12 chromosomes of *Tradescantia* were subjected to damages that could result in a chromosome breakage, hence, in micronuclei. By contrast, only one locus in one chromosome could bear the mutation for pink pigmentation in the cells of the stamen hair (Ma et al., 1978). It must be pointed out that micronuclei frequency in the Trad-MCN assay is greater at the earlier stage of the pollen tetrad, and one may assume that some micronuclei are incorporated into nuclei as the pollen matures. This suggests that some micronuclei may result from lagging chromosomes or laggings of unpaired chromosomes, because it is known that associations of homologous chromosomes in meiosis are not always complete, especially in clone 4430.

The great sensitivity and simplicity of the Trad-MCN assay was demonstrated further in experiments in which X-rays at a low dose were compared with two well-known chemical mutagens, ethyl methanesulfonate (EMS) and sodium azide (NaN_3) in both liquid and gaseous forms (Ma, 1979a). A low dose of X-rays induced high frequencies of micronuclei, that is, 23 MCN/100 tetrads at a 20-rad X-ray exposure level when compared with 1.8 MCN/100 cells of human lymphocytes at 50-rad of X-rays (Countryman and Headdle, 1976) or 2.5 MCN/100 cells of mouse erythroblasts of bone-marrow culture at 35-rad of X-rays (Jenssen and Ramel, 1976). Although there were 0.2% pink mutations per rad in the Trad-SHM assay, there were 1.6% MCN per rad for the Trad-MCN assay. The dose-response relationship of the Trad-MCN assay to X-rays gave a correlation coefficient of 0.99. The results obtained for the chemical agents verified these findings, both in relation to sensitivity and the dose-response relationships (Ma, 1979a).

One additional advantage of the Trad-MCN assay was the short-term exposure needed for completion of a test — only 6 h, followed by a 24-h recovery period to allow the cells treated at prophase to reach the

scorable tetrad stage. This meiotic timetable was tested in a study of the stage of sensitivity using X-ray exposure of *T. paludosa* (Ma et al., 1980). Groups of plant cuttings received a single 35-rad X-ray exposure, after which inflorescences were removed and fixed at 3-h intervals for 48 h. A peak of sensitivity occurred after 24 h postirradiation, which agrees with the observations of Taylor (1950). A second peak appeared at about 39 h postirradiation, suggesting that the earlier prophase I and/or premeiotic stages are also very sensitive.

The utilization of the Trad-MCN assay for *in situ* monitoring of environmental clastogens was proposed after studies involving promutagens (benzo-α-pyrene) and polluted sites (Ma, 1979b; Ma, 1981a). No external enzymatic activation was needed, because the enzymatic apparatus was fully functional in the exposed plant cuttings.

A series of limitations of the Trad-MCN assay has been presented. The test obviously provided a relative index of genetic damage. Translocations, inversions, and other types of chromosome and chromatid rearrangements and exchanges would not be revealed as micronuclei. No carcinogenicity information could be easily extrapolated from the frequencies of micronuclei, and the metabolic pathways of mutagenic and promutagenic agents may be quite different in *Tradescantia* and other subjects (especially mammalian species). Also, the high sensitivity of the system results in day-to-day variation in spontaneous micronuclei frequencies, requiring careful control of experimental conditions and simultaneous control samples (Ma, 1981a).

One additional disadvantage of the Trad-MCN test was the labor-intensive and time-consuming procedure for micronucleus scoring in the tetrads (Ma, 1990b). In order to overcome this limitation and to facilitate and standardize the scoring process, a micronucleus image analysis system was devised (Ma et al., 1992d; Xu et al., 1989). The computerized system scoring speed was 3.5 times faster than manual observations, with a 96% congruity in the frequencies scored.

A relatively recent overview of the *in situ* monitoring of environmental clastogens (Ma, 1990b) revealed that through 1990 about 300 tests had been conducted with the Trad-MCN assay in a variety of categories. Around 50% of these tests exhibited genotoxicity. In the following sections, the results obtained to date with the Trad-MCN assay in the evaluation of environmental genotoxicity are reviewed and discussed.

II. ASSESSMENT OF ENVIRONMENTAL MUTAGENESIS

A. Air Pollution

T. paludosa was exposed at several polluted sites in Illinois and to gaseous agents commonly found in polluted atmospheres in a combination of *in situ* and *in vivo* laboratory tests (Ma et al., 1982a; Ma and Harris, 1985; Ma et al., 1989). *In situ* assays carried out in public parking garages revealed a correlation between the rate of micronuclei production and the volume of traffic, with a positive dose-response (time of exposure, from 2 to 6 h) relationship ($\alpha = 0.01$). A series of *in situ* exposures of *T. paludosa* and of clone 4430 to industrial sites, farms, and laboratory atmospheres resulted in positive results ($\alpha = 0.01$) in most categories. Atmospheres of an office, a livestock farm, and a residential area gave negative results. A high frequency of micronuclei occurred at a site where gaseous pollutants from an agricultural chemical production plant were prevalent (Ma et al., 1982a).

Tradescantia plants were fumigated with the atmospheric pollutants NO_2, SO_2, and O_3, as well as gaseous hydrazoic acid (HN_3) and EMS (Ma et al., 1982a). With the exception of O_3, all agents proved clastogenic to *Tradescantia*. Both NO_2 and SO_2 required long exposure times (24 and 22 hours,

respectively), while gaseous HN_3 and EMS gave positive results after 6-h exposures. In a recent experiment, O_3 was shown to be clastogenic in the Trad-MCN assay in concentrations as low as 100 ppb (Rodrigues et al., 1996).

As a consequence of its versatility, *Tradescantia* was proposed as a monitor of indoor pollution. Several studies have assessed the proficiency of the Trad-MCN assay for the low level of contaminants customarily present in home environments. Among the positive responses found are several common commercial air fresheners, tobacco smoke, *p*-dichlorobenzene (moth balls) and other insecticides listed for residential use, and diesel exhaust gases (Harris and Ma, 1983; Ma et al., 1982b; Ma and Harris, 1987a; Ma and Harris, 1987b; Ma et al., 1983b).

A somewhat unusual group of atmospheric pollutants evaluated *in situ* for mutagenicity with the Trad-MCN assay were the chemical smokes employed by the U.S. Army. These tests also involved other assays, including chromosome breakage and sister chromatid exchange in a native rodent (Schaeffer et al., 1987). The smokes were generated from fogoil, tank diesel, and hexachloroethane. All of them induced a higher rate of genotoxic events for at least one dose when compared with the controls in the Trad-MCN assay ($\alpha = 0.1$). The production of chromosome breakage in rodents was depressed. There was a higher degree of variability (expressed as larger standard deviations from the mean micronuclei production) for all *in situ* treatments relative to controls (Schaeffer et al., 1987). Even though this statistical effect was discussed merely as obscuring the dose-dependence of the results, it might be indicative of a characteristic of the *Tradescantia* system. The smaller buds of a young inflorescence are hidden beneath larger buds and bracts, which may result in their being protected from unrestricted exposure, especially in atmospheric exposures, an effect anticipated by Ma, (1979b).

In an *in situ* study with *Tradescantia* clone 4430 in Mexico (Ruiz et al., 1992), the micronuclei frequency peaks for one heavily industrialized, one residential, and one mixed occupation area were evaluated. Plants exposed to the industrial site always showed higher levels of micronuclei than the controls ($\alpha = 0.01$) throughout the year, whereas the residential area samples tended to be higher than the controls only in specific months.

The Trad-MCN assay gave positive results in assessing the mutagenic risks posed by gaseous emissions from a municipal waste incinerator (Ma et al., 1993b; Ma et al., 1996; Ma et al., 1992b) and from a landfill vent pipe (Ma, 1994b; Ma et al., 1993a; Ma et al., 1996).

From the studies referred to earlier, it may be concluded that the Trad-MCN is well suited for assessing atmospheric contamination, whether from heavily polluted industrial or urban areas or under diurnal indoor environmental conditions. Weather conditions such as variations in wind speed and direction normally led to high levels of statistical variability in the data.

A summary of the results obtained with *Tradescantia* in assessments of atmospheric pollution and gaseous agents is presented in Table 1.

B. Water Pollution

Nearly all studies for evaluating the presence of mutagenic agents in natural waters must incorporate a step to concentrate possible active agents to be evaluated by bioassay or chemical analysis. This happens because of the intrinsic low mutagenicity of the putative agents most frequently present in natural waters, because of the very low concentrations at which they are found, or both.

The necessity for sample concentration was clearly illustrated in an evaluation of the probability of detecting a mutagen in natural

TABLE 1
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Trad-MCN Assay of Polluted Atmospheres and Atmospheric Pollutants

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
In situ Monitoring						
Air pollution	4–6 h		–	$p < 0.01$	Parking garage Chicago, IL Clear wind from lake	Ma et al., 1982a
	1–4.5 h		–	$p < 0.01$	Parking garage, Decatur, IL	
	2–6 h		+	$p < 0.01$	Parking garage, Peoria, IL	
	2–4 h		–	$p < 0.01$	Truck and bus stop	
	2–3 h		–	$p < 0.01$	Truck and bus stop	
	2.5–5 h		+	$p < 0.01$	Truck and bus stop	
	3 months		+	$p < 0.01$	Industrial site, Granite City, IL	
	3.5 h		+	$p < 0.01$	Industrial site, Granite City, IL	
	4.5 h		–	$p < 0.01$	Residential area, China	
	4–6 h		+	$p < 0.01$	Agrochemical industry site, China	
	5 h		–	$p < 0.01$	Bus station, China	
	6 h		+	$p < 0.01$	Bus station, China	
	6 h		+	$p < 0.01$	Rubber company, China	
	4 h		–	$p < 0.01$	Office environment, China	
	3–6 h		+	$p < 0.01$	P-dichlorobenzene treated herbarium, China	
	6 h		–	$p < 0.01$	Livestock farm, swine house exhaust	
Diesel exhaust fumes	23–70 min	0.3–4.2 ppm	–	$p < 0.01$	Concentration measured as hydrocarbons; exhaust generated by running engine	
	23–70 min	6–13 ppm	+	$p < 0.01$	Concentration measured as hydrocarbons; exhaust generated by running engine	
Gases in chambers						
NO_2	6–24 h	5.0 ppm	+	$p < 0.01$	Positive for longer exposure only	
SO_2	6–22 h	1.0 ppm	+	$p < 0.01$	Positive for longer exposure only	
O_3	5.5 h	5.0 ppm	–	$p < 0.01$	Longer exposure not attempted	

TABLE 1 (continued)
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Trad-MCN Assay of Polluted Atmospheres and Atmospheric Pollutants

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
O_3	6 h	100 ppb	+	$p < 0.01$		Rodrigues et al., 1996
HN_3	6 h	136–272 ppm	+	$p < 0.01$	Single application of gas, without replenishment	Ma et al., 1982a
EMS	6 h	1000 ppm	+	$p < 0.01$	Same as above	
Benzo-α-pyrene	6 h	0.05–0.10 m*M*	+	$p < 0.01$		Ma, 1981a
1,2-dibromo ethane	6 h	5–80 ppm	+		Dose response correl. coefficient = 0.99	Ma et al., 1978
Industrial district	6–12 h		+	$p < 0.01$	Seasonal variability, Mexico	Ruiz et al., 1992
Residential district	6–12 h		+	$p < 0.01$	Seasonal variability, Mexico	
Mixed district	6–12 h		+	$p < 0.01$	Seasonal variability, Mexico	
Chemical smokes						
Fogoil	30 min	15–100 m from smoke source	+	$p < 0.01$	Gas concentrations reported in relative terms; all distances produced positive results	Schaeffer et al., 1987
Tank diesel	30 min	15–100 m	+	$p < 0.01$	Same as above	
Hexachloro	30 min	15–100 m	+	$p < 0.01$	Same as above	
Landfill gaseous emissions	4–6 h		+		Positive responses in 5 of 13 monitoring trips; gases burned at emission source	Ma et al., 1993a; Ma et al., 1996
Municipal incinerator	4–6 h	50–500 m from source	+		Positive results obtained with stagnant atmosphere	Ma et al., 1993b; Ma et al., 1996
Indoor pollutants						
Dry cleaning	15 h		–	$p < 0.05$	Night hours	Ma and Harris, 1987a; Ma and Harris, 1987b
House	16 h		+	$p < 0.05$	After carpet shampooing	
House	17 h		–	$p < 0.05$	Clean air	
Pipe smoke	24 h		+	$p < 0.05$	Within office room	
Tobacco smoking room	10 h		+	$p < 0.05$	In a public school	
Air fresheners	1–6 h		+		Several brands	
Formaldehyde fumes	1–6 h		+		Positive dose-response relationship	

waters with the Ames assay (Johnston and Hopke, 1980). The analysis considered a weight-dependent variable, taking into account the mutagenic potency and the average concentration of organic mutagens commonly found in natural waters and the amount of chemical needed to induce a doubling in revertants in the Ames assay. Considering that (1) generally only 1-ml aliquots are assayed in a test plate, (2) organic compounds typically occur in water at µg/l concentrations, and (3) 95% of the chemicals tested so far have a doubling dose of at least 1500 µg, the average environmental sample that would permit the detection of contaminants with 95% confidence should contain about 1500 l. This means that a concentration factor of six orders of magnitude is required to reduce the volume of such a sample for testing. It was concluded that the lifetime exposure of a human to mutagens present in drinking waters could be appreciable despite the failure to detect them in environmental samples (Johnston and Hopke, 1980).

Perhaps the most important feature of the Trad-MCN assay, as well as of certain other plant assays, is its capability to detect low level genotoxicity in either short-term *in situ* exposures or *in vivo* tests with unconcentrated water samples (Fang, 1981a; Fang, 1981b). This was demonstrated in a 2-year genotoxicity study of the surface waters of Spring Lake reservoir and of the municipal drinking water obtained from the reservoir in Macomb, IL (Ma et al., 1985). Water samples were drawn from the reservoir biweekly and tested for genotoxicity and for the presence of nutrient elements and metals. The most prominent result of this study was a recurring seasonality in the expression of micronuclei frequency peaks ($\alpha = 0.05$) that occurred following the periods of intense precipitation and runoff from corn and soybean fields upstream from the reservoir. Micronuclei production for the drinking water tended to follow the patterns observed for the reservoir ($\alpha = 0.05$), although the peaks were lower.

In a follow-up investigation, tests were conducted with a chronic mouse-erythrocyte-micronucleus test, and additional samples of a shallow- and a medium-depth well located in the same rural area as the reservoir (Ma et al., 1987). A similar pattern of micronucleus frequency ($\alpha = 0.01$) following heavy precipitation or snow thaw were found for the Trad-MCN assay, with delays matching the times supposedly required for the arrival or accumulation of clastogens at the sampling sites. Analyses of water samples for organic compounds from the shallow well exhibited elevated levels of methylene chloride, dichlorobromoethane, trichloroethylene, and tetrachloroethylene. The mouse assays confirmed these results, although 6-month exposures to the mutagenic samples (when compared with 30-h exposures for *Tradescantia*) were required for a significant ($\alpha = 0.03$) increase in micronuclei frequencies to occur.

The quickness and simplicity of the Trad-MCN test (Ma, 1994a; Ma and Cabrera, 1986) have prompted its application to the study of a variety of water samples, for example, industrial effluents (Chen et al., 1984; Chen and Xiang, 1983; Ruiz et al., 1987; Zheng, 1985), surface waters and landfill leachates (Ma et al., 1992c), sea water and marine pollution (Chen, 1982; Chen et al., 1983; Chen and Fang, 1981; Chen et al., 1988; Chen et al., 1989; Chen and Zheng, 1982; Chen and Zhou, 1985), groundwater (Helma et al., 1992), and drinking water (Helma et al., 1994a; Lo, 1985).

The experiments described for the detection of genotoxicity in natural waters cannot be qualified as *in situ*, because in all cases samples were brought to the laboratory and assayed under controlled conditions. Appropriate evaluation of *in situ* mutagenicity in aquatic environments became possible after the introduction of the "aquatoon", a floating device specifically designed to hold plant material for exposure to water bodies. The aquatoon was employed successfully in an *in situ* genotoxicity study of the effluents of a pulp and paper mill on the north shore

of Lake Superior (Grant et al., 1992). The *Tradescantia* (clone 4430) micronucleus and stamen-hair-mutation assays, and the *Vicia faba* L. root tip chromosomal aberration assay were performed in the creek containing the raw effluents and in the bay into which the creek emptied. The Trad-MCN and *V. faba* assays showed positive responses after 24-h exposures at both sites ($\alpha = 0.05$). There was partial agreement between the genotoxic effects and the optical density of the samples. In addition to being more sensitive, the two tests that showed the best results were much better adapted to *in situ* studies. The material could be fixed immediately after exposure, whereas the stamen hair mutation assay requires a long recovery period under conditions that cannot be attained in the field or during transportation.

In a study of industrial wastewater in Mexico, a higher level of micronuclei production was found relative to tapwater controls ($\alpha = 0.01$), even after a dilution to 1/3 strength (Ruiz et al., 1992). Seasonal variations observed in the data could not be correlated to any environmental parameter measured. One interesting aspect of these experiments was the lower than normal spontaneous frequency of micronuclei found in *Tradescantia* (0.8 to 1.5 MCN/100 tetrads). It was postulated that the high elevation and subtropical climate typical of the region could be more favorable to growth of clone 4430 due to its genetic relationship to the alpine species *T. hirsutiflora*.

The leachates of an abandoned 20-year-old landfill were tested for genotoxicity with the Trad-MCN assay (Ma et al., 1993a). The high toxicity of the samples precluded tests with solutions diluted less than tenfold, whereas genotoxicity could still be detected in 20-fold dilutions of the leachate samples.

The genotoxicity of contaminated groundwaters treated in a purification plant designed to clean one of the most important aquifers in Austria were assessed in a series of experiments with the Trad-MCN assay (Helma et al., 1992; Helma et al., 1993; Helma et al., 1994b). The purification methods consisted of activated charcoal filtration and UV irradiation. Samples drawn before any treatment exhibited positive, dose-dependent clastogenic effects ($\alpha = 0.05$) after a 24-h exposure. When treated in the laboratory with increasing amounts of UV light (up to 1500 J/m^2), these samples induced micronuclei formation in a dose-dependent fashion relative to UV applied. Results for irradiated clean tapwater were negative. Chemical parameters routinely measured at the purification plant indicated that the activated charcoal-filtered samples were of drinking water quality. In many cases, however, higher micronuclei frequencies were found for these samples before or after UV irradiation. The mechanism responsible for the effects observed was postulated to be activation of water pollutants to genotoxic compounds by UV irradiation. The enhancement of clastogenicity by UV light decreased after storage, with an estimated half-life of approximately 1 d. The authors concluded that similar UV light treatment of waters for drinking could produce hazardous compounds that might pass undetected in the treatment plants.

Studies on water pollution demonstrate the capabilities and advantages of the Trad-MCN assay for the *in situ* assessment of environmental genotoxicants. The ability to detect biological effects in samples considered clean by most chemical standards and the absence of any requirements for tedious concentration procedures that may result in loss and chemical alteration of the compounds are undoubtedly desirable characteristics of this system.

Table 2 presents a summary of results obtained with *Tradescantia* in the evaluation of water pollutants.

C. Soil Contaminants and Soil Amendments

Several studies have evaluated the mutagenicity of soils *in situ*, of extracts of soils from contaminated sites (Ho et al., 1983) be-

TABLE 2
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Trad-MCN Assay of Water Pollutants

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Water pollution						
Drinking water	30 h		+	$p < 0.05$	Both lake and tap-water produced peaks in MCN frequency following rainy season	Ma et al., 1985
Drinking water	30 h		+		Lake, shallow- and medium-depth well waters analyzed; MCN frequency increased following rainy season	Ma et al., 1987
In situ exposure to lake water	24 h		+	$p < 0.05$	Lake Superior and inlet polluted by pulp and paper mill effluent	Grant et al., 1992
Wastewater	30 h	Threefold dilution	+	$p < 0.01$	Industrial effluents presents; positive responses over entire year	Ruiz et al., 1992
Landfill leachates		20-fold dilution	+			Ma et al., 1993a
Groundwater	30 h	Threefold dilution	+	$p < 0.05$	PAH-contaminated groundwater; UV treatment increased mutagenicity	Helma et al., 1993; Helma et al., 1994b

fore and after the application of remediation measures, and of soil amendment materials themselves. Perhaps the most voluminous soil amendment used throughout the world is municipal sewage sludge. Generally, the impact of this material on the environment has been judged by its heavy metal contamination (L'Hermite and Dehandtschttler, 1980). However, complex organic compounds are sometimes introduced into the sewage treatment systems. The possible mutagenicity of such sludges from Chicago was evaluated utilizing two higher plant assays (*Zea mays* L. and *Tradescantia*) and two strains of *Salmonella* in the histidine reversion test (Hopke et al., 1982). Laboratory tests with *T. paludosa*, showed that sludge dilutions above 1:4 showed increased micronuclei frequency. This result was in agreement with results obtained for other species.

The clastogenicity of several chemicals commonly found in hazardous-waste sites were evaluated in a series of experiments aimed at elucidating the possible synergistic or antagonistic behavior of chemical mixtures in the Trad-MCN assay (Ma, 1989; Ma, 1990a; Sandhu et al., 1989). Initially, seven chemicals selected from the U.S. EPA Superfund Priority 1 chemical list (Waters et al., 1987) were tested to determine their minimum effective doses (MED). Five of seven chemicals tested produced positive results ($\alpha = 0.05$), and could be ranked in descending order according to their MED in the

Trad-MCN as follows: lead tetraacetate in DMSO (0.4 ppm), heptachlor in DMSO (2.0 ppm), dieldrin in DMSO (3.8 ppm), arsenic trioxide in NaOH (4.0 ppm), and 1,2-benz[*a,h*]anthracene in ethanol (12.5 ppm). Both tetrachloroethylene (TCE) and aldrin were immiscible with water, precluding adequate exposure in solution. When exposed in the gaseous state at 30 ppm for 2 h, TCE gave a positive response ($\alpha = 0.05$), but aldrin did not (Sandhu et al., 1989).

Armed with these data, Ma and co-workers (1992a) assessed the clastogenicity of chemical mixtures. All mixtures of TCE (a nonclastogen) and dieldrin (at a concentration below the MED) gave positive results ($\alpha = 0.05$), suggesting a synergistic relationship. Surprisingly, all mixtures of lead tetraacetate and arsenic trioxide (both potent clastogens) proved negative, suggesting an antagonistic relationship. This effect was believed to result from a neutralization of the acid (from acetate) and the base (from sodium hydroxide) that dissolved arsenic trioxide. The other combinations were generally slightly antagonistic, especially for the combined action of three agents. Some mixtures were toxic, preventing the normal development of tetrads. This complex and frequently unpredictable response induced by chemical mixtures led the authors to conclude that *in situ* evaluations are warranted when multiple compounds interact, such as normally happens in hazardous-waste sites.

Gill and Sandhu (1992) expanded on these findings by testing the same chemicals after soil incorporation using rooted *Tradescantia* plants. Most of the results agreed with those previous, but in some instances interactions within soil altered the expression of clastogenicity. For example, arsenic trioxide and lead tetraacetate did not induce increased micronuclei production in solution (as in Ma et al., 1992a), but did so in soil. In general, rooted plants showed higher micronuclei frequencies for the same chemical mixtures than cuttings treated in solution. A possible enhancement of metabolic activation of such mixtures in the root systems and/or by soil microorganisms was suggested as a likely reason. These results demonstrated once again that predicting the genotoxic effects of chemical mixtures from their components was not feasible and emphasized the importance of *in situ* assessments.

The significance of these conclusions was accentuated by the demonstration that tannic acids may act as synergistic compounds in the induction of clastogenicity in *Tradescantia* (Knasmuller et al., 1992). Exposure of *Tradescantia* for 24 h to increasing amounts of tannic acids caused a dramatic dose-dependent increase in the clastogenic effects of X-rays (35 rad), whereas tannic acids alone showed only moderate genotoxicity. Similar results were obtained with tannic acids in combination with other chemicals. This result may be important because tannic acids are present in many foods and beverages, as well as in natural waters and soils. Therefore, practically all chemicals released into the environment may interact with, and be potentiated by, tannic acids.

The value of the Trad-MCN assay as a tool in environmental assessment was emphasized once again in an evaluation of bioremediation measures at a hazardous waste site (Baud-Grasset et al., 1993a; Baud-Grasset et al., 1993b). Heavily creosote-contaminated soils (more than 5000 ppm polyaromatic hydrocarbons) were incubated with the lignin-degrading fungus *Phanerochaete chysosporium* Burdsall for 8 weeks. Aqueous extracts of soils before and after incubation were evaluated for clastogenicity with the Trad-MCN. Extracts of the original soils were highly clastogenic, with the lowest effective concentration being 0.25% for a 30-h exposure. *Phanerochaete* caused a decrease in soil contamination, doubling the concentration of extract needed to induce a micronuclei frequency similar to that before incubation (from 1 to 2%). The Trad-MCN assay again proved to be extraordinarily sensitive,

permitting the detection of differences between closely comparable samples.

The results obtained with *Tradescantia* in the assessment of soil contaminants are summarized in Table 3.

D. Pesticides and Health-Related Agents

Plants are the main biologic receptors of pesticides applied in the field. Thus, it is not surprising that a great deal of attention has been directed to the genotoxicity of pesticides in plants (Mohammad and Ma, 1983). An extensive review of the genetic toxicology of pesticides in higher plant systems (Sharma and Panneerselvan, 1990) listed a total of 178 active ingredients that have been tested in at least one of 31 different plant species, utilizing a variety of organs and genetic endpoints. Approximately 30% of these compounds were found to be genotoxic, whereas only 6% could be unequivocally considered to be free of such genetic hazards.

Tradescantia appeared only once in that review, indicating that this assay has not been among the preferred systems for pesticide evaluation of genotoxicity despite its sensitivity and amenability for field testing. The earliest references in which *Tradescantia* was employed to test pesticide genotoxicity involved the cytological effects of the insecticide mevinphos and the herbicide cyanazine (Ahmed and Grant, 1972b), and the mercury-containing seed treatment fungicide Panogen 15®(methylmercury dicyandiamide) (Ahmed and Grant, 1972a). In these cases, chromo-

TABLE 3
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Trad-MCN Assay of Soil Amendments and Contaminants

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Soil contaminants						
Sewage sludge	24 h	4-fold dilution	+			Hopke et al., 1982
Aldrin	30 h	2.0–36 ppm	–	$p < 0.05$		Sandhu et al., 1989
Tetrachloro ethylene	2 h	30 ppm	+	$p < 0.05$	Positive only when exposed in gaseous form	
Arseninc trioxide	30 h	3.96 ppm	+	$p < 0.05$	Diluted in NaOH	
1,2-benz[*a*,*h*] anthrnent	30 h	12.5 ppm	+	$p < 0.05$	Diluted in ethanol	
Dieldrin	30 h	3.81 ppm	+	$p < 0.05$	Diluted in DMSO	
Heptachlor	30 h	1.88 ppm	+	$p < 0.05$	Diluted in DMSO	
Lead tetra-acetate	30 h	0.44 ppm	+	$p < 0.05$	Diluted in DMSO	
Hazardous-waste site soil	30 h	0.5% aqueous extract	+	$p < 0.05$	Over 5000 ppm mixed PAHs	Baud-Grasset et al., 1993a; Baud-Grasset et al., 1993b
			+	$p < 0.05$		

somal aberration in root tip mitosis was tested. Ahmed and Grant (1972b) reported that mevinphos and cyanazine induced augmented aberration frequencies, but exposures were very high (200 to 600 ppm), considered to be too extreme in terms of environmental contamination. They found that concentrations of Panogen 15® as low as 10 ppm caused cytotoxicity, whereas clear genotoxicity was noticed with just 1 ppm (Ahmed and Grant, 1972a). These results might have been significant for human exposure in mixing and spraying operations.

Acknowledging the special merit of *in situ* studies of agricultural chemicals, Grant, (1982) opined that no organisms were as useful as *Tradescantia* or was any test as adequate as the Trad-MCN for the evaluation of the genetic hazards *in situ*. These assertions were soon tested in a study of the genotoxicity of the insecticide malathion used for pest control in a greenhouse (Ma et al., 1983a). In one treatment, intact potted plants were sprayed with malathion in the greenhouse, simulating conventional pest control treatment. Additional treatments involved absorption of malathion solutions through stems (with and without prior DMSO dissolution or treatment with S-9 microsome fraction of Arochlor™-induced mouse liver macerate) and exposure of intact plants to heat-generated malathion fumes in air-tight chambers. All exposures to malathion solutions, whether sprayed or absorbed through the stems, were negative. Exposure of cuttings to fumes of malathion resulted in a striking increase in micronuclei frequencies, suggesting that gaseous forms of some pesticides may be particularly effective in the Trad-MCN assay (Ma et al., 1983a).

The clastogenicity of Benlate® (benomyl) and thiophanate, two fungicide products used in fruit storage, were evaluated with the Trad-MCN assay at concentrations of 0.05 and 0.07%, respectively (Huang and Chen, 1993b; Huang and Chen, 1993c). Both agents induced high levels of micronuclei. Several pesticides were evaluated in a large scale study of the genotoxicity of health related agents (Ma et al., 1984) (see below). Among the positive results were dicamba, dichlorvos fumes, chlorpyrifos, gaseous malathion, *p*-dichlorobenzene, and Tordon® (picloram). Atrazine, 2,4-D, liquid malathion, and simazine were among the negative results. Eleven of the 18 pesticide agents tested in this study gave positive results. Of eight pesticides tested, both in the Trad-MCN and in the Ames test, only one (simazine) gave different results in the two tests.

Rodrigues (1995) assessed *in situ* the abatement of pesticide genotoxicity in an integrated pest management program for corn/soybean. All three pesticides applied — cyanazine, metolachlor, and chlorpyrifos — showed clastogenic activity both *in situ* and under laboratory conditions. Extracts of pesticide-sprayed soils were also positive.

Results obtained to date for the clastogenicity of pesticides in *Tradescantia* are summarized in Table 4.

Ma and co-workers (1984) presented the results of 140 Trad-MCN assays performed with a variety of chemical and physical agents. The agents were classified in nine categories (numbers in brackets indicate number of agents tested in the category): (1) known carcinogens/mutagens [15], (2) common beverages [8], (3) common chemicals [30], (4) common drugs [32], (5) pesticides (discussed above) [18], (6) common household chemicals [16], (7) ionizing radiation and radioisotopes [3], (8) *in situ* monitoring [13], and (9) complex environmental mixtures [8]. Some positive results within these groupings were (1) benzo-α-pyrene (50 m*M*), EMS (50 m*M*), and sodium azide (0.2 m*M*); (2) ethanol (5%), decaffeinated coffee (25%), and cola (50%); (3) formaldehyde fumes, nitrous oxides, and sulfur dioxide; (4) saccharin, aspirin; (5) some air-fresheners and cosmetics; (6) all ionizing radiation; (7) several polluted sites; and (8) several types of combustion exhausts and unconcentrated

TABLE 4
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Trad-MCN Assay of Selected Pesticides

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Pesticides						
Mevinphos	3–12 h	200–600 ppm	+	$p < 0.001$	Root tip mitosis	Ahmed and Grant, 1972a; Ahmed and Grant, 1972b
Cyanazine	3–12 h	200–600 ppm	+	$p < 0.001$	Root tip mitosis	
Panogen 15® (mercurial)	1–3 h	1–5 ppm	+	$p < 0.05$	Root tip mitosis	Ahmed and Grant, 1972a
Malathion	6 h	5.5–1650 ppm	–	$p < 0.05$	Stem absorption and spray application	Ma et al., 1983a
Malathion	6 h	0.25–0.65%	+	$p < 0.05$	Heat-generated fumes	
Malathion	6 h	4125 ppm	–	$p < 0.05$	Applied as liquid Negative in the Ames test	Ma et al., 1984
Malathion acetate	6 h	1.27%	+	$p < 0.05$	Applied as a gas	
Atrazine	6 h	200 ppm	–	$p < 0.05$	Positive in the Ames test	
Cyanazine	6 h	400 ppm	+	$p \cong 0.05$	Negative in some trials	
Simazine	6 h	200 ppm	–	$p < 0.05$	Negative in the Ames test	
Dicamba	6 h	200 ppm	+	$p < 0.05$	Toxic above 50 ppm (MED)	
Dichlorvos	6 h	0.5%	+	$p < 0.05$	Applied as gas; positive in the Ames test	
2,4-D	6 h	200 ppm	–	$p < 0.05$	Negative in the Ames test	
Chlorpyrifos	6 h	400 ppm	+	$p \cong 0.05$	Negative in some trials	
Picloram	6 h	200 ppm	+	$p < 0.05$	MED 200 ppm	
Maleic hydrazide	6 h	50 ppm	+	$p < 0.05$	Positive in the Ames test	
Cyanazine	30 h	10 ppm	+	$p < 0.05$	50-ppm solutions were toxic	Rodrigues, 1995
Metolachlor	30 h	50 ppm	+	$p < 0.05$		
Chlorpyrifos	30 h	50 ppm	+	$p < 0.05$	A combination of these last three compounds applied to soil produced positive results *in situ*	

TABLE 4 (continued)
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Trad-MCN Assay of Selected Pesticides

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Dichlorvos	1–6 h		+		Insecticide	Ma and Harris, 1987b
Benlate®		0.05%	+			Huang and Chen, 1993b Huang and Chen, 1993c
Thiophanate		0.07%	+			

contaminated waters. Among the negative results were some potent mutagens, for example, (1) 1,2-benzanthacene, methyl methanesulfonate (overdose occurred by exposure to 1 to 50 m*M*), and dinitrotoluene; and (4) mitomycin C (1 to 30 ppm). Of 39 agents tested with the Trad-MCN assay and for which results of Ames tests were available, 26 gave the same results in both tests, representing a congruity of 67% (Ma et al., 1984). *Tradescantia* has been employed also in the evaluation of genotoxicity of medicines (Chen and Guan, 1988; Zhang et al., 1994), and various chemicals of environmental concern (Helma et al., 1995; Ma, 1993).

The results available to date with *Tradescantia* in the evaluation of the clastogenic properties of selected chemical agents and physiological stresses are presented in Table 5.

E. Radiation, Cosmic Rays, and Radiowaves

T. palludosa was used to study the potential effects of factors associated with space flight, such as acceleration, vibration, weightlessness, and ionizing radiation (Delone et al., 1986). Inflorescences were fixed chemically at several times from take-off to post-landing, and the mitotic figures of the microspores were analyzed for aberrations. One very special aberration was observed in this material, especially for microspores exposed at early prophase. It consisted of complex nonreciprocal translocations involving spherical fragments. The appearance of such rearrangements was not associated with the duration of flight or with take-off or landing. It was speculated that the causative agent of these aberrations was a heavy bombardment by cosmic radiation (Delone et al., 1986). In another study (Marimuthu et al., 1972), the effects of space flight on the development of the female gametophytes of *Tradescantia* clone 02 were evaluated. Misorientation of the nuclei suggested that malfunction of the spindles could be associated with exposure to free flight.

The sensitivity of *Tradescantia* to radiation has been demonstrated for X-rays (Ma et al., 1982c), external and internal radioisotope sources (Anderson and Ma, 1981; Anderson and Ma, 1982; Kirby-Smith and Daniels, 1953), and cosmic rays. Likewise, long-wave radio frequencies and short-wave electromagnetic fields occurring in the vicinity of broadcasting antennae have been shown to be harmful

TABLE 5
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Effects of Physiological Stresses and Selected Chemical Agents on the Trad-MCN Assay

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Selected chemicals						
EMS	24 h	50–100 m*M*	+		Aqueous solution absorbed through the stems	Ma, 1979a
9 chemical categories					140 chemicals assayed, 52 were positive, 20 were borderline, and 5 were toxic	Ma et al., 1984
Physiological stresses						
Anaerobiosis	12–48 h	Max. 2%	+		Increase chromosome breakage, including micronuclei in microspores	Steinitz, 1944
Magnesium deficiency	Continuous	<1 ppm	+	$p < 0.001$	Abnormal chromosome replication and micronuclei at meiosis	Steffensen, 1953
Sulfate deficiency	Continuous	4.0 ppm	+	$p < 0.001$	Same as above	Steffensen, 1954
Calcium deficiency	Continuous	2.5 ppm	+	$p < 0.001$	Same as above	Steffensen, 1955

to replicating chromosomes. In a series of experiments *in situ* (Haider et al., 1994), *Tradescantia* cuttings were exposed at five distances from the antennae, in Faraday (electromagnetic shielding) and plastic (nonshielding) cages distributed around sites that exceeded the International Radiation Protection Association standards for electric field strength. All treatments resulted in high micronuclei frequencies when compared with laboratory controls ($\alpha = 0.05$) and, more importantly, comparison between unshielded and Faraday cage-shielded groups showed highly significant differences ($\alpha = 0.004$) (Haider et al., 1994). This result is particularly interesting, because both groups were exposed to exactly the same environmental conditions except for the influence of electromagnetic radiation. A dose-response relationship over distance supports the results that the effects observed were due to the electromagnetic fields.

The clastogenic effects of X-rays and other ionizing radiation on *Tradescantia* are summarized in Table 6.

II. *TRADESCANTIA* STAMEN HAIR ASSAY

The stamen-hair mutation assay (Trad-SHM) is a point mutation (mitotic) assay in which expression of the heterozygous dominant blue character of the stamen hair cells is prevented, resulting in the appearance of the

TABLE 6
Summary of Environmental Genotoxicity Results Using Chromosome Breakage in *Tradescantia*, with Special Reference to the Trad-MCN Assay of X-rays and Other Radiation

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Radiation						
X-rays	8 min	75–200 rad	+		Chromosome breaks, mostly at mitosis	Sax, 1938
X-rays	~5 min	77–416 rad	+	$p < 0.05$	Chromatid aberrations in pollen tubes and microspores, and significance measured for coefficients in exponential fit of data	Kirby-Smith and Daniels, 1953
^{60}Co γ-rays	30 min	100–400 rad	+	$p < 0.01$		
^{32}P β-rays	20 min	100–400 rep	+	$p < 0.01$		
^{60}Co γ-rays	16 d	0.41 rad	+	$p < 0.05$	Micronuclei in microspores	Sparrow and Singleton, 1953
X-rays on 5-FUdR treated cells	36 h 5-FUdR + 2 min X-rays	100 rad + 10^{-6} *M* 5-FUdR	+	$p < 0.05$	Mitotic delay resulting in reduced number of exchanges and chromatid breaks	Rushton, 1969
X-rays	Seconds	20–40 rad	+		Positive dose response relationship in MCN	Ma, 1979a
X-rays	Seconds	10–58 rad	+		Correlation coefficient for dose response = 0.995	Ma et al., 1980
X-rays plus tannic acids	12 h	35 rad X-rays	+		Synergistic interaction, positive dose-response relationship	Krasmuller et al., 1992
Space flight	*In situ* exposure in space satellites		+		Several developmental effects observed in the embryo sacs	Marimuthu et al., 1972
Cosmic rays	*In situ* exposure in space satellites		+		Unusual chromosome aberrations, i.e., nonreciprocal translocations and spherical fragments	Delone et al., 1986

recessive pink color (Emmerling-Thompson and Nawrocky, 1982; Mericle and Mericle, 1967; Mericle and Mericle, 1971; Nayar and Sparrow, 1967). Early studies with this system centered on the assessment of the genotoxic and cytotoxic effects of ionizing radiation and employed the meristematic cells of the stamen hairs of *Tradescantia* clone 02 as

a higher organism surrogate for microbial cultures. In this assay, full growth of the hair was considered as equivalent to colony formation and stunted hairs as equivalent to nonsurvivors in cell cultures due to severe, highly deleterious or lethal events. In addition to mutation (color change) being used as an endpoint, genotoxic changes such as the expression of giant, twin, or triplet cells, branching of the hair and other growth anomalies were recorded along with loss of reproductive integrity as indicators of genotoxicity (Nayar and Sparrow, 1967).

The genetic basis for the expression of pink cells in the stamen hairs of *Tradescantia* clone 4430 was established by means of reciprocal test-crosses with the parental pink- and white-colored *T. subacaulis* Bush (Emmerling-Thompson and Nawrocky, 1980). Pink pigmentation was determined to depend on a pair of alleles at a single locus, with blue (B) being dominant to pink (b); and clone 4430 was shown to be homozygous dominant for the white locus. The identity of both blue and pink pigments of four different clones of *Tradescantia* have been determined microspectrophotometrically (Sanda-Kamigawara and Ichikawa, 1993).

Pink mutation as well as loss of reproductive integrity in the stamen hairs of several species and hybrids of *Tradescantia* (Ichikawa and Sparrow, 1967a; Ichikawa and Sparrow, 1967b; Ichikawa and Sparrow, 1968; Ichikawa and Sparrow, 1969; Ichikawa et al., 1969; Sparrow and Ichikawa, 1967) became important endpoints in the study of the genotoxic effects of radiation (Alvarez and Sparrow, 1965; Kappas et al., 1972; Nauman et al., 1976; Nauman et al., 1974; Sparrow et al., 1973; Underbrink et al., 1973a; Underbrink et al., 1971).

Sparrow and co-workers (1972) studied the effects of neutrons and X-rays in the Trad-SHM assay (clone 02), defining a linear dose-effect relation for both agents and a doubling dose as low as 1 rad for X-rays. The spontaneous mutation frequencies of several species and hybrids of *Tradescantia* were determined based on many years of experimentation at Brookhaven National Laboratory (Sparrow and Sparrow, 1976). Hybrids (i.e., clone 4430) and putative hybrids (i.e., clone 02) showed lower frequencies and a narrower variability in spontaneous mutation when compared with clones of pure species and were considered more suitable subjects for experimentation.

The effects of background radioactivity were studied during orbital flight (Sparrow et al., 1968) and by cultivating *Tradescantia* on monazite sand (Nayar et al., 1970). Mutation was found to increase in all exposed samples, and radionuclides absorbed into the plants were much more effective than external radiation alone. These results were later confirmed by exposing plants to soil samples drawn from the nuclear bomb experimental site at Bikini Island (Ichikawa and Ishii, 1991). Soil samples that caused significant increases in mutation frequency were shown to contain ^{137}Cs and ^{60}Co, among other radionuclides. Other studies involving the absorption of radionuclides include tritiated chemicals (Nauman et al., 1979; Tano et al., 1984) and ^{131}I (Tano and Yamaguchi, 1979).

The Trad-SHM assay was used *in situ* as a monitor for ionizing radiation in the vicinity of nuclear power plants in a large study carried out in Japan (Ichikawa, 1981). Significantly increased mutation frequencies were correlated with wind direction and operation periods of the nuclear facilities. Cebulska-Wasilewska (1992) observed an increase in the spontaneous mutation frequencies of *Tradescantia* correlated with the contamination caused in Cracow by the blowout of the nuclear reactor in Chernobyl (a 700-km distance). Similar increase in somatic mutation frequencies were also recorded in May to June 1986 in Japan (more than 8000 km distance) (Ichikawa et al., 1996). In this case, variations in spontaneous mutation rates in a

10-year period (1982 10 1992) could always be correlated to temperature factors, but the significantly high mutation frequency in this particular period of 1986 could not. Exposure to radiation also enabled the standardization of the Trad-SHM assay as related to temperature variations (Nauman et al., 1977a; Nauman et al., 1977b), dose (Nauman et al., 1977c; Nauman et al., 1975), and other variables of the experimental conditions (Underbrink and Sparrow, 1974; Underbrink et al., 1975a; Underbrink et al., 1975b).

The applicability of the Trad-SHM assay to chemical mutagenesis studies was proposed by Underbrink et al. (1973b) and tested in a comparison of the effects of ionizing radiation and gaseous EMS and DBE (Nauman et al., 1976). The responses to chemical agents showed characteristics similar to X-rays (exponential rise followed by saturation in mutant cell frequencies), and clone 4430 was more sensitive than clone 02. These results were later confirmed with a variety of chemicals and radionuclides (Tano, 1987; Tano, 1990; Tano and Yamaguchi, 1985). In these studies, mutagens were applied topically, directly onto the inflorescences. Doses as low as 5 to 20 pg for *N*-nitroso-*N*-methylurea and *N*-nitroso-*N*-ethylurea, and 100 pg for EMS were effective and detectable in the Trad-SHM test. The detection limit for external radiation was below 1 rad.

This high sensitivity of the Trad-SHM assay to chemical mutagens was first shown after the accidental exposure of plants (clone 02) to fumes entering the air supply of a building at Brookhaven National Laboratory. A sudden increase in spontaneous mutation frequency raised the suspicion that led to the discovery of the contamination (Sparrow and Schairer, 1971). Additional studies with the Trad-SHM assay evaluating the mutagenesis of chemical agents involve maleic hydrazide (Gichner et al., 1982b), MMS, EMS, DMS (dimethyl sulfate) (Ichikawa et al., 1990; Ichikawa and Takahashi, 1978; Sanda-Kamigawara et al., 1991), *N*-nitroso compounds, and several organic solvents, among other agents, as well as evaluations of synergistic action between chemicals and between chemicals and radiation (Badaev et al., 1989; Gichner et al., 1994; Gichner et al., 1982a; Gichner et al., 1988; Ichikawa, 1992; Ichikawa et al., 1990; Ichikawa et al., 1993; Kuglik et al., 1994; Sanda-Kamigawara et al., 1991; Shima and Ichikawa, 1994; Shima and Ichikawa, 1995a; Shima and Ichikawa, 1995b; Veleminsky et al., 1987; Villalobos-Pietrini et al., 1986).

The Trad-SHM has been shown to be capable of activating promutagens into direct-acting mutagens (Gichner et al., 1980). Benzo-α-pyrene, atrazine, and several *N*-nitroso compounds were mutagenic when tested without prior treatment with microsomal fractions (Veleminsky and Gichner, 1988). Xiao and Ichikawa (1995, 1996) reported activation of maleic hydrazide (MH) into a mutagen by peroxidase, and showed that MH could act synergistically (Cebulska-Wasilewska et al., 1981) and antagonistically with X-rays when X-rays were delivered before and after MH treatments, respectively. X-rays suppressed the activation of MH in the latter case. A review of the mutagenicity of ionizing radiation and chemical agents in the Trad-SHM assay was presented by Ichikawa (1992).

Perhaps the most important contribution of the Trad-SHM assay was in the series of studies on atmospheric pollution carried out with a mobile laboratory (Schairer, 1979; Schairer and Sautkulis, 1982; Schairer et al., 1982; Schairer et al., 1979; Schairer et al., 1983). Air drawn from polluted sites around the U.S. induced higher mutation frequencies than filtered air samples from the same locations or air samples from the control site in the Grand Canyon. The mutagenicity of polluted atmospheres (Sparrow and Schairer, 1974) has been detected also in the vicinity of an oil refinery and petrochemical complex (Lower et al., 1983a), a lead smelter (Lower et al., 1978; Lower et al., 1983b), a pharma-

ceutical factory (Cebulska-Wasilewska and Guminska, 1987), and a municipal waste incinerator (Ma, 1994b; Ma et al., 1993b; Ma et al., 1996).

The mutagenic effects of chemical smokes used by the U.S. Army were evaluated with the Trad-SHM assay. Positive responses were found for fogoil and tank diesel, as well as for their combination (Schaeffer et al., 1987). Ozone at concentrations occasionally found in polluted areas (300 to 800 ppb) was not mutagenic in the Trad-SHM assay (Gichner et al., 1992; Rodrigues et al., 1996), even though it had been reported positive at higher concentrations (Schairer, 1979). A review of the Trad-SHM as an assay for gaseous mutagens was presented under the U.S. EPA Gene-Tox Program (Van't Hof and Schairer, 1982).

In addition to studies of gaseous mutagens, the Trad-SHM assay has been used to assess the mutagenicity of aquatic environments (Lower et al., 1985; Tano, 1989). Episodes of mutagenicity in the water of a reservoir in Missouri were shown to be correlated with events facilitating the transfer of mutagens from the contaminated sediment to the water column (Lower et al., 1985). Grant et al. (1992) evaluated *in situ* the genotoxicity of the water in an area of Lake Superior in the vicinity of a pulp and paper mill, using the Trad-SHM, the Trad-MCN (micronuclei) and the *V. faba* chromosome aberration assays. Even though the Trad-SHM was sensitive enough to detect mutagenic effects, it was inferior to the other assays in terms of amenability for field manipulation. Remotely located field sites cause difficulties in cultivating the plants for the long (14 d) recovery period required in this test.

There is a scarcity of information on the mutagenic effects of pesticides in the Trad-SHM assay (Mohammad and Ma, 1983). Tomkins and Grant (1972) studied the mutagenic effects of menazon (an *s*-triazine aphicide), metobromuron (substituted urea herbicide), and Daconil 2787® (chlorothalonil, chlorinated aromatic hydrocarbon fungicide) by wrapping the inflorescences of *Tradescantia* with cotton soaked in pesticide solutions (1500 ppm). No positive responses were found. The mutagenicity of the herbicide and growth regulator maleic hydrazide has been demonstrated (Gichner et al., 1982b; Xiao and Ichikawa, 1995; Xiao and Ichikawa, 1996). The benzimidazole-derived fungicide Benlate® (benomyl) was tested for mutagenicity with the Trad-SHM assay (clone KU 20) at doses used commonly in agriculture (0.5 to 4.0 g/l) (Sakamoto and Takahashi, 1981). Again, no positive responses were recorded. Contrasting with these negative results, seven out of nine insecticides were positive when tested with the Trad-SHM assay using clone 4430 (Huang and Chen, 1993a). Dichlorvos (0.1%), omethoate (0.04%), methamidophos (9.05%), Meobal® (3,4-xylyl methylcarbamate) (0.05%), mevinphos (0.006%), Amobem® (chloramben, actually an herbicide) (0.045%) and thiophanate-methyl (0.07%) gave positive results, whereas trichlorfon (0.1%) and Bassa® (2-*sec*-butylphenyl methylcarbamate) (0.02% toxic) did not. Atrazine was reported mutagenic after chronic exposure (Schairer and Sautkulis, 1982), and cyanazine also has been reported to be mutagenic in the Trad-SHM system (Veleminsky and Gichner, 1988).

A summary of the results obtained with the Trad-SHM in the assessment of environmental mutagenesis is presented in Table 7.

III. CONCLUSION

A wealth of basic genetic and developmental information available on *Tradescantia* provides a solid framework in support of its use as a biomonitor in environmental genotoxicity assays (Ma and Grant, 1982). Micronuclei in the pollen mother cells are easily recognizable, permitting a low degree of uncertainty in scoring, and diminishing the subjectivity in the recognition of chromo-

TABLE 7
Summary of Environmental Mutagenesis Results Obtained with the Stamen Hair Mutation Assay in *Tradescantia* (Trad-SHM)

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Air pollution						
In situ *monitoring*						
Several polluted sites throughout the U.S.	10 d		+	$p < 0.05$	Highest mutation rates associated with petroleum processing	Schairer, 1979; Schairer and Sautkulis, 1982; Schairer et al., 1982
Lead smelter	Chronic exposure	0.3–11 km from source	+/–	$p < 0.001$	No correlation occurred between mutation rate and distance from the smelter	Lower et al., 1978; Lower et al., 1983b
Oil refinery	Chronic exposure	100–500 m from source	+	$p < 0.001$	All tests were statistically significant for one location in Texas when compared with the green-house control	Lower et al., 1983a
Landfill gaseous emissions	4–6 h		+		Positive responses in 7 of 13 monitoring trips; gases burned at emission source	Ma et al., 1993a
Municipal incinerator	4–6 h		+		Positive results obtained with stagnant atmosphere	Ma et al., 1993b
Chemical smokes	30 min	15–100 m from source	–	$p > 0.9$		Schaeffer et al., 1987
Selected gases in chambers						
NO	6 h	250 ppm	+	$p < 0.01$		Schairer and Sautkulis, 1982; Van't Hof and Schairer, 1982
NO_2	6 h	50 ppm	+	$p < 0.05$		
SO_2	6 h	40 ppm	+	$p < 0.01$		
DBE	6 h	1 ppm	+	$p < 0.01$		
EMS	6 h	5 ppm	+	$p < 0.01$		
Vinyl chloride	6 h	75 ppm	+	$p < 0.02$		
O_3	6 h	5 ppm	+	$p < 0.02$		
O_3	11 h/d	800 ppb	–	$p = 0.68$		Gichner et al., 1992

TABLE 7 (continued)
Summary of Environmental Mutagenesis Results Obtained with the Stamen Hair Mutation Assay in *Tradescantia* (Trad-SHM)

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
O_3	6 h/d	100 ppb	–	$p > 0.05$	Three days cumulative exposure	Rodrigues et al., 1992
Water pollution						
In situ *monitoring*						
Lake water	24 h		–	$p < 0.05$	Lake polluted by pulp and paper mill effluent Results were equivocal	Grant et al., 1992
Bottom sediment	Chronic exposure		+	$p < 0.005$	Mutation rates were high in all 91-d experimental periods	Lower et al., 1985
Selected chemicals in solution						
Benzo-α-pyrene	24 h	3.9×10^{-5} *M*	+	$p < 0.01$		Schairer and Sautkulis, 1982; Van't Hof and Schairer, 1982
Caffeine	Chronic	10^{-3} *M*	–	$p > 0.05$		
NaN_3	3 h	10^{-3}	+	$p < 0.01$		
Benzidine	24 h	5.4×10^{-7} *M*	+	$p < 0.01$		
EMS	6 h	10 ppm	+		Positive dose-response relationship from 10 to 500 ppm	Nauman et al., 1976
DBE	6 h	10 ppm			Same as above	
EMS	Acute	100 ng	+	$p > 0.9$	Chemical solutions applied directly onto the inflorescence	Ichikawa et al., 1990; Ichikawa and Takahashi, 1978; Sanda-Kamigawara et al., 1991; Tano, 1987; Tano, 1990; Tano and Yamaguchi, 1985
N-nitroso compounds	Acute	100 ng	+			Gichner et al., 1982a

TABLE 7 (continued)
Summary of Environmental Mutagenesis Results Obtained with the Stamen Hair Mutation Assay in *Tradescantia* (Trad-SHM)

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Maleic hydrazide	Acute		+		Synergism occurred with X-rays delivered before MH treatment; and suppression of MH effects by X-rays applied after MH treatment	Xiao and Ichikawa, 1995; Xiao and Ichikawa, 1996
N-nitroso compounds	24 h	Several	+			Gichner et al., 1982a
Pesticides						
Atrazine	Chronic	0.045 mg		$p < 0.01$	Dose applied per pot	Van't Hof and Schairer, 1982
Menazon	24 h	1500 ppm	–	$p > 0.05$	Inflorescence wrapped with pesticide-soaked cotton	Tomkins and Grant, 1972
Metobromuron		1500 ppm	–			
Tetrachloro isophthalonitrile		1500 ppm	–			
Benomyl	2 h	4 g/l	–	$p > 0.05$	Dose is equivalent to recommended use	Sakamoto and Takahashi, 1981
Nine insecticides		Several	+/–		Positive responses in seven of nine compounds	Huang and Chen, 1993a
Radiation						
^{60}Co γ-rays	16 h	500 R	+		Mutation frequencies decreased above 300 R	Nayar and Sparrow, 1967
Monazite sand	Chronic	1.3 mR/h	+	$p < 0.05$		Nayer et al., 1970
X-rays	mins	160 R	+		Positive dose-response relationships with doses from 10 to 160 rad	Nauman et al., 1976
X-rays	mins	160 R	+	$p < 0.01$	Positive dose-response relationships with doses from 0.25 to 5 rad	Sparrow et al., 1972
Neutrons	mins	10 R	+	$p < 0.01$	Positive dose-response relationships with doses 0.01 to 8 rad	

TABLE 7 (continued)
Summary of Environmental Mutagenesis Results Obtained with the Stamen Hair Mutation Assay in *Tradescantia* (Trad-SHM)

	Dose range			Result		
Agent	Exposure time—max	Concentration	+/–	Statistical significance	Remarks	Ref.
Internal radiation	Acute	100 nCi	+		Tritium-labeled compounds and ^{131}I; positive dose-response relationships occurred	Tano, 1987
In situ exposure to nuclear power plants	Chronic		+	$p < 0.05$	Significant increases in mutation rates were correlated with operation plants and wind direction	Ichikawa, 1981
Bikini Island soils	76 d	150 μR/h	+	$p < 0.01$	Main radionuclides were ^{137}Cs and ^{60}Co	Ichikawa, and Ishii, 1991
In situ exposure to contaminated atmosphere	Chronic		+	$p < 0.05$	Shortly after the Chernobyl accident; plants exposed in Cracow, 700 km from the radiation source	Cebulska-Wasilewska, 1992
In situ exposure to contaminated atmosphere	Chronic		+	$p < 0.05$	Shortly after the Chernobyl accident; plants exposed in Japan, 8000 km from the radiation source	Ichikawa, et al., 1996

somal breakage, while the induction of pink mutations in the stamen hairs provide a sensitive somatic indicator of mutagenesis. Additionally, the *Tradescantia* assays have proven to be suitable for studies on synergism between chemicals and between chemicals and other genotoxic agents such as radiation, a valuable property for the assessment of genotoxic risks in complex environmental situations (Shima and Ichikawa, 1995b).

Grant (1994) assessed the present status of higher plant bioassays for the detection of environmental mutagens, stressing the advantages of plant systems with regard to the possibility of performing *in situ* evaluations. In a recent study sponsored by the International Programme on Chemical Safety the utility of the two *Tradescantia* bioassays discussed here (along with three other plant bioassays) was evaluated with four known genotoxic chemicals (Grant and Salamone, 1994) in five different laboratories (Sandhu et al., 1994a; Sandhu et al., 1994b). The results obtained in this study substantiated the Trad-SHM assay as a reliable system for screening chemicals for their mutagenicity (Ma et al., 1994a). Regarding the Trad-MCN assay, even though the results for the four

chemicals tested were not identical, there was good agreement among all laboratories, suggesting that the Trad-MCN assay is a reliable short-term bioassay for clastogens (Ma et al., 1994b), and as has been reviewed in the present literature survey it is specially appropriate for the *in situ* monitoring of genotoxic chemicals.

In conclusion, the studies reviewed here demonstrate that *Tradescantia* plants, particularly the Trad-MCN and Trad-SHM assays, provide a very sensitive, easily manipulated system for the study of genotoxicity, especially under the *in situ* conditions indispensable for environmental studies.

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