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Pollen Grain Bioassay for Environmental Pollution Screening

By

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Summary

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Pollen grain assays in *Vicia faba* and naturally growing plants indicated increased genotoxic effects in habitats with elevated radiation levels. However, severe cytological damage in test plants could not be attributed solely to in situ genotoxic effect of prolonged irradiation, but to ageing of plants and complex clastogenic effects in the environment, including harsh weather conditions. Pollen grain assay in different plant species indicated that perennials could be used as biological indicators of long-term genotoxic exposure.

Introduction

Plants have been used for the detection of genotoxic agents in the environment for decades. In short-term testing procedure, traditionally the *Tradescantia* micronucleus assay (GILL & SANDHU 1992), *Tradescantia* stamen-hair mutation assay (GICHNER & al. 1994a), *Arabidopsis* assay (GICHNER & al. 1994b), *Vicia faba* root tip assay (GRANT & SALAMONE 1994), *Allium* test (SMAKA-KINCL & al. 1996), and Tobacco mutagenicity assay (GICHNER & PLEWA 1998) are utilised. Test plants have been used for the detection of mutagens in industrial areas (RUIZ & al. 1992, GRANT & al. 1992) as well as for the mutagenicity screening of radioactive contamination after the Chernobyl accident (CEBULSKA-WASILEWSKA 1992). But recently the relevant strategy lies in the use

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of cytogenetic methods in natural vegetation (TOMKINS & GRANT 1976, SHEVCHENKO & GRINIKH 1990, MIČIETA & MURIN 1996, JABEEN & ABRAHAM 1996, BESSONOVA & al. 1996, KORDYUM & SIDORENKO 1997) for the in situ evaluation from environmental pollution.

We performed pollen grain bioassay in surrounding of the former uranium mine and yellow cake production plant at Žirovski Vrh as well as at the Krško Nuclear Power Plant (NPP). *Vicia faba* as test plants was used for the determination of short-term radiation effects as well as naturally growing plants as biomonitors of long-term exposure effects to assess in situ genotoxic hazards.

Material and Methods

In the surroundings of Žirovski Vrh, three experimental sites were chosen, where radionuclide levels were constantly measured (Table 1). Site I was on the slope of the hill called Boršt in a drainage ditch about 20 m down the dry-tailings pile. Site II was beyond the valley, opposite to site I and supposedly contaminated by radioactive particles from the Boršt deposit. Site III was close to the former yellow-cake production plant in Todraž. The Botanical Garden of the University of Ljubljana was the control location with no anthropogenic radiation sources.

Routine discharges from the Krško NPP are known to contribute only local environmental radioactivity, and according to measured activities in the environment, the radiological impact is of minor significance (BREZNIK 1996, MIKLAVŽIČ & al. 1996). Inside the Krško NPP area, one experimental site was chosen; the one was outside the NPP area. The habitats of wild experimental plants were about 1 km SE from the NPP.

Table 1. Radionuclides (Bq/kg dry sample) in soil and absorbed dose rate in air (μGy/h) at experimental sites in the area of the Žirovski Vrh.

	U-238	Ra-226	Th-232	K-40	Cs-137	Dose rate
Site I	1770	1900	41	480	12	0.35
Site II	51	55	62	670	367	0.13
Site III	5600	840	65	560	22	0.18

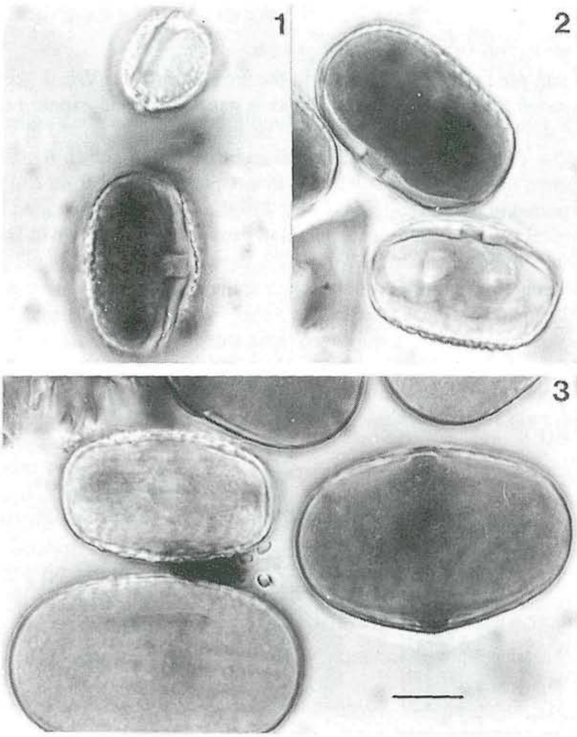
Ten soaked seeds of *Vicia faba* were germinated in soil taken from each experimental site as well as in the normal garden soil. When about 20 cm high, potted seedlings were transferred to experimental sites. At each site, two experimental series of plants, those potted in the soil from the site and those in garden soil, were exposed to naturally occuring conditions. Additionally, all were grown in the Botanical Garden. After 3, 6, and 8 weeks of exposure, the flower buds were sampled.

From naturally growing plants, flower buds were taken from *Berberis communis, Brassica oleracea, Cornus sanguinea, Crataegus monogyna, Plantago lanceolata, Plantago media, Polygonatum multiflorum, Ranunculus acris, Ranunculus lanuginosus, Sambucus nigra,* and *Symphytum officinale.* For the controls, flower buds from the same plant species were sampled on sites with no anthropogenic radiation sources.

The flower buds were fixed in 3:1 ethanol-acetic acid, then kept at -20°C. The slides were prepared by squashing anthers in aceto-carmine. In fully developed anthers, aberrant shapes and sizes of pollen grains as well as empty pollen grains were scored (Figs. 1-3). In each sample, 500 pollen grains were observed.

Results and Discussion

Pollen deformation in *Vicia faba* (Figs. 4 and 5) grown in soil from experimental sites was more marked than in plants grown in the garden soil. In the botanical garden, the frequency of pollen deformation in plants grown in the soil from experimental sites was higher than in those grown in the garden soil. With prolonged exposure, the degree of pollen deformation increased in all plants at experimental sites as well as in the botanical garden. An extremely high degree of pollen deformation was predominantly due to numerous sterile flower buds with anthers with no pollen grains.



Figs. 1-3. Deformation of pollen grains in *Vicia faba*. Bar represents 10 micrometers.

Vicia faba pollen grain assay results indicated the impact of radionuclides from soil as well as their possible transfer and accumulation in plant organs, but the influence of environmental conditions on the results could not be excluded.

The average frequency of pollen deformation was 33.7% in *Plantago lanceolata,* 22.9% in *Ranunculus lanuginosus,* and 16.8% in *Crataegus monogyna*; however, similarly high frequencies in the control plants were observed as well (Fig. 6). Variability of the results in all experimental plants was obvious; the degree of variation likely reflect instability of cytomorphological features and physiological properties in irradiated natural plant populations (POZOLTINA 1996).

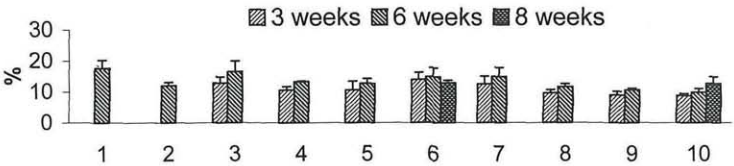


Fig. 4. *Vicia faba* pollen grain assay in the area of Žirovski Vrh. 1 – Plants potted in soil from site I and exposed to site I. 2 – Plants potted in garden soil and exposed to site I. 3 – Plants potted in soil from site I and grown in Botanical Garden. 4 – Plants potted in soil from site II and exposed to site II. 5 – Plants potted in garden soil and exposed to site II. 6 – Plants potted in soil from site II and grown in Botanical Garden. 7 – Plants potted in soil from site III and exposed to site III. 8 – Plants potted in garden soil and exposed to site III. 9 – Plants potted in soil from site III and grown in Botanical Garden. 10 – Plants potted in garden soil and grown in Botanical Garden.

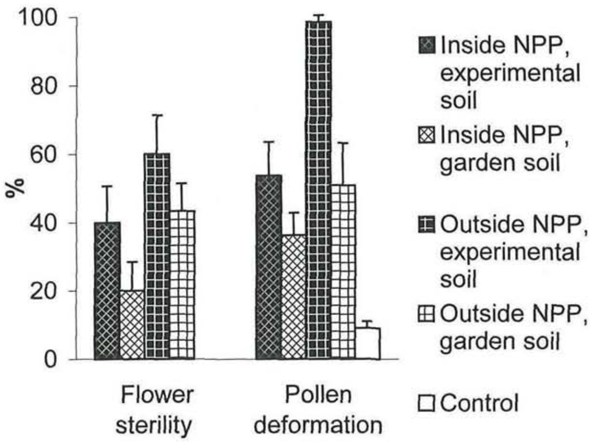


Fig. 5. *Vicia faba* pollen grain assay in the area of the Krško NPP.

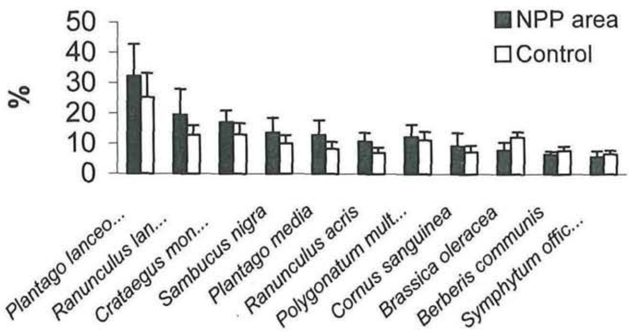


Fig. 6. Pollen grain assay in naturally grown plants close to the Krško NPP.

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