ACTA BIOLOGICA CRACOVIENSIA Series Botanica 42/2: 125–133, 2000

REPRODUCTION OF *VICIA CRACCA* L. IN THE POLLUTED ENVIRONMENT OF THE LEGNICA-GŁOGÓW COPPER BASIN (POLAND)

ROMANA IZMAIŁOW*

Department of Plant Cytology and Embryology, Jagiellonian University, ul. Grodzka 52, 31-044 Cracow, Poland

Received June 12, 2000; revision accepted August 29, 2000

The effects of stress conditions in a polluted environment on the reproductive processes of plants were studied for two successive years. *Vicia cracca* is a component of the spontaneous green belt at the base of a copper processing post-flotation waste reservoir (Żelazny Most, Legnica-Głogów Copper Basin, Silesia, Poland). Plants from the site were compared with plants derived from seeds harvested from the contaminated site which grew in non-polluted soil on an experimental plot near Cracow. Almost all of the studied flowers showed degenerative tapetal processes in ~50% of the anthers, starting simultaneously in various stages of meiotic divisions in the pollen mother cells. At the tetrad stage nearly 50% of the anthers contained wholly degenerated tetrads. Disturbances in meiosis increased the amount of degenerated pollen grains. The proportion of potentially functional pollen grains was 47% in 1997 and 56% in 1998; in the control material the corresponding proportions were 87% and 84% (1998 and 1999). Various kinds of developmental disturbances and degenerative processes eliminated some of the ovules from the seed production. The percentages of ovules forming seeds was 56% and 59% in successive years (85% and 87% in the control). Most of the disturbances and degeneration can be attributed to the combined negative impacts of specific environmental factors.

Key words: *Vicia cracca*, polluted environment, stress conditions, reproduction in stress conditions, tapetum, pollen, seed/ovule ratio.

INTRODUCTION

Pollution of the biosphere resulting from mining and metallurgy poses serious environmental and human problems. Industrial activity means gas and dust emissions and the production of millions of tons of wastes, a source of toxic metals and substances which penetrate and accumulate in soils and groundwater. The wastes, largely uncolonized by plants, become dangerous to large adjacent areas due to wind erosion of their components. In laboratory studies, heavy metals which can be present in the substratum, particularly cadmium (Cd), mercury (Hg), lead (Pb), copper (Cu), zinc (Zn), molybdenum (Mo), manganese (Mn), iron (Fe), nickel (Ni) and cobalt (Co), have been proven to be potentially toxic to plants when applied in higher concentrations.

Failure to colonize wastes has been attributed to heavy metals content in soil, and air pollution. Other field conditions such as low fertility, low available soil moisture, and unfavorable soil texture and pH, also determine a plant's ability to inhabit such sites (Baker, 1987; Tomsett and Thurman, 1988; Antosiewicz, 1992). Some factors affect each other; for example, changes in pH alter the mobility of nutrients and toxins (Kirk and Bajita, 1995; Saleque and Kirk, 1995).

In Poland the areas most contaminated by mining and smelting are located in the Black Triangle, an area shared with Germany and the Czech Republic which is one of the most polluted regions in Europe. The present study was conducted in the close vicinity of Żelazny Most near Rudna in the Legnica-Głogów Copper Basin. The reservoir covers

* e-mail: izmailow@grodzki.phils.uj.edu.pl

PL ISSN 0001-5296

an area of 1410 hectares; it is the largest one in Europe (285 million cubic meters). Since 1977 it has been loaded with post-flotation wastes from copper ore processing. The pollution factors impacting the area are as follows:

- high wind erosion of the reservoir edge and slopes, which are up to 50 m high (some parts of slopes are poorly colonized by vegetation);
- infiltration of water from the reservoir, which fails Polish water quality standards because of the content of some components.

The soils east of Żelazny Most show increased content of metals (Pb, Cu, Cd, Co, Hg) and metalloid arsenic (As) (Krajewski, 1993; Kijewski, 1998). Water infiltrating from the reservoir contains the ions Cl^-, SO_4^{2-}, Na^+, Ca^{2+} and increased content of heavy metals (Cu, Pb, Mn) as well as flotation substances (detergents, xanthates, ether extract, phenols and cyanides (Czaban and Maślanka, 1998). The upper, biologically active soil layer has a pH of 5.5–6.0, resulting in increased availability of metal ions to plant roots when heavy metals are present.

Confronted with polluted and contaminated environments, populations or individuals of some plant taxa present non-tolerant genotypes, whereas others become tolerant to different extents. Plants from the latter group are able to colonize such sites.

This study addressed whether or not the environment of the Żelazny Most area sufficiently favors embryological and reproductive processes in plants. *V. cracca* was chosen as a taxon represented by individuals at the base of the reservoir.

MATERIALS AND METHODS

Flowers and pods of various ages were collected at random from specimens of *V. cracca* growing at the east end of the Żelazny Most reservoir. The soils of this area contained elevated levels of three elements: Cu (up to 112.5 mg/kg; Polish standards permit up to 50 mg/kg Cu content in light soils); Pb (up to 60.5 mg/kg; 50 mg/kg permitted); As (up to 27.6 mg/kg; 20 mg/kg permitted) (data from Piątkowski and Skibicki, 1997).

The material for embryological study was collected in two successive years (1997–1998). The flowers and pods from the oldest inflorescences were immediately fixed in ethanol/acetic acid (3:1). Paraffin-embedded material was cut to 10 µm thickness and stained in Heidenhain's hematoxylin with alcian blue.

A total 740 ovules were studied (410 in 1997 and 330 in 1998). The results for developmental stages in ovules were noted for each pistil separately. Germination of the seeds harvested at the reservoir was tested on soil taken from their habitat and in petri dishes without medium.

The results of embryological study were compared with results from plants derived from seeds harvested at Żelazny Most and sown the following spring (1998 and 1999) in unpolluted standard soil on an experimental plot in Modlnica near Cracow.

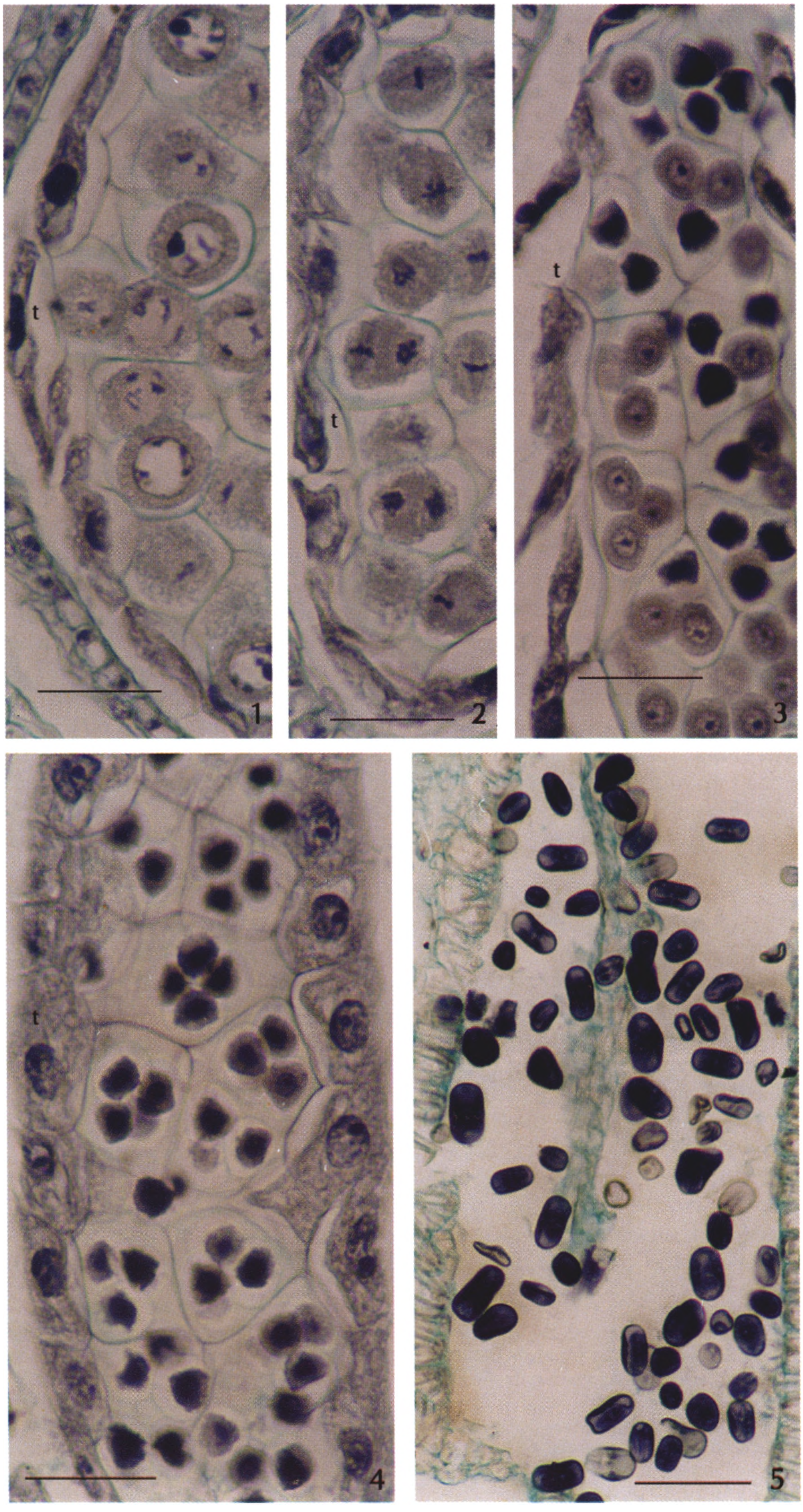
RESULTS

ANTHERS AND POLLEN GRAINS

Anther tapetum of secretory type showed great sensitivity to unfavorable environmental conditions. Almost all of the studied 80 flowers contained precociously degenerated tapetum layer in ~50% of their anthers. Signs of tapetal disintegration were observed in anthers throughout all stages of meiotic division in pollen mother cells, from prophase I to the tetrad stage (Figs. 1–3). The tapetal cells contracted and flattened, frequently separated from each other along their radial walls, and had smaller amounts of cytoplasm; their nuclei gradually became irregular in outline.

A small proportion of pollen mother cells showed disturbances in meiotic division, generally involving asynchronous and irregular distributions of chromosomes in anaphase I and II, producing unequal ploidy of microspores and possibly influencing their viability. At the tetrad stage, nearly 50% of the anthers per flower were filled with wholly degenerated tetrads; only sporadically, 1–2 microspores in a tetrad looked alive. The intensity of tapetal and tetrad degeneration was higher in 1997. In some anthers the degenerated tetrads were accompanied by tapetum of normal appearance (Fig. 4), but in others tetrad abortion was associated with precocious deterioration in the tapetum.

Figs. 1–5. *Vicia cracca* L. from polluted environment; degenerative processes in anthers. **Fig. 1.** Degeneration of tapetum during prophase I. **Fig. 2.** Degeneration of tapetum during meiotic division II. **Fig. 3.** Degeneration of tapetum at tetrad stage. Tetrads composed of all viable, some degenerated or all degenerated microspores. **Fig. 4.** Tapetum of normal appearance; most microspores are degenerated. **Fig. 5.** Mature pollen grains and microsporocytes. Bars = 30 µm in Figs. 1–4 and 50 µm in Fig. 5. t – tapetum.



Pollen samples collected from the inflorescences of a few plants showed large amounts of small irregular microspores (Fig. 5). In 1997, 47% of the pollen grain samples were apparently typical in size, shape and acetocarmine staining; 56% were typical in 1998. Typically formed pollen grains germinated on stigmas, and the pollen tubes grew into the embryo sacs.

The control material handled the same way did not show signs of precocious degeneration of tapetum; typically this tissue could be observed even at the 1-nucleate microspore stage (Fig. 6). In addition to the prevailing, morphologically normal tetrads there were tetrads with 1–2 degenerated microspores. In 1998, 87% of the pollen grain samples from the control plants were morphologically normal and tested positively with acetocarmine; in 1999 the figure was 84%.

OVULES

In pistils of *V. cracca* 4–7 ovules are usually formed; they are campylotropous, crassinucellate and bitegmic. A single megaspore mother cell gives rise to a linear tetrad of megaspores after meiotic division. The monosporous embryo sac develops according to the Polygonum type (Figs. 7, 8). The egg cell lies in a slightly lateral position and is adjacent to one of the two synergids (Fig. 9). The antipodals are ephemeral. During growth of the mature embryo sac the nucellar cells gradually disintegrate and the integumentary tapetum is formed (Fig. 7). Embryogenesis follows the Caryophyllad type, Medicago variation (Fig. 10).

Some of the ovules in the studied control material were eliminated from the process of seed production; besides those in which the young embryo sac degenerated, there were ovules containing mature but not yet fertilized embryo sacs, though the flower was after anthesis. The proportion of ovules developing into seeds (seed/ovule ratio) in the control plants was 85% in 1998 and 87% in 1999.

The plants growing at the foot of the waste reservoir showed various kinds of disturbances and degeneration processes, found in ~41% of the 410 (1997) and ~44% of the 330 (1998) ovules. A significant number of ovules showed arrested nucellar growth in early stages of ovule development, not observed in the control material. The top of the short nucellus in such ovules did not reach the vicinity of the micropylar canal; moreover, remnants of degenerated cells or degenerated embryo sac were usually visible in its central region (Fig. 11).

Another kind of developmental disturbance consisted in degeneration of extensive parts of the integumentary tapetum and the inner cell layer of the integument around the embryo sac. These changes did not resemble the signs of typical destruction of the nucellus or integumental tapetum in older ovules. The consequences of widespread degeneration around the embryo sac were abortion of the egg apparatus and destruction of polar nuclei (Figs. 12, 13). The egg cell also could degenerate in ovules not manifesting signs of degeneration of nucellar or integumental cells (Fig. 14). In such ovules the pollen tube often was visible in the micropylar canal.

A proembryo consisting of a few cells was the oldest developmental stage in which degeneration processes were observed (Fig. 15). Abortion of young embryos at early embryogenesis was found in ~8% in both years, in series of 65 and 97 ovules, respectively; it could occur in embryo sacs with normal endosperm development and also simultaneously with its degeneration. Globular proembryos as well as older embryos showed typical structure.

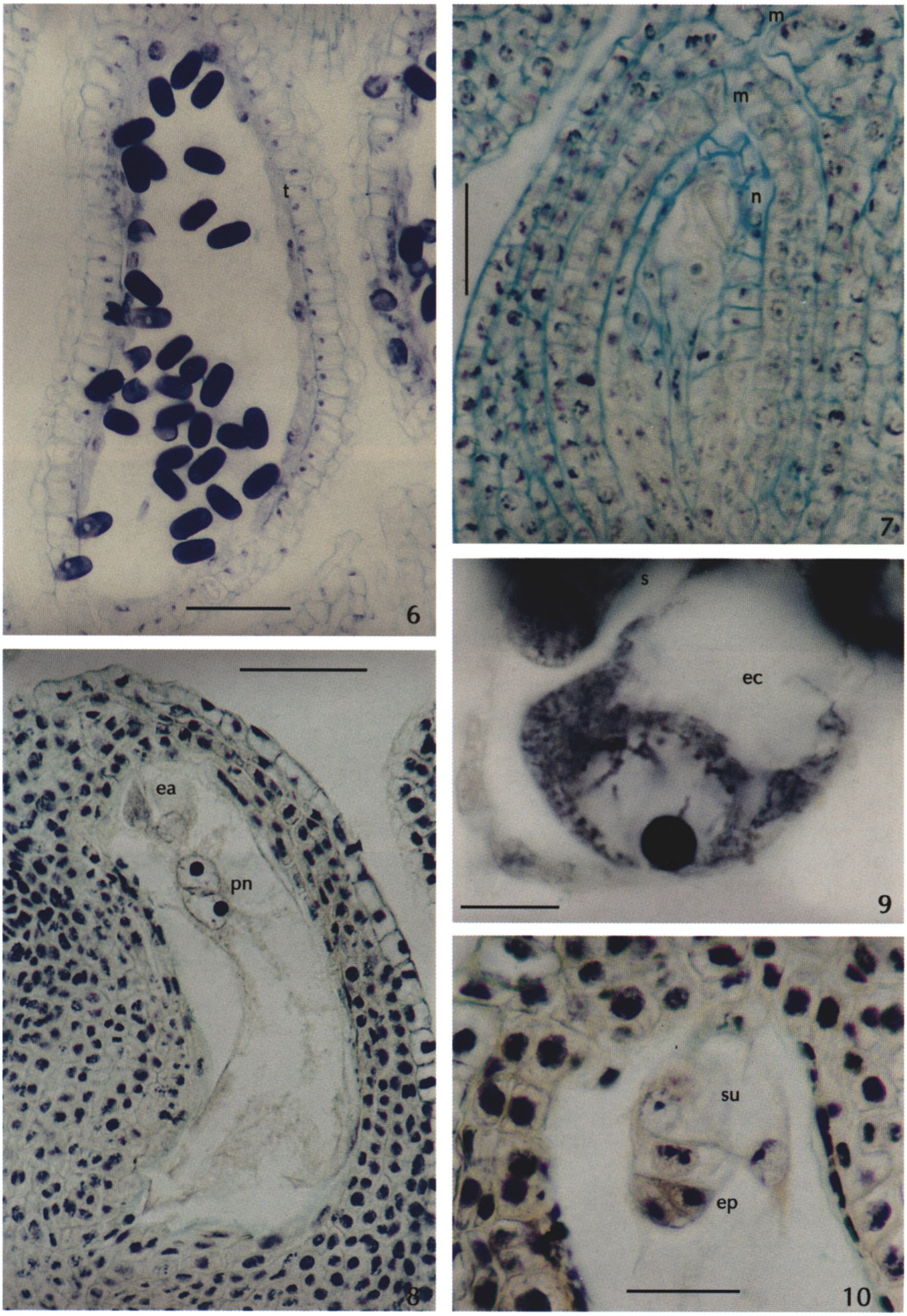
In plants from the polluted habitat the seed/ovule ratio was lower than in the control, as a result of developmental disturbances in ovule tissues and abortion of the gametophyte or of the embryo: 56% in 1997 and 59% in 1998. Degeneration processes were independent of ovule localization in the pistil.

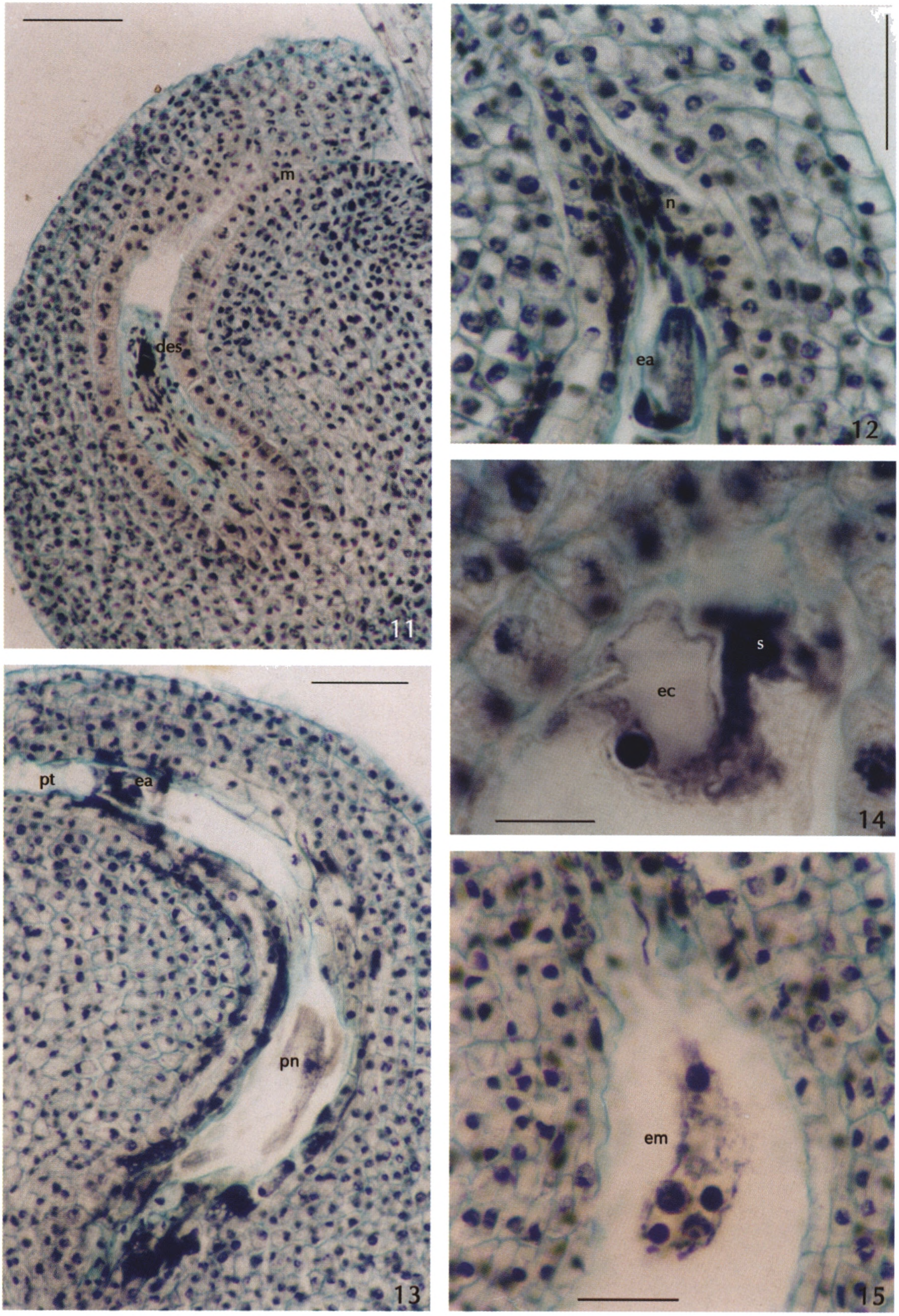
Of the well-developed seeds harvested from the polluted habitat, 99% germinated both in petri dishes and on soil taken from the contaminated habitat.

DISCUSSION

It has been well documented in many laboratory studies that some of the heavy metals essential for life processes in plants are very toxic at increased

Figs. 6–10. *Vicia cracca* L., control material. Some of developmental stages without disturbances or degenerations. **Fig. 6.** Mature pollen grains still accompanied by tapetum of normal appearance. t – tapetum. **Fig. 7.** Longitudinal section of ovule with 4-nucleate embryo sac (one nucleus visible in the section) surrounded by nucellus. Micropylar end of nucellus is seen near bottom of micropylar canal. n – nucellus; m – micropyle. **Fig. 8.** Longitudinal section of mature embryo sac with egg apparatus and polar nuclei. ea – egg apparatus; pn – polar nuclei. **Fig. 9.** Micropylar region of embryo sac with egg cell during fertilization and degenerated synergid. ec – egg cell; s – degenerated synergid. **Fig. 10.** Section of proembryo; embryo proper composed of quadrants, suspensor in initial stage of development. ep – embryo proper; su – suspensor. Bars = 50 µm in Figs. 6 and 8; 30 µm in Figs. 7 and 10; 10 µm in Fig. 9.


t
6
m
m
n
7
ea
pn
8
s
ec
9
su
ep
10


m
des
11
n
ea
12
pt
ea
pn
13
s
ec
14
em
15

concentrations. On the other hand, environmental studies in metalliferous regions have found metal-tolerant and metal-sensitive taxa, populations or individuals.

Plants growing on soils contaminated by heavy metals accumulate them in different amounts, and their distribution varies inside the organism. This is explained by reference to the sites of inactivation in excluders (in the roots) and accumulators (in the stems). Several authors (Wierzbicka, 1995a; Woźny, 1995, 1997; Ernst, 1999; and others) present mechanisms preventing the migration of metals from the roots to above-ground organs, differences in the mobility of various metals in a plant, and the ways toxic heavy metals are immobilized (detoxified). Lead concentrations much lower in shoots than in roots were found in seedlings of four test species in soil-water extracts (Whitby and Hutchinson, 1974).

Studies comparing the content of some metals in various plant organs of three species from zinc dumps (Godzik, 1993) showed that the highest amount of metals was accumulated in the leaves and stolons of *Biscutella leavigata*, in the stolons, leaves and perianth of *Rumex thyrsiflorus,* and in the underground parts of *Thymus pulegioides*. It is known, however, that the apical meristems and the region of the quiescent center in the root are highly protected against heavy metal accumulation (Lane and Martin, 1977; Przymusiński and Woźny, 1985; Wierzbicka, 1987); generative organs are usually classed together with these tissues. According to Shaw (1990; cited in Wierzbicka, 1995b), as a rule the ovules and particularly embryo sacs – and in consequence the seeds – are protected against lead even at high concentrations in vegetative organs. Seed coats in most plants effectively protect their interiors against direct contamination until they are damaged (Wierzbicka et al., 1986). In some species, however, in the final stage of imbibition the seed coats are permeable to metal ions (e.g., lead and barium ions), which penetrate the embryos and delay the germination process (Wierzbicka and Obidzińska, 1998). A number of studies show that metals can be transported in lower concentrations to flowers, fruits and seeds, in spite of mechanisms reducing the quantity of metals in above-ground organs. Merry et al. (1981) found that wheat grain from a polluted area near a lead-zinc smelter had higher concentrations of Zn, Pb and Cd than grain from non-polluted soils. Reeves and Baker (1984) established the presence of Ni in seeds of *Thlaspi geosingense* harvested on contaminated soil.

The results from studies of metal-tolerant plants of *Silene dioica* and *Mimulus guttatus* growing in nutrient solution with copper/zinc added indicated that seed abortion could be affected by toxic metals in the pistil (Searcy and Mulcahy, 1985b). The authors stated that in the treated plants the percentage of potentially functional pollen was significantly lower than in the control plants (Searcy and Mulcahy, 1985a).

Particularly interesting results were obtained in PIXE microanalysis of the distributions of several elements in seed tissues (Mesjasz-Przybyłowicz et al., 1998, 1999) of *Silene vulgaris* and *Gypsophila fastigiata* from zinc dumps. Zn was relatively homogeneously distributed throughout *Silene* seeds, with slightly higher amounts in the testa, hilum and endosperm. *Gypsophila* seeds accumulated much higher amounts of Zn, mainly around the hilum and in the radicle. Cu and Rb were found mainly in the embryo. According to the authors, the restricted allocation of Zn in embryonic tissues may allow *S. vulgaris* to maintain reproductive success in an environment of high Zn availability (Mesjasz-Przybyłowicz et al., 1999). Ernst (1999) suggests that "for the maintenance of metal-tolerant populations it is necessary to protect the seeds from a surplus of metals. As long as the metal exposure does not exceed the metal tolerance, metal-tolerant plants have no specific demand for the protection of the seeds." However, in view of the possible transport of small amounts of pollutants into the flower and seed tissues, not only metallophytes are interesting in this respect. One can suppose that reproduction of plants growing in areas of industrial polluted may be affected by contamination.

Observations concerning the succession of *Convolvulus arvensis* in the emission zone of a copper smelter showed that flower formation capacity

Figs. 11–15. *Vicia cracca* L. from polluted environment; degenerative processes in ovules. **Fig. 11.** Longitudinal section of ovule with nucellus arrested in growth, not reaching micropyle region. Remnants of degenerated young embryo sac visible in nucellus. m – micropyle; des – degenerated embryo sac. **Fig. 12.** Degenerated micropylar part of nucellus and aborted egg apparatus. n – nucellus; ea – egg apparatus. **Fig. 13.** Embryo sac with aborted egg apparatus and polar nuclei; degenerated cells of nucellus and of inner integument visible at chalazal pole and border of embryo sac. Remnants of pollen tube still visible in micropyle. ea – egg apparatus; pn – polar nuclei; pt – pollen tube. **Fig. 14.** Micropylar part of embryo sac with degenerating egg cell. ec – egg cell; s – degenerated synergid. **Fig. 15.** Abortion of young proembryo. em – proembryo. Bars = 50 μm in Figs. 11 and 13; 30 μm in Figs. 12 and 15; 10 μm in Fig. 14.

depended on the distance from the smelter. The plants nearest to it had retarded flowering, and seeds were seldom formed (Fabiszewski, 1983). Investigations of heavy metal tolerance in *Agropyron repens* from the same area (Brej, 1998) indicated that the stress conditions did not reduce seed production in this taxon. However, the population nearest the smelter had decidedly limited seed fertility and germination. Similarly, seeds of *Silene vulgaris* from a calamine waste heap had 45% germination capacity, indicating their poor quality (Wierzbicka and Panufnik, 1998).

Disturbances in embryological processes in the specimens of *V. cracca* growing at the Żelazny Most reservoir must be associated with the difficult conditions of the habitat, where a number of unfavorable factors including increased heavy metals content are present. Elimination of some ovules in early stages after arrested nucellar development or after degeneration of the young embryo sac may be the result of the action of pollutants or may be a life strategy providing nutrition to other ovules. Degeneration of embryo sac content, of the integumental tapetum or of the young proembryo, not found in the control material, probably is attributable to the unfavorable conditions of the site. Similarly, degeneration processes in some of the anthers, reducing the pollen/ovule ratio, probably were caused directly by environmental conditions. The effects of phytotoxic agents could have been intensified by decreased soil pH during a long period of rainy weather in 1997.

In a polluted area, species with short life cycles and strong reproductive powers can respond more rapidly than those with long life cycles (trees), and can achieve maximum tolerance in a few generations (Bradshaw, 1976). The studied specimens of *V. cracca* under stress conditions were able to complete the life cycle. Disturbances in embryological processes decreasing the seed crop may indicate that pioneer individuals of *V. cracca* entering this contaminated habitat spontaneously did not reach full tolerance to stress conditions. The present results suggest that the studied population of *V. cracca* is undergoing successful selection for tolerance to its contaminated environment. Selected plants may be dispersed continuously in the habitat by seed spread.

ACKNOWLEDGEMENTS

This study was funded by the Polish Committee for Scientific Research (KBN), project no. 6 PO4C 028 14.

REFERENCES

ANTOSIEWICZ DM. 1992. Adaptation of plants to an environment polluted with heavy metals. *Acta Societatis Botanicorum Poloniae* 61: 282–299.

BAKER AJM. 1987. Metal tolerance. *New Phytologist* 106 (Suppl.): 93–111.

BRADSHAW AD. 1976. Pollution and evolution. In: Mansfield TA [ed.], *Effects of air pollutants on plants*, 135–159. Cambridge University Press, Cambridge.

BREJ T. 1998. Heavy metal tolerance in *Agropyron repens* (L.) P. Bauv. populations from the Legnica copper smelter area, Lower Silesia. *Acta Societatis Botanicorum Poloniae* 67: 325–333.

CZABAN S, and MAŚLANKA W. 1998. Hydrologiczne i geotechniczne problemy eksploatacji składowiska odpadów poflotacyjnych "Żelazny Most". In: Przybylski T, Kurzydło H, Merkel B, and Althus M [eds.], *Rekultywacja i ochrona środowiska w regionach górniczo-przemysłowych*, 73–85. Towarzystwo Przyjaciół Nauk w Legnicy, Legnica.

ERNST WHO. 1999. Evolution of plants on soils anthropogenically contaminated by heavy metals. In: van Raamsdonk LWD, and den Nijs JCM [eds.], *Plant Evolution in Man-made Habitats. Proceedings VIIth Symposium IOPB*, Amsterdam 1998, 13–27. Hugo de Vries Laboratory, Amsterdam.

FABISZEWSKI J. 1983. Reagowanie populacji roślin na stresy jonowe. In: Fabiszewski J [ed.], *Bioindykacja skażeń przemysłowych i rolniczych*, 47–56. Ossolineum, Wrocław.

GODZIK B. 1993. Heavy metals content in plants from zinc dumps and reference areas. *Polish Botanical Studies* 5: 113–132.

KIJEWSKI P. 1998. Charakterystyka geochemiczna utworów powierzchniowych w zasięgu oddziaływania zakładów przemysłu miedziowego. In: Przybylski T, Kurzydło H, Merkel B, and Althus M [eds.], *Rekultywacja i ochrona środowiska w regionach górniczo-przemysłowych*, 49–61. Towarzystwo Przyjaciół Nauk w Legnicy, Legnica.

KIRK GJD, and BAJITA JB. 1995. Root-induced iron oxidation, pH changes and zinc solution in the rhizosphere of lowland rice. *New Phytologist* 131: 129–137.

KRAJEWSKI J. 1993. Ocena stopnia akumulacji wybranych metali ciężkich (Cu, Pb, As, Zn) w glebach Legnicko-Głogowskiego Okręgu Miedziowego. *Archiwum Ochrony Środowiska* 3-4: 221–237.

LANE BSD, and MARTIN ES. 1977. A histochemical investigation of lead uptake in *Raphanus sativus*. *New Phytologist* 79: 281–286.

MERRY RH, TILLER KG, DE VRIES MPC, and CARTWRIGHT B. 1981. Contamination of wheat crops around a lead-zinc smelter. *Environmental Pollution* Ser. B. 2: 37–48.

MESJASZ-PRZYBYLOWICZ J, GRODZINSKA K, PRZYBYLOWICZ WJ, GODZIK B, and SZAREK-ŁUKASZEWSKA G. 1998. Comparison between Zn distribution in seeds from a zinc dump in Olkusz, Southern Poland. *Proceedings Microscopy Society of Southern Africa* 28: 61.

MESJASZ-PRZYBYŁOWICZ J, GRODZIŃSKA K, PRZYBYŁOWICZ WJ, GODZIK B, and SZAREK-ŁUKASZEWSKA G. 1999. Micro-PIXE studies of elemental distribution in seeds of *Silene vulgaris* from a zinc dump in Olkusz, southern Poland. *Nuclear*

Instruments and Methods in Physics Research B 158: 306–311.

PIĄTKOWSKI J, and SKIBICKI K [eds.]. 1997. *KGHM Polska Miedź S.A. Ochrona Środowiska. Biuletyn 1997*, 1–61. Stowarzyszenie Ochrony Środowiska "BMS - Ekologia", Wrocław.

PRZYMUSIŃSKI R, and WOŹNY A. 1985. The reactions of lupin roots on the presence of lead in the medium. *Biochemie und Physiologie der Pflanzen* 180: 309–318.

REEVES RD, and BAKER AJM. 1984. Studies on metal uptake by plants from serpentine and non-serpentine populations of *Thlaspi geosingense* Hálácsy (Cruciferae). *New Phytologist* 98: 191–204.

SALEQUE MA, and KIRK GJD. 1995. Root-induced solubilization of phosphate in the rhizosphere of lowland rice. *New Phytologist* 129: 325–336.

SEARCY KB, and MULCAHY DL. 1985a. Pollen tube competition and selection for metal tolerance in *Silene dioica* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). *American Journal of Botany* 72: 1695–1699.

SEARCY KB, and MULCAHY DL. 1985b. Pollen selection and the gametophytic expression of metal tolerance in *Silene dioica* (Caryophyllaceae) and *Mimulus guttatus* (Scrophulariaceae). *American Journal of Botany* 72: 1700–1706.

TOMSETT AB, and THURMAN DA. 1988. Molecular biology of metal tolerances of plants. *Plant Cell and Environment* 11: 383–394.

WHITBY LM, and HUTCHINSON TC. 1974. Heavy-metal pollution in the Sudbury Mining and Smelting Region of Canada. II. Soil toxicity tests. *Environmental Conservation* 1: 191–200.

WIERZBICKA M. 1987. Lead accumulation and its translocation barriers in roots of *Allium cepa* L. – 0autoradiographic and ultrastructural studies. *Plant Cell and Environment* 10: 17–26.

WIERZBICKA M. 1995a. How lead loses its toxicity to plants. *Acta Societatis Botanicorum Poloniae* 64: 81–90.

WIERZBICKA M. 1995b. Oddziaływanie metali ciężkich na rośliny. *Kosmos* 44: 639–651.

WIERZBICKA M, and OBIDZIŃSKA J. 1998. The effect of lead on seed imbibition and germination in different plant species. *Plant Science* 137: 155–171.

WIERZBICKA M, and PANUFNIK D. 1998. The adaptation of *Silene vulgaris* to growth on a calamine waste heap (S. Poland). *Environmental Pollution* 101: 415–426.

WIERZBICKA M, ŁOBODZIŃSKA B, and GODZIK B. 1986. Seed coats as barriers for lead in plants resistant and nonresistant to heavy metals. *Postępy Biologii Komórki* 13: 377.

WOŹNY A [ed.]. 1995. *Ołów w komórkach roślinnych, pobieranie, reakcje, odporność.* [*Lead in plant cells uptaking reactions, resistance*], 1–56. Sorus, Poznań.

WOŹNY A. 1997. Odpowiedzi komórek roślinnych na obecność metali śladowych w ekosystemie. [Responses of plant cells to trace (heavy) elements of ecosystems]. In: Burchardt L [ed.], *Teoretyczne i praktyczne aspekty badań ekologicznych*, 35–57. Idee Ekologiczne 10. Ser. Szkice 6.