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Anatomy of abscission zone of *Betula pendula* (Roth.) leaves from trees growing under different levels of pollution

Abstract: A study was carried out on the leaf abscission zone from birch trees growing on polluted sites (two) and a non-polluted site (one). Samples for anatomical investigation were collected from six trees on each site, during three succeeding vegetation seasons. It was observed that in trees growing at the polluted sites: 1) maturation of the abscission zone was faster, 2) the protective layer was thinner and 3) the formation of leaf scar periderm was delayed in comparison with trees from the non-polluted site. The results obtained suggest that environmental pollution influences the formation of the abscission zone and the protection of the leaf scar.

Additional key words: anatomy of the leaf abscission zone, urban/industrial pollution

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Introduction

Abscission is the sequence of events whereby a multicellular organ (e.g. leaf, flower, fruit, branch) becomes separated from the parent body. This process is one of the most universal characteristics of plants and takes place in a region called the abscission zone. Separation of cells occurs in an area within the abscission zone termed the separation layer (Webster 1968, Kozlowski 1973, Osborne 1984, 1989, Leshem et al. 1986, Addicott 1991).

Seasonal shedding of leaves depends on climate and is a consequence of the natural process of leaf senescence (Moline and Bostrack 1972, Noodén and Leopold 1988, Kozlowski et al. 1991). Environmental conditions such as low light intensity, low or height temperatures, water stress or flooding accelerate the process of leaf abscission (Tang and Kozlowski 1982, Jankiewicz 1985, Park and Thimann 1990, Rosenthal and Camm 1996). Environmental pollution also acts as a factor accelerating senescence and leaf abscission (Noble and Jensen 1980, Kargiolaki et al. 1991, Tjoelker and Luxmoore 1991, Günthardt-Goerg et al. 1993, Pell et al. 1995, Bortier et al. 2000). Premature leaf abscission is usually preceeded by injury to the leaf blade. Most studies on the influence of environmental pollution on leaf abscission focus on physiological and biochemical aspects of metabolism, photosynthesis or growth of leaves (Constantinidou and Kozlowski 1979, Noble and Jensen 1980, Darral 1989, Eamus et al. 1990, Bücker and Ballach 1992, Führer et al. 1993, Günthardt-Goerg et al. 1993, Landolt et al. 1994, Rantanen et al. 1994). Studies concerning the anatomy and ultrastructure of the leaf blade under conditions of pollution have been also carried out (Mudd and Kozlowski 1975, Soikkeli 1981, Huttunen and Laine 1983, Barnes et al. 1988, Ebel et al. 1990, Zobel and Nighswander 1991, Mayo et al. 1992). It is known that under industrial pollution green leaves are abscised (Dugger and Ting 1970,

Mudd and Kozlowski 1975, Wiltshire et al. 1996). Abscission of green leaves suggests that pollution could influence the abscission zone directly. The purpose of this study was to follow anatomical changes during the development of the abscission zone of leaves from trees growing in sites differing in their degree of pollution.

Materials and methods

Studies were carried out on three sampling sites localised within Upper Silesia along an air pollution gradient (Fig. 1). At the first site, marked "I" (industrial pollution) trees grow in the direct vicinity of a huge steel mill (the Katowice Steel Mill). At the second site, marked "U" (urban pollution) trees grow along a busy road (Kochłowicka street in Katowice town). At the last site, marked "N" (non-polluted area) trees grow in a community with a domination of birch localised on the border of a meadow (in the vicinity of Mikołów-Mokre town). Although the site "N" is surrounded by an industrial area, the trees look typical, without any signs of environmental stress. At all three sites the trees are not growing in forest stands.

Sampling sites were established in relatively uniform and representative portions of the stands. Plant material for anatomical analysis was collected from six trees (20–25-year-old; *Betula pendula* Roth.) on each site during successive vegetation seasons (1998, 1999, 2000): from April to August – once a month, from September to November – twice a month (every two weeks). Samples included the base of the oldest leaf. Samples were fixed in 3% glutaraldehyde buffered at 6.8 pH with 0.2 M phosphate buffer overnight at room temperature, washed three times in phosphate buffer, dehydrated gradually in an alcohol series, transferred through propylene oxide and embedded in Epon 812. After Epon polymerisation, samples were cut as cross and longitudinal sections (3 mm thick) with an ultramicrotome. Sections were attached to microscope slides with Houpt's glue. Sections were then stained: with leucofuchsin (PAS-reaction + toluidine blue) to view cell walls; with Sudan IV for localisation of lipid substances; phloroglucinol + HCl for localisation of lignin. Fresh, hand-cut sections were stained with toluidine blue O. Sections were analysed under light, fluorescence and differential-interference contrast microscopes (Olympus-Provis) with a photo-camera (Olympus). Cell size and thickness of the protective layer were assessed statistically (Student t-test, $p<0.05$).

Results

Anatomy of the mature abscission zone

The abscission zone of the birch leaf is situated in the basal part of the petiole. The mature abscission zone consists of three cell layers: a separation layer, a protective layer and the periderm of the abscission zone (Fig. 2). The separation layer is composed of several rows of thin-walled cells. Sometimes the number of rows in the separation layer on the adaxial

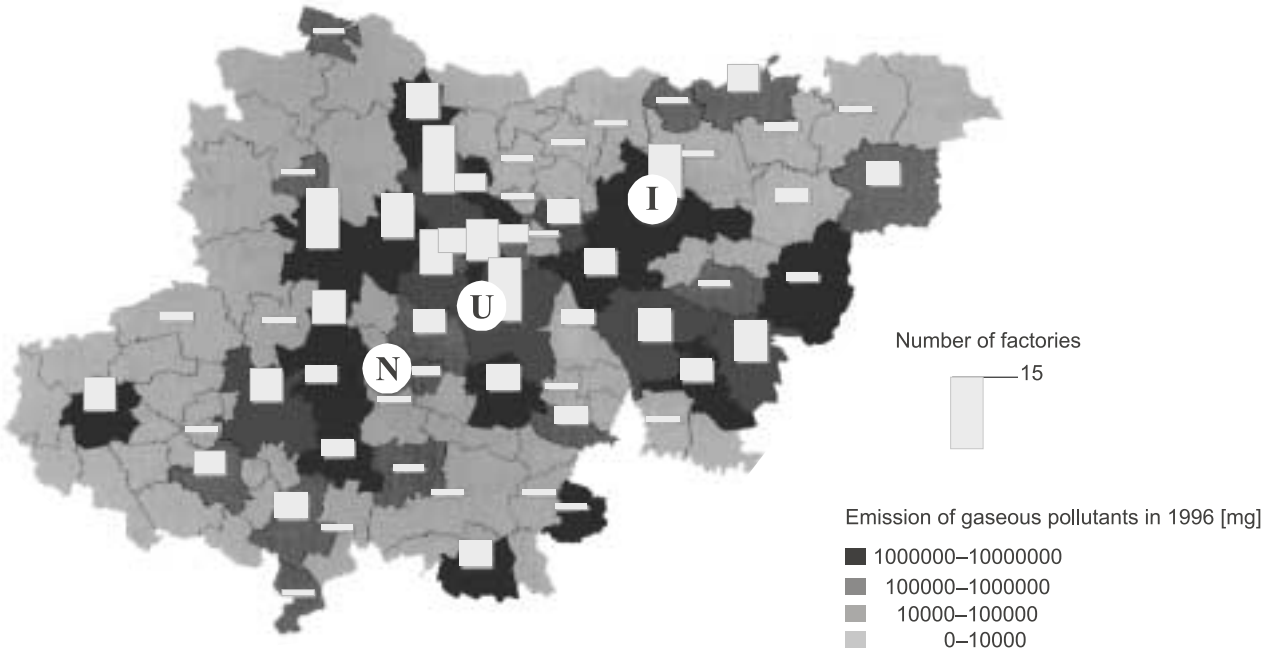

Fig. 1. Localisation of the investigation sites within Upper Silesia, the emission of gaseous pollutants and the number of factories; I – industrial pollution, U – urban pollution, N – not polluted area (modifiedafter Jarzębski 1997)

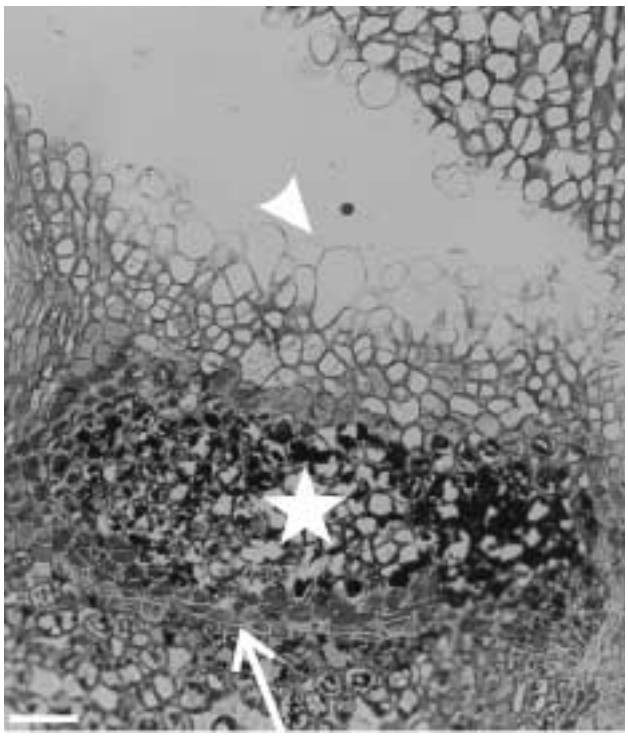

Fig. 2. Anatomy of mature abscission zone of birch leaf (arrowhead = separation layer, asterisk = protective layer, arrow = periderm; bar = 50 μm)

side of the leaf base is different from that on the abaxial side. The separation layer distally is limited by parenchyma cells of the petiole whose walls are positively stained with phloroglucinol. The protective layer is composed of cells with lignified and suberized walls. The cell walls of the abscission zone periderm are characterised by suberin lamella. The periderm of the abscission zone is connected to the periderm of the stem. In the mature abscission zone the cytoplasm of cells in the separation layer is stained blue with toluidine blue. Just before separation of cells in the abscission zone, the cytoplasm of cells in the separation layer is stained green with toluidine blue. The cytoplasm of cells in the protective layer and in the periderm does not show a positive reaction on staining with toluidine blue at any stage of abscission zone development. Vascular tissue in the abscission zone is characterised by short tracheary elements. Cell ends of neighbouring tracheary elements are localised at the same level, namely at the level of the separation layer just beneath the protective layer.

Marker of the area predestined for formation of the leaf abscission zone

Studies on the maturation of the abscission zone were undertaken to find an anatomical marker to indicate at an early stage the area in which the abscission zone would be formed. This marker does exist. Epidermal external walls together with the cuticle localised on the adaxial side of the leaf base are

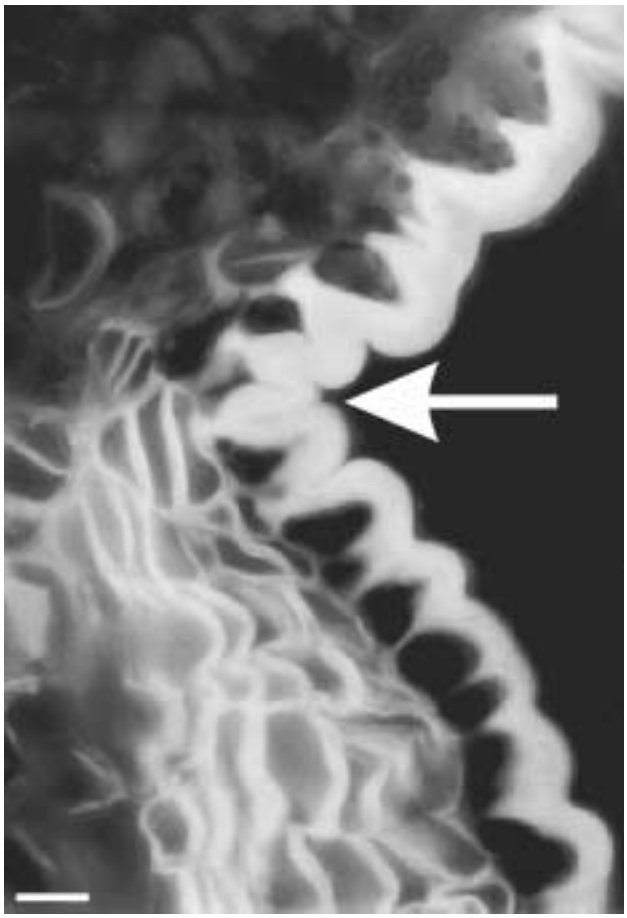

Fig. 3. Longitudinal section through the leaf base. The thickness of epidermal external walls with cuticle as a marker of the area destined for formation of the abscission zone. Epidermis of petiole is characterised by thicker walls and epidermis of stem is characterised by thinner walls (the border is indicated by arrow; bar = 10 μm)

thicker than the epidermal walls on the stem (Fig. 3). This difference is evident during the whole vegetative season starting from April, when the first samples were collected. For this reason, the thickness of the epidermal external walls together with the cuticle was our marker of the area predestined for formation of the abscission zone.

Maturation of abscission zone

The first anatomical sign of the commencement of maturation of the abscission zone was the size and shape of the cells. Namely, in samples collected in April, cells from the area predestined for formation of the abscission zone differed in size and shape from parenchyma cells of the petiole and stem. Cells of the future abscission zone were isodiametrical, while parenchyma cells of the petiole and stem had longitudinal walls longer than the transverse walls. The longitudinal walls of cells in the abscission zone were shorter (14.8 mm) than the longitudinal walls of petiole cells (21 mm) or stem cells (17.7 mm) and these differences were statistically significant. There was

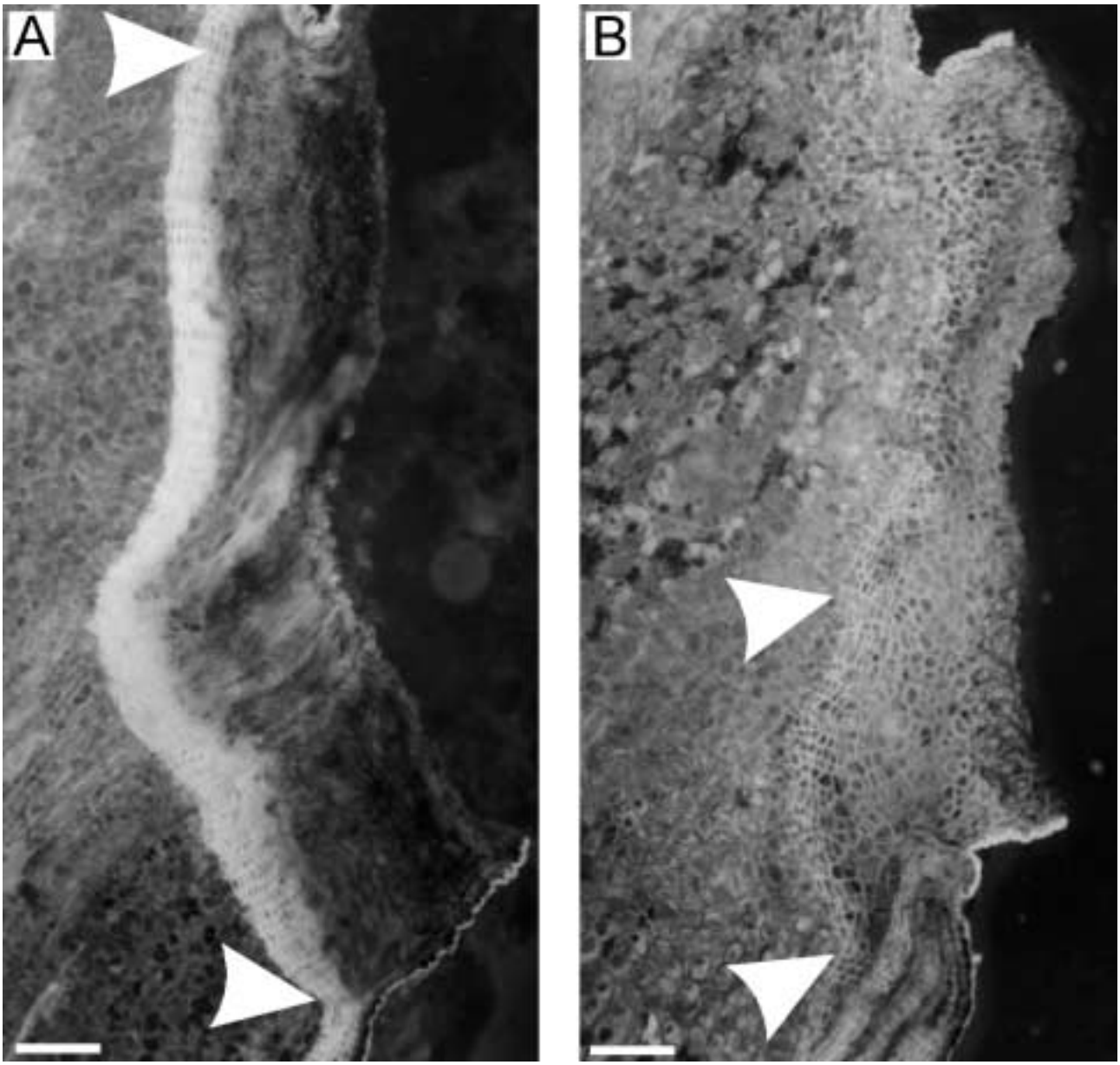

Fig. 4. Leaf scar just after leaf abscission (A) from the not polluted site and (B) the polluted sites (the range of periderm is indicated by arrowheads; bar = 100 μm)

no significant difference between the mean transverse dimension of cells in the abscission zone (13.8 mm) and petiole cells (12.9 mm) or stem cells (13.4 mm). Arrangement of cells in the area of the future abscission zone was different in comparison with the arrangement of parenchyma cells in the petiole and stem. Namely, parenchyma cells in petiole and stem were arranged in regular columns, whereas it was difficult to set any order of cells in the future abscission zone. Moreover, cells in the area predestined for abscission zone formations were characterised denser cytoplasm.

The next step of maturation of the abscission zone was the formation of the protective layer. The first mature (lignified and suberized) cells of the protective layer were visible in samples collected from trees growing at site "I" at the end of July. In samples from trees growing at sites "U" and "N", mature cells of the protective layer were visible from the middle of August.

In samples collected from trees growing at sites "I" and "U" maturation of cells in the protective layer of the abscission zone was finished in the first half of September, while in samples from trees growing at site "N" maturation of the protective layer was finished by the beginning of October. The mature protective layer of the abscission zone on the adaxial side of the leaf base was significantly thicker in leaves from site "N" (292.5 mm) than those from sites "I" (169.1 mm) and "U" (186.9 mm). The difference in thickness of the protective layer was the result of decreased cell number in the protective layer. Another

step in the maturation of the abscission zone was the formation of a periderm. This process started in September in trees growing at both the polluted and non-polluted sites. The last step in maturation of the abscission zone was the formation of a separation layer. The separation layer was formed when the protective layer was fully mature (when the protective layer was present across the whole leaf base). It was observed that in leaves from trees growing at the polluted sites the separation layer was formed earlier in comparison with the non-polluted site.

The development of protective tissues continued after abscission of the leaf and finally the scar was protected by a mature periderm. In samples of leaf scar from trees growing at the non-polluted site, the periderm was present across the whole scar just after abscission of the leaf (Fig. 4A). In samples from both polluted sites the periderm of the leaf scar was not fully formed (Fig. 4B). In these samples just after leaf abscission the peridem of the scar was visible only on the adaxial side.

Discussion

Our anatomical analysis of the leaf abscission zone shows that the development of the abscission zone was different in trees growing at the polluted and non-polluted sites. The most important differences were: faster maturation of abscission zone, thinner abscission zone, and delayed formation of leaf scar periderm in trees growing at the polluted sites in comparison with trees from the non-polluted site.

Information about the influence of pollution on abscission zone is important as it is known that in a polluted environment green leaves are abscised (Dugger and Ting 1970, Mudd and Kozlowski 1975, Wiltshire et al. 1996), which suggests that pollution influences the abscission zone directly. In the literature there is only one (according to the author's knowledge) report on the influence of pollutants on the development of the abscission zone. Namely, studies on bean plants showed that treatment with cadmium accelerates the formation of the abscission zone (Vázquez et al. 1989). This result seems to be compatible with our observation that in polluted sites differentiation of birch leaf abscission zone is faster that in control sites. However, in Vázquez et al. (1989) studies the pollutant (cadmium) was supplied to the roots whereas in our investigations the whole plants were exposed to pollution.

In the present study it was showed that the abscission zone in trees from polluted sites was thinner than in trees from the non-polluted site. The difference in the thickness of the abscission zone was due to the decreased cell number in the protective layer. Moreover, in trees growing at the polluted sites the formation of the leaf scar periderm was delayed in comparison with trees from the non-polluted site. Those observations indicate that in trees from the polluted sites cell divisions in protective tissues were inhibited in comparison with the non-polluted site. This suggests that pollution decreased the number of cell divisions and influenced the differentiation of cells in protective tissues. The influence of pollution on cell division and differentiation is well known: sulphur dioxide inhibited cell divisions in conifer needles (Halbwachs 1984), ozone decreased cell divisions in stems (Matyssek et al. 1993, Kurczyńska et al. 1998) and suppressed formation of stem periderm (Günthardt-Goerg et al. 1993), urban/industrial pollutants decreased the cell divisions in stems (Robitaille 1981, Thompson 1981, Kurczyńska et al. 1997).

Under normal conditions the protective tissues of the leaf scar are mature before leaf abscission (Osborne 1984). If this process is not completed then trees are not sufficiently protected against pathogen attack and desiccation (Leshem et al. 1986). It is known that in polluted environments trees suffer because of pathogens (James et al. 1980). It seems possible that predisposing trees to pathogens in polluted environments could be explained by the suppressed formation of leaf protective tissues observed in the studies.

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